Alicante, 24 | 27 de mayo de 2022

VIII Congreso Red Española de Canales Iónicos

RECI

Red Española de Canales Iónicos

PRESENTATION

Dear friends,

I am very pleased to write this introductory note to announce the face-to-face organization of the RECI biennial meeting in its eighth edition. As you know, RECI VIII, originally scheduled in Granada on May 2021, had to be postponed to 2022 due to the mobility restrictions imposed by the Covid pandemic that we are suffering. This has also been the reason for the change of venue to Alicante.

Fortunately, the health crisis has substantially improved, which has made it possible to relax the conditions for mobility and the organization of events, which allows us to schedule the meeting. Although we still have a degree of uncertainty, now increased by President Putin's decision to start an unusual and inexplicable war in Europe. I hope that common sense imposes and paralyzes this senseless aggression, and the word is used to resolve the conflict created.

Hoping for a prompt resolution, we are grateful to have the opportunity to meet this year and share excellent moments that strengthen scientific and personal ties. With this objective we have tackled the challenge of organizing RECI VIII, i.e. the meeting of a network whose first meeting was at the Institute of Neurosciences (UMH-CSIC) in San Juan on May 16-18, 2007. Fifteen years have passed, and the Network maintains the same spirit and desire to make visible and promote research on ion channels at a national and international levels. Since then, we have grown, maintaining an open network, without fees, but committed, to the best of its ability, to promoting and supporting both research and training in ion channels in an interdisciplinary manner. And I trust that it will be so in the coming years.

And 15 years later, we return to San Juan. This time to the San Juan Complex, a complex that brings together all the facilities in an integral way for the organization of a meeting like RECI. The complex has a range of services to enjoy scientific discussions in relaxing environment. The integration of its services has made it possible to organize RECI VIII in record time, with guarantees of comfort and tranquility for those attending. I hope you find it pleasant, and you can enjoy the center.

We propose a quality scientific program that the organizing committee has prepared in record time and to whom I am very grateful for their dedication and enthusiasm. We start the Italo-Spanish-Portuguese-French course on Ion channels and transporters with the course, which will have 4 speakers from the countries involved. This course typically brings together 30-40 students, who enjoy the latest advances in ion channel and transporter research. It is usually a course also organized biennially, lasting 2 days, which we have adapted to the RECI VIII meeting so as not to miss the opportunity to offer it. As you will see, we have made a pack for students so that they can enjoy both events. I encourage students to take advantage of the offer and attend the course and the RECI meeting. And I also encourage seniors to encourage their students to participate.

After the course, we continue with the RECI VIII meeting that has been organized to give young people a leading role. Thus, the 2-hour symposia will have 2-3 25-minute talks, and the rest will consist of short presentations selected from the poster communications received. For the posters we have reserved 3 hours with the aim of making them the main meeting point and discussion of results and a stimulus for young researchers. Of course, we have the 3 plenary sessions. It is worth noticing that Dr. Luis Enjuanes sesions on Covid is apparently distant from ion channels, but I think it will surprise us since this virus also has vital proteins that form ion channels.

Of course, we have also incorporated relaxing moments at the welcome reception, lunches and dinners, which we will enjoy in the San Juan Complex. These moments with a beer or wine will allow to enjoy more personal relationships, with a weather that is still very pleasant in Alicante.

Noteworthy, we have kept the registration fees affordable for everyone who wants to attend and participate. I trust that you will find the program and venue very attractive to attend. I trust to see you all and greet you personally in San Juan on May 24-27. Many thanks to all! And see you soon...

Antonio Ferrer

COURSE PROGRAM

Tuesday, May 24th

13:00-15:30 h **REGISTRATION ISPF**

16:00-16:30 h **OPENING CEREMONY**

16:30-17:30 h **PLENARY 1. FRANCE**

Gain and loss of function in channelopathies: a focus on sodium channels in epilepsy and migraine.

Chair: Florian Lesage
Speaker: Massimo Mantegazza (France)

17:30-18:00 h **COFFEE BREAK**

18:00-19:30 h **PLENARY 2. SPAIN**

Conformational plasticity of ion channels: a fluorescence-based approach

Chair: Antonio Ferrer
Speaker: José Antonio Poveda Larrosa y Lourdes Renart

19:30-20:00 h **DISCUSSION**

20:30-22:00 h **DINNER**

Wednesday, May 25th

09:30-11:00 h **PLENARY 3. ITALY**

Investigating the retinal network ex-vivo

Chair: Felix Viana
Speaker: Stefano Di Marco

11:00-11:30 h **COFFEE BREAK**

11:30-13:00 h **PLENARY 4, SPAIN**

Modulation of TRPA1 channels by the chaperone Sigma-1Recepetor as a neuroprotective strategy against peripheral neuropathy by chemotherapy

Chair: Ana Gomis
Speakers: Elvira de la Peña and Jorge Fernández Trillo

13:00-13:30 h **DISCUSSION AND CLOSING CEREMONY**

13:30-16:00 h **LUNCH FOR ISPF**

CONGRESS PROGRAMME

Wednesday, May 25, 2022

15:00 h **REGISTRATION RECI**

16:00-16:30 h **OPENING CEREMONY**

16:30-17:30 h **OPENING LECTURE**

Chair: Juan A. Rosado
Speaker: Oscar Casis. *Universidad del País Vasco*

17:30-19:30 h **SYMPOSIUM 1. ION CHANNELS IN PHYSIOLOGY AND PATHOLOGICAL CONDITIONS**

Chairs: Asia Fernández Carvajal and Antonio Felipe

Presentations (20 +5 min)

- **Anticonvulsant and neuroprotective roles of SGK1.1**
  Teresa Giráldez. *U.La Laguna*
- **Dominant negative effect of an *SCN5A* variant in native cardiac sodium channels**
  Fabiana S. Scornik. *U. Girona*
- **The Volume-Regulated Anion Channel VRAC: recent physiological and pathological findings**
  Rosa Planells. *Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP)*

Short presentations (9+2 min)

- **Adenosine modulates Kv1.3 by complementary PKC and PKA pathways in dendritic cells**
  Irene Estadella Perez. *U. Barcelona*
- **Electrophysiological effects of IQM-266 on Itof. Role of cardiac beta subunits**
  Angela de Benito. *Instituto de Investigaciones Biomédicas 'Alberto Sols' (CSIC-UAM)*
- **Revealing the role of the mechanosensitive Piezo1 channel in controlling endosome trafficking for efficient cytokinetic abscission**
  Julia Carrillo Garcia. *U. Pompeu Fabra*
- **Oxaliplatin time course effect on a pre-clinical in vitro model of male and female rat DRG neurons**
  Eva Mª Villalba Riquelme. IDiBE, *U. Miguel Hernández*

20:30-22:00 h **RECEPTION**

Thursday, May 26, 2022

9:00-11:00 h **SYMPOSIUM 2. VIRAL PROTEIN CHANNELS**

Chair: Vicente Aguilella

Presentations (20 +5 min)

- **Viroporins from a historical perspective: still a bone of contention?**
  José Luis Nieva. *UPV-Biofisika, Bilbao*
- **The envelope protein channel of SARS-CoV-2: structure-function and future perspectives.**
  Jaume Torres*. Nanyang Tech. U. Singapur*
- **Electrophysiological characterization of viroporin ion channels regulated by lipid-protein interactions**
  Antonio Alcaraz. *U. Jaume I, Castellón*

Short presentations (9+2 min)

- **Controlling the release of apoptogenic factors; the role of viral Bcl2s' transmembrane domains**
  Luis Martínez Gil. *UVA*
- **Effects of Sars-Cov-2 envelope protein on store-operated channels and calcium homeostasis in human lung microvascular endothelial cells**
  Sendoa Tajada. *IBMG UVA/CSIC*
- **The viroporin SARS-CoV-2 envelope protein induces calcium release from intracellular stores and apoptosis in rat hippocampal neurons**
  Sara López Vázquez. *IBMG UVA/CSIC*

11:00-12:00 h **COFFEE AT POSTERS**

12:00-14:00 h **SYMPOSIUM 3. Ca^{2+} IN HEALTH AND DISEASE**

Chair: Juan Antonio Rosado

Presentations (20 +5 min)

- **Orai1 facilitates post-ischemic angiogenesis after myocardial infarction through Notch1 signaling pathway**
  Tarik Smani. *IBiS-U. Sevilla*
- **Store Operated Calcium Entry remodelation in Breast cancer**
  José Javier López. *U. Extremadura*
- **Calcium Channel remodeling in rat hippocampal neurons aged in vitro**
  Lucía Nuñez. *UVA*

Short presentations (9+2 min)

- **Polyamine depletion reverses transcriptomic as well as calcium remodeling in colon cancer cells**
  Enrique Pérez Riesgo. *IBGM (CSIC-UVa)*
- **Orai1α controls TRPC1 channel location and function in HeLa cells.**
  José Sánchez-Collado. *U. Extremadura*

- **Calmodulin is critical for folding of the Kv7.2 calcium responsive domain as the nascent peptide exits the ribosome**
  Arantza Muguruza-Montero. *Instituto Biofisika*
- **The effects of aging on pancreatic β-cell function involve multiple events in the regulation of insulin secretion**
  Iván Quesada. *IDiBE-UMH*

14:00-16:30 h **LUNCH AND POSTERS**

16:30-18:30 h **SYMPOSIUM 4. ION CHANNELS AND PAIN**

Chair: Ana Gomis

Presentations (20 +5 min)

- **Piezo2 channels: from structure to nociception**
  Paco Taberner. *Inst Neurociencias-UMH/CSIC*
- **Clues to understanding TRPC5 as a cold-activated channel and its inhibition by the analgesic duloxetine**
  Lucie Zimová. *FGU-CAS, Praga*
- **Skin sensory glia as detector cells for pain and touch**
  Laura Calvo-Enrique. *U Salamanca*

Short presentations (9+2 min)

- **The endocrine disruptor bisphenol A regulates sodium Nav1.7 ramp currents in mouse dorsal root ganglion neurons and increases nociception**
  Juan Martínez-Pinna. *U. Alicante- IDiBE*
- **Discovery of new TRPM8 modulators and their therapeutic potential**
  Alicia Medina. *IDiBE-UMH*
- **Light-gated channel Channelrhodopsin-2 improves neuroregenerative potential of Neural Precursors Cells after Spinal Cord Injury (SCI)**
  M. del Mar Sanchez Martín. *CIPF-Valencia*
- **Testosterone-TRPM8 interactions drive pain resilience in a mouse model of chronic migraine**
  David Cabañero Ferri. *IDiBE-UMH*

18:30-19:30 h **RECI PLENARY**

Chair: Vicente Aguilella
Speaker: Luis Enjuanes. *CNB-CSIC, Madrid*

20:30-23:00 h **CONGRESS DINNER**

Friday, May 27, 2022

09:00-11:00 h **SYMPOSIUM 5. PHOTOPHARMACOLOGY AND DRUG DISCOVERY**

Chairs: Rosario González Muñiz and Francisco Ciruela

Presentations (20 +5 min)

- **Enantiopure lactams as NMDA receptor antagonists**
  María M M Santos. *U. Lisboa*
- **Controlling receptor activity with photoswitchable drugs: basic research and future therapies**
  Pau Gorostiza. *Inst. for Bioengineering of Catalonia*
- **Purinergic photopharmacology: towards new therapeutic opportunities**
  Francisco Ciruela. *U. Barcelona*

Short presentations (9+2 min)

- **Evaluation of the EAR-20 peptide as a positive allosteric modulation of NMDA receptors**
  Roberto García. *U. Barcelona*
- **Ion channel based development of biosensors for discovering improved treatments for amyotrophic lateral sclerosis**
  Sara Alicante. *Instituto de Biofisika, UPV*
- New chiral heterocyclic compounds with potent TRPM8 antagonist activity
  Carolina Izquierdo. *Instituto de Química Médica, IQM-CSIC*
- **Structural and functional insights into TRPM8 modulation mediated by the immunosuppressant macrolide Rapamycin**
  Khalid Oudaha. *Instituto de Neurociencias, UMH-CSIC*

11:00-12:00 h **COFFEE AT POSTERS**

12:00-13:00 h **CLOSING PLENARY**

Chair: Rosario González Muñiz
Speaker: Robin S. Bon.

- **Understanding TRPC1/4/5 channels through chemical/structural biology**

13:00-13:30 h **CLOSING CEREMONY**

13:30-15:30 h **LUNCH AND FAREWELL**

ORGANIZING AND SCIENTIFIC COMMITTEES

Vicente Aguilella
Universidad Jaume I Castellón de la Plana

Asia Fernández Carvajal
Universidad Miguel Hernández de Elche

Antonio Ferrer Montiel
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Ana Gomis
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Antonio Felipe
U. Barcelona

Felix Viana
UMH-CSIC

Paco Ciruela
U. Barcelona

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COURSE PLENARY SESSIONS

01. Gain and loss of function in channelopathies: a focus on sodium channels in epilepsy and migraine

Massimo Mantegazza
Institute of Molecular and Cellular Pharmacology (IPMC)
University Côte d'Azur, CNRS UMR7275 and Inserm
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Ion channels are major targets of genetic neurological diseases. Understanding the functional effect of genetic variants is important for shedding light on pathological mechanisms, stratify patients and select/develop treatments. Notably, pathologic genetic variants of the same ion channel can often induce either gain- or loss-of-function, leading to different phenotypes, sometimes with counterintuitive mechanisms.

I will address these issues focusing on sodium channels, in particular the voltage-gated sodium channel $Na_V1.1$ (*SCN1A* gene) which is a major target of human variants implicated in epilepsy and migraine.

02. Conformational plasticity of ion channels: a fluorescence-based approach

Jose Antonio Poveda Larrosa Lourdes Renart
IDiBE, U. Miguel Hernandez de Elche

X-ray crystallography has been fundamental to provide high-resolution snapshots of the atomic structure of proteins, including the superfamily of ion channels. However, due to the harsh experimental conditions used in this and other high-resolution techniques (e.g., NMR), protein dynamics could be hampered. This way, complementation with milder biophysical techniques is highly required.

Our research group has been studying the molecular basis of the functional properties of K+ channels (ion conduction, selectivity, and inactivation) by using simpler model prokaryotic representatives and applying different fluorescent approximations. Steady-state intrinsic fluorescence from tryptophan residues turned out to be a good reporter of the conformational plasticity of the selectivity filter (SF), the domain mainly responsible for the selection and permeation of K+ and considered as one of the gates that modulates the channel activity. A deeper study was performed by a time-resolved characterization of single-Trp mutants. Here, the presence of a homo-Förster Resonance Energy Transfer (homo-FRET) process allowed for the characterization of the interplay between the SF and the pore-helices conformation and to calculate the Trp-Trp intersubunit distances in different experimental conditions. Furthermore, the selective labelling of the activation (intracellular) gate of the protein with an extrinsic probe, together with the occurrence of a homo-FRET process among these dyes, allowed us to study the allosteric communication between this domain and the selectivity filter, a key process to understand the function and modulation of K+ channels. The description at the molecular level of this interplay is mandatory to develop new "allosteric drugs", able to modulate this communication, which has proven to be very selective and potent.

Both the steady-state and time-resolved fluorescence approaches, combined with our own functional characterization and the available NMR and X-ray data, has allowed us to propose new hypothesis on the molecular basis of ion conduction, selectivity and inactivation.

03. Investigating the retinal network ex-vivo

Stefano Di Marco

Fondazione Istituto Italiano di Tecnologia, Center for Synaptic Neuroscience and Technology (NSYN)
IRCCS Ospedale Policlinico San Martino, Genova

Although located in the periphery, the retina is a part of the Central Nervous System (CSN): a small piece of the brain that evolution has placed at the back of our eyes. Retina shares with the rest of CSN the same neurotransmitters, basic synaptic mechanisms, receptors and circuitry organization.

Moreover, the retina is easily accessible to the experimenter, and it is possible to study its physiology ex-vivo, at conditions proximal to those in-vivo. The system has a defined input: photoreceptors, which can be stimulated using the very natural stimulus: light, and a defined output: retinal ganglion cells, which encode the stimulus using sparse spiking codification. Between the input and the output, there is the retinal network: a black box that can be dissected electrophysiologically and morphologically using different techniques.

In the talk, we will dissect, using the patch-clamp technique, a phenomenon of short-term plasticity of the retina: contrast adaptation, to observe how excitatory and inhibitory inputs concur to preserving the ability to filter stimuli space and time, or, in other words, the spatial receptive field properties of retinal ganglion cells.

Finally, I will introduce a high-density multi-electrode array platform (3Brain Biocam X) to simultaneously record the extracellular light-evoked activity from thousands of retinal ganglion cells from pieces or entire whole-mount retinas. This technique will be exploited to demonstrate the efficacy of a photochromic molecule: Ziapin, in restoring light responsivity from blind retinas. Interestingly, we demonstrate for the first time that a single molecule can restore the variety of specific and complex patterns of response to light of the different physiological classes of retinal ganglion cells.

04. Modulation of TRPA1 channels by the chaperone Sigma1Receptor as a neuroprotective strategy against peripheral neuropathy by chemotherapy

Jorge Fernández-Trillo y Elvira de la Peña

Instituto de Neurociencias, UMH-CSIC, San Juan de Alicante, Spain

Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent, adverse side effect of many anticancer drugs. Oxaliplatin, a platinum compound used in the treatment of advanced colorectal cancer and other solid tumors, often leads to a form of painful peripheral neuropathy characterized by mechanical and cold hypersensitivity.

TRPA1 channels play a fundamental role in chemonociception, as molecular transducers of reactive irritants, stress and tissue damage. Their role in cold and mechanical nociception has also being described. Sigma-1 Receptor (σ-1R) is a molecular chaperone that modulates both trafficking and function of various ion channels. In mice, the σ-1R antagonist, S1RA, is able to reduce the symptoms of neuropathic pain. Since the molecular determinants of this antinociceptive effect remain unknown, we studied a possible modulation of TRPA1 by σ-1R.

We performed calcium imaging and patch-clamp experiments in HEK293 cells transfected with hTRPA1 tagged with tGFP (hTRPA1-tGFP). Incubation of these cells with S1RA decreased the amplitude of [Ca2+]i responses and of the membrane currents, evoked by AITC. Similar results were obtained with BD1086, another σ-1R antagonist. FRET experiments in cells transfected with hTRPA1-tGFP and mCherry- σ-1R revealed that both proteins are localized at a distance compatible with a physical interaction that was supported by co-immunoprecipitation experiments. Treatment of these cells with S1RA decreased FRET levels, suggesting an impairment or conformational change of this putative interaction. Finally, TIRF-FRAP experiments indicate that S1RA reduces the trafficking of TRPA1 towards the plasma membrane, resulting in a reduction of TRPA1 expression at the plasma membrane which was confirmed by cell surface biotinylation assays.

These results suggest a role TRPA1 in the anti-nociceptive effects of σ-1R antagonists.

Funding source: SAF2016-77233-R, PID2019-108194RB-100, co-financed by ERDF, Severo Ochoa Program SEV-2017-0723 and GRISOLIA/2015/034. MINECO project PID2019-108194RB-100 and Generalitat Valenciana PROMETEO/2021/031.

VIII Congreso Red Española
de Canales Iónicos

OPENING LECTURE

Role of inflammation in cardiac electrical remodeling in diabetes

Oscar Casis, Julián Zayas-Arrabal, Amaia Alquiza, Leyre Echeazarra, Víctor Fernández-López, Mónica Gallego.

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Diabetes is a chronic metabolic disease characterized by hyperglycemia in the absence of treatment. Although it is one of the most ancient known diseases, described almost 4000 years ago, its incidence is far from diminishing and in the last 50 years it has multiplied by five and continues to grow. In the last century, the efficacy of the treatment improved exponentially, prolonging the life expectancy and the quality of life of patients, changing a lethal to a chronic disease. However, while life spam lengthened, long-term complications appeared.

Among the diabetes-associated complications, cardiovascular disease is the major cause of mortality and morbidity. Diabetic cardiomyopathy is a complex dysfunction in which mechanical and electrical abnormalities appear independently. Type 1 and type 2 diabetic patients often show a cardiac electrical remodeling, characterized by a prolonged ventricular repolarization, visible in the electrocardiogram as a lengthening of the QT interval duration. QT prolongation strongly correlates with the risk of developing torsade de pointes, a ventricular tachycardia that can degenerate into ventricular fibrillation and sudden death. At the cellular level, the prolonged repolarization is due to alterations in the expression and activity of several ion channels and regulatory proteins, mainly a reduction of repolarizing potassium channels.

Although a mechanistic link between the metabolic alterations and the electrical remodeling has been elusive, there is now growing evidence that both disturbances share a common inflammatory origin. Furthermore, inflammation is currently considered a key factor in the development of Type 2 diabetes. Diabetic patients have elevated circulating cytokines, such as TNFa or IL1b, that are responsible for the characteristic insulin resistance. At the same time, in the heart, these cytokines reduce potassium currents compromising the ability of cardiac myocytes to repolarize.

Therefore, a strategy to improve the metabolic defects and prevent the long-term cardiac electrical dysfunction could be the modulation of the inflammatory system. In this context, the potassium channel Kv1.3 is a particularly interesting target. This channel expresses in cytokine releasing cells such as adipocytes, macrophages and lymphocytes. Inhibition of Kv1.3 channel, which reduces cytokine secretion, has given encouraging results in animal models of diabetes.

Funding: This work was supported by grants from the Gobierno Vasco (PIBA2018-58 and GIC18/150), and MICINN (PID2020-118814RB-I00). AA is a predoctoral fellow of the Gobierno Vasco. JZA and VFL were predoctoral fellows of the UPV/EHU. JZA had a STSM from the EU-CARDIOPROTECTION COST Action CA16225.

SYMPOSIUM 1. ION CHANNELS IN PHYSIOLOGY AND PATHOLOGICAL CONDITIONS

Chair: Asia Fernández Carvajal and Antonio Felipe

INVITED

S1I-01. Anticonvulsant and neuroprotective roles of SGK1.1

Teresa Giráldez
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38010 La Laguna, Spain.

Epilepsy is a neurological disease that affects more than 50 million people around the world. It is characterized by recurrent seizures, the most extreme form of synchronous brain activity. In the nervous system, Kv7 channels are responsible for the M-current, which is important to regulate neuronal excitability. In this talk I will present the work from our laboratory in recent years, where we described a new modulator of Kv7.2/3 channels, the neuronal isoform of SGK1 kinase, SGK1.1, which up-regulates channel activity and counteracts hyperexcitability. Using a kainic acid-induced model of temporal lobe epilepsy with transgenic mice expressing a constitutively active form of SGK1.1, we have demonstrated that this kinase is a potent anticonvulsant factor, shortening seizure severity and duration independently of age, sex and genetic background. Furthermore, we show that SGK1.1 drastically reduces seizure-induced neuronal death and associated gliosis through M current-dependent and -independent mechanisms. Finally, our results demonstrate that constitutively active SGK1.1 is able to up-regulate Kv7 channels harboring epilepsy-causing mutations. Altogether, our results establish SGK1.1 as a potential therapeutic target for epilepsy treatment.

S1I-02. Dominant negative effect of an *SCN5A* variant in native cardiac sodium channels

Fabiana S. Scornik
Departament de Ciències Mèdiques.
Universitat de Girona, Girona, Spain.
Cardiovascular Genetics Center, Institut d'Investigació Biomèdica de Girona
Dr. Josep Trueta, Girona, Spain.

SCN5A is the major gene linked to Brugada syndrome (BrS), an inherited cardiac arrhythmogenic disease that predisposes patients to sudden cardiac death. BrS has been historically considered a monogenic disease with an autosomal dominant pattern of inheritance and incomplete penetrance. *SCN5A* is the only gene suggested to present definitive evidence of causality. This gene encodes the pore-forming α subunit of the cardiac voltage-gated sodium (Na_V) channel, $Na_V1.5$, which is responsible for initiation and propagation of the action potential in the heart. Family-based genetic studies have revealed that approximately 25% of BrS cases carry a rare deleterious variant, either a single-nucleotide variant (SNV) or an insertion/deletion in the *SCN5A* coding region of the gene. Most of these genetic variants have been associated with $Na_V1.5$ loss-of-function, either by affecting channel gating or reducing the number of channels at the plasma membrane.

In recent years, several studies have suggested that BrS has a complex pattern of inheritance and a heterogeneous genetic basis, and that it may be caused by the presence of multiple susceptibility

variants acting synergistically through one or more pathways, thus following an oligogenic or polygenic model of inheritance.

On the other hand, a growing body of evidence is shifting the conventional paradigm with respect to $Na_V1.5$ channel assembly and function. It has been recently demonstrated that Na_V channels exist and function as dimers rather than as monomers. This requires direct or indirect α-α subunit interactions within multimeric complexes, which have profound mechanistic implications.

This α-α interaction may be at the root of the incomplete penetrance and variable expressivity observed in BrS. Altogether, this evidence points to a role of patient-specific genetic background on the cellular and clinical phenotype among carriers of $Na_V1.5$ variants.

To investigate the role of the genetic background in the phenotypical expression of a BrS-associated variant (V1525M), we performed patch clamp studies to measure sodium currents from patient-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM). We included carriers and non-carriers, members of a family with a history of BrS. In addition, we determined gene and protein expression by qPCR and western blot and performed a genetic panel for arrhythmogenic diseases.

Our results showed a large reduction in I_{Na} density in hiPSC-CM derived from two V1525M carriers compared with hiPSC-CM derived from a non-carrier. Because this current reduction was larger than 50%, it is likely that the mutant channel exerts a dominant negative effect over the WT subunit. In addition, we observed that I_{Na} was not affected in hiPSC-CM derived from a V1525M carrier who also carried the $Na_V1.5$_p.H558R polymorphism. This is in agreement with previous studies that showed that $Na_V1.5$_p.H558R rescues the loos of sodium current produced by BrS associated *SCN5A* variants. To address this observation, we performed heterologous expression experiments which showed that heterozygous expression of V1525M produced a loss of I_{Na} function when it was expressed alone, but not when this variant was expressed together with H558R. In addition, treatment of hIPSC-CM with the antiarrhythmic drug mexiletine rescued I_{Na} function.

In Conclusion, our results in patient-specific hiPSC-CM point to a DN effect of NaV1.5_p.V1525M, which can be reverted by the presence of NaV1.5_H558R. Overall, our data points to a role of patient-specific genetic background as a determinant for incomplete penetrance in BrS. Further experiments need to be done to determine the molecular mechanism of the observed DN effect of this mutation.

Fundació La Marató de TV3, by Obra social “la Caixa”, Centro de Investigación Biomédica en Red de Enfermedades Cardiovasculares (CIBERCV).

S1I-03. The Volume-Regulated Anion Channel VRAC: recent physiological and pathological findings

Rosa Planells-Cases

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and Max-Delbrück-Centrum für Molekulare Medizin (MDC) Berlin
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Title ANTICONVULSANT AND NEUROPROTECTIVE ROLES OF SGK1.1

Volume-regulated anion channels (VRACs) play an essential role in vertebrate cell volume regulation by transporting halide ions and organic osmolytes to counteract osmotic imbalances. VRACs are formed by LRRC8 hetero-hexamers composed by the obligatory LRRC8A subunit combined with at least one of its paralogs LRRC8B to LRRC8E. The subunit composition of VRACs not only determines the electrophysiological properties of the swelling-induced Cl^- current ($I_{Cl,vol}$) but also its substrate specificity. For instance, while LRRC8D-containing VRACs transport organic osmolytes like taurine and Pt-based drugs and determines cancer drug resistance and survival expectancy of ovarian cancer patients, LRRC8E-containing VRACs transport negatively charged osmolytes like aspartate[1]. Here, I will review recent physiological findings on VRACs obtained with both *ex vivo* and in vivo mouse models. First, I will review the role of VRACs in endocrine pancreas (prominently

expressing LRRC8A and LRRC8D). I will show that VRAC influences glucose-induced Ca^{2+}-response in β-cells, reduces first-phase insulin secretion and impairs glucose tolerance *in vivo*[2]. Second, I will summarize the cellular and subcellular localization of different LRRC8 subunits in mouse kidney and explore their role by targeting LRRC8 subunit expression in different nephron segments. Rather than finding a key role of VRAC in renal medulla, well known to experience large osmolarity changes depending on the hydration status of the animal, VRAC is most highly expressed in proximal tubules which express metabolite-conducting LRRC8A/D channels. Targeted disruption of either subunit entails proximal tubular injury and Fanconi-like symptoms. We propose that VRAC may mediate non-specific exit of organic compounds in this highly transporting nephron segment. Thirdly, I will explore the role of VRAC in immunity. LRRC8E-containing VRACs have the particular ability to transport negatively charged substrates like 2'3'-cyclic-GMP-AMP (cGAMP), an intracellular immune signaling molecule that links the detection of dsDNA from diverse pathogens or cell damage to the activation of innate immunity. Indeed we have shown that *Lrrc8e*$^{-/-}$ mice exhibits impaired Interferon responses and compromised immunity to DNA-based virus. Our findings suggest that cell-to-cell transmission of cGAMP via LRRC8/VRAC channels is central to effective anti-viral immunity[3].

Funding

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Deutsche Forschungsgemeinschaft (SFB 1365, B02)

References

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ORAL

S1O-01. Adenosine modulates Kv1.3 by complementary PKC and PKA pathways in dendritic cells

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The voltage-gated potassium channel Kv1.3 is crucial for the activation and proliferation of leukocytes, playing an essential role during the immune response. An altered expression of the channel is at the onset of several autoimmune diseases. Therefore, Kv1.3 is considered a potential therapeutic target against autoimmune pathologies such as multiple sclerosis, psoriasis or rheumatoid arthritis. In this context, the study of the mechanisms involved in the modulation of Kv1.3 deserves considerable attention. The down-stream signal produced by the channel is the result of a balance between positive and negative inputs targeting the channel to the cell surface. Therefore, the Kv1.3 turnover, caused by channel internalization and degradation, fine-tunes the inflammatory response. Thus, understanding endocytosis is crucial for the knowledge of the Kv1.3 physiology. Adenosine (Ado), a potent endogenous immunomodulator, induces Kv1.3 endocytosis in HEK-293 by stimulating PKC. In addition, Ado also activates PKA-signaling pathway. In this study we investigated the role of PKC and PKA in the Kv1.3 turnover. We analyzed the channel internalization, membrane abundance, ubiquitination, and endocytic mechanisms. Our results showed that both PKC and PKA-dependent signalling induce Kv1.3 ubiquitination and decrease channel expression. While PKC-dependent activation induced a massive endocytosis, targeting the channel to lysosomal degradation, PKA activation, without internalizing Kv1.3, targeted the channel to the proteasomal compartment. Moreover, Kv1.3 is crucial during CY15 dendritic cells (DC) activation. Lipopolysaccharide (LPS) enhanced Kv1.3 expression at the plasma membrane. However, activation of Ado A1 and A2A receptors in CY15 cells counteracts LPS-dependent Kv1.3 induction. Therefore, Ado, exerting an effective anti-inflammatory mechanism, mediated two alternative and redundant PKC and PKA-mediated molecular mechanisms fine-tuning the abundance of Kv1.3 at the cell surface. Our results bring light to the effective Ado-dependent immunosuppression in leukocytes.

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S1O-02. Electrophysiological effects of IQM-266 on I_{tof}. Role of cardiac beta subunits.

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The transient outward potassium current (I_{tof}), responsible for the repolarization of cardiac action potential, is generated by the activation of K_V4 channels assembled with KChIP2 and other accessory subunits, such as DPP6 and KCNE2. To test the hypothesis that these subunits modify

the pharmacological response of the channel, we have analyzed the electrophysiological effects of IQM-266 on the currents generated by K_V4.3/KChIP2, K_V4.3/KChIP2/DPP6, K_V4.3/KChIP2/KCNE2 channels and on the total I_K and I_{tof} in cardiac myocytes. CHO cells were transiently transfected (K_V4.3/KChIP2, K_V4.3/KChIP2/DPP6 or K_V4.3/KChIP2/KCNE2), and the potassium currents were recorded using the whole-cell patch-clamp technique. In mice cardiac myocytes, potassium currents were recorded by using the perforated patch-clamp technique. Our results indicate that IQM-266 exerts an activating effect on the I_K and I_{tof} peak amplitude in cardiac myocytes. To decipher which beta subunits were involved in these effects, IQM-266 was studied in CHO cells transfected with the above-mentioned cDNAs. IQM-266, at 3 µM, induced an increase in the K_V4.3/KChIP2 current charge, which augmented in the presence of DPP6, whereas it was abolished when KCNE2 was expressed. However, IQM-266 decreased the peak amplitude in transfected cells. In this study we concluded that: i) IQM-266 is a new activator of the I_{tof} in mice cardiac myocytes, ii) DPP6 and KCNE2 modify the pharmacological response of K_V4.3/KChIP2 channels to IQM-266, and iii) the presence of either DPP6 or KCNE2 is not enough to reproduce the activator effect observed when IQM-266 was tested on Ito recorded from mouse ventricular myocytes.

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S1O-02. Revealing the role of the mechanosensitive Piezo1 channel in controlling endosome trafficking for efficient cytokinetic abscission

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Cell division is a highly mechanically regulated process[1,2]. However, we still poorly understand how mitotic forces and the external mechanical environment regulate the different steps of cell division. Here, we present a novel role for the mechanosensitive Piezo1 channel at the intercellular bridge (ICB) connecting daughter cells during the final step of cell division to regulate abscission. We demonstrate that this channel is localized at the ICB in a Pacsin3-dependent manner. Pharmacological and genetic inhibition of Piezo1 or Pacsin3 cause the mislocation of endosomal trafficking proteins and proteins of the ESCRT machinery resulting in multinucleation. Furthermore, we identify the calcium-dependent protein FIP3 as the link between Piezo1-generated Ca^{2+} signals and the delivery of components of the abscission machinery, promoting the final cut of the membrane. These results provide a step further in understanding how mechanical forces participate in cytokinesis and identify Piezo1 as a key modulator of endosome trafficking.

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S1O-04. Oxaliplatin time course effect on a pre-clinical *in vitro* model of male and female rat DRG neurons

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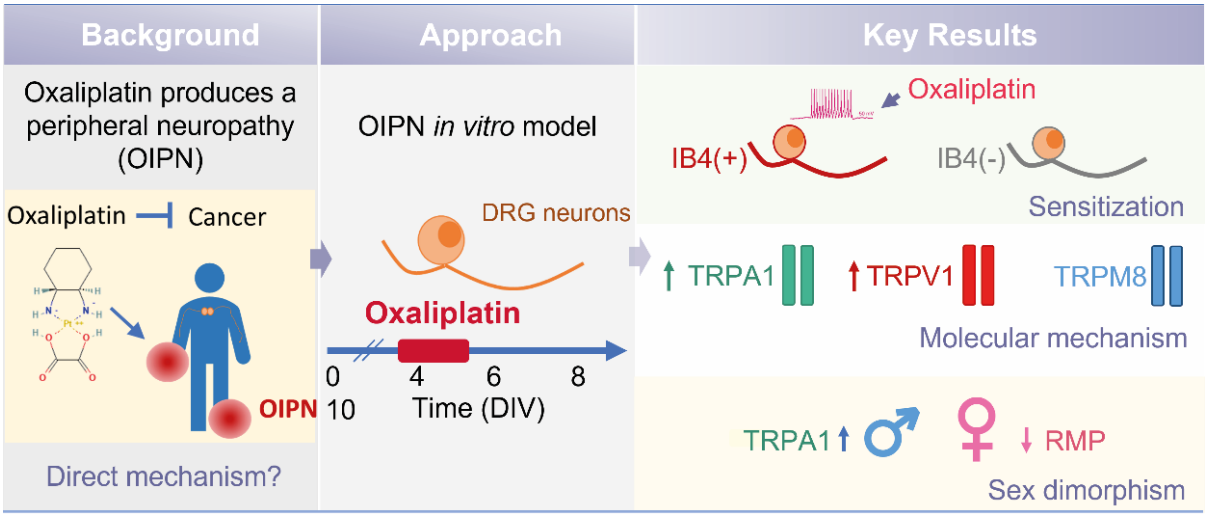

Oxaliplatin is one of the most common drugs used to treat colorectal, gastric, and pancreatic cancers. Nevertheless, oxaliplatin induces a peripheral neuropathy (OIPN) that translates into acute and chronic pain in the patients. The severity of these symptoms forces the reduction and compromises the continuation of the treatment, having a drastic impact on patients' survival and quality of life. Previous literature identified the alteration of the sensory neurons as a key player for the neuropathy development [1]. Mechanistically, TRP channels have been proposed as major contributors to the pain symptoms; however, the exact mechanism, the time course and the sex differences of this effect remain unclear, resulting in a lack of effective treatments. To address this issue, we investigated the time course effect of the direct oxaliplatin exposure on the sensory neurons. We adapted a previously *in vitro* model developed with the chemotherapeutic paclitaxel to study this condition [2]. Oxaliplatin was applied for 48h and the electrical and TRP activities were measured 0h, 48h and 96h after removing the agent. As a result, 0h post-treatment oxaliplatin significantly increased the spontaneous and evoked activity and reduced the rheobase of the neuronal subpopulation IB4(+). No significant changes were found on IB4(-) neurons, although a tendency towards an enhanced activity could be noted. These changes were correlated to increased TRPV1 activity and higher percentage of TRPA1 expressing cells whereas TRPM8 activity remained unaltered. TRPV1 and TRPA1 potentiation were time-dependent dissipating at 48h and 96h. Furthermore, a sex-dimorphic response could be observed. The percentage of neurons expressing TRPA1 was higher in male treated neurons whereas female treated neurons showed reduced RMP. Our data suggest that oxaliplatin could have a major impact on IB4(+) neurons with a sex-dependent effect. Moreover, TRPV1 and TRPA1 could be important players in the development of OIPN, through modulation of the functionality of IB4(+). Altogether, our *in vitro* model allowed us to identify potential therapeutic targets of oxaliplatin-induced pain symptoms, providing a powerful tool for investigating the mechanisms underlying neuropathies.

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SYMPOSIUM 2. VIRAL PROTEIN CHANNELS

Chair: Vicente Aguilella

INVITED

S2I-01. Viroporins from a historical perspective: still a bone of contention?

José L Nieva

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The Spanish virologist Luis Carrasco coined the term "Viroporin" in the early 1990s to describe a family of small and hydrophobic proteins encoded by animal viruses. Initially discovered as key factors for the progression of cytopathic RNA virus infection, the viroporin universe has expanded over the last two decades to include members with an increasing diversity of structures and sequences. Despite this diversity, viroporins share a common functional trend: their capacity to assemble oligomeric membrane channels during the replication cycle of the virus. Their specificity spectrum ranges from low-pH activated, unidirectional proton channels, to pores allowing passive diffusion of solutes only limited by their size, a mechanistic generalization that caused some debate in the field. Expression of viroporins facilitates virion assembly and release from infected cells and alters a number of cellular functions including calcium homeostasis and glycoprotein trafficking, and can induce rearrangement of endomembrane systems. However, a unifying theory linking the various membrane permeability alterations induced by viroporins to the pathogenic outcome of viral infection is still lacking. The observation that viroporins can also establish interactions with cellular partners that alter the innate antiviral response and modulate virulence adds another level of complexity to the resolution of this problem. Here, we describe the context in which the viroporin concept originated, trace its evolution over the past two decades and finally discuss the pertinence of qualifying viroporins as members of the "membrane miniprotein" class.

S2I-02. The envelope protein channel of SARS-CoV-2: structure-function and future perspectives.

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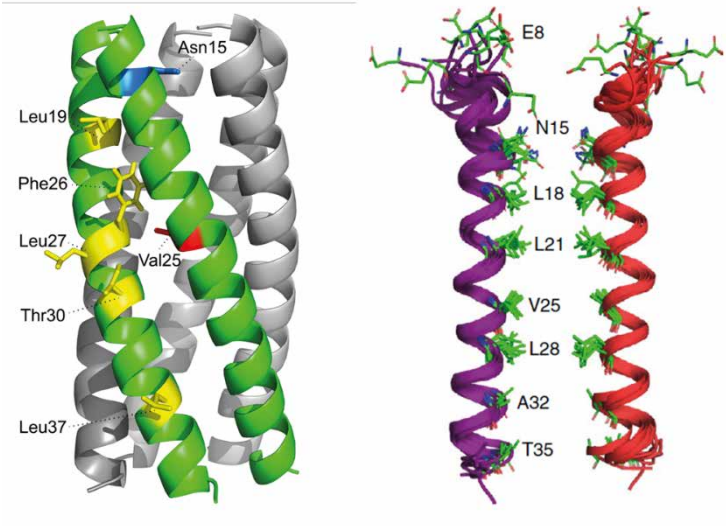
A global pandemic is underway caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a close relative to the SARS virus that caused the 2003 SARS pandemic. There is an urgent global need for antivirals to prepare rapid-response strategies for future coronavirus pandemics. One potential – yet often overlooked – SARS-CoV 2 target is the ion channel formed by the 75-amino acid long envelope (E) protein. While mice infected with mouse-adapted SARS virus showed lung epithelial cell damage and died, a single E channel-inactivating mutation led to survival (1). Escape mutations were found at the E channel domain and correlated with enhanced virulence. Pathogenicity has been linked to activation of the NLRP3 inflammasome by Ca^{2+} efflux from internal compartments (2). Preliminary models of the E channel have been determined by both solution (3) and solid state NMR (4), although lipids and extracellular C-terminal domain may be involved in modulating structure and channel activity. The intracellular localization of E has challenged the use of cellular measurements and channel activity has been characterized in synthetic planar bilayers. The channel appears to have modest cation-selectivity, if any, and a role in permeabilizing

membranes to protons has been suggested, but not proven. Two drugs directly contact and inhibit the channel (e.g., hexamethylene amiloride and amantadine) at micromolar concentrations, but overall no pharmacologically useful inhibitors have been confirmed, questioning its druggability. A deeper understanding of the E protein channel is needed before rational design of therapeutic agents.



Models of the SARS E protein channel in solution NMR (1) (left) and ssNMR (4) (right).

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S2I-03. Electrophysiological characterization of viroporin ion channels regulated by lipid-protein interactions

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We use high-resolution conductance measurements and current fluctuation analysis to demonstrate that some viroporin channels (CSFV swine fever virus p7, FMDV foot and mouth disease virus 2B, SARS-CoV E) are ruled by equilibrium conformational dynamics involving protein-lipid interactions. We show that these viroporin form subnanometric channels involved in virus propagation, but also much larger pores (1 ~ 10 nm in diameter) with potentially significant roles in virus pathogenicity. In the case of CSFV p7, atomic force microscopy (AFM) confirms the existence of a variety of pore sizes and their tight regulation by solution pH. Our findings provide new insights into the sources of noise in protein electrochemistry and demonstrate the existence of slow complex dynamics characteristic of crowded systems like biomembrane surfaces.

ORAL

S2O-01. Controlling the release of apoptogenic factors; the role of viral Bcl2s' transmembrane domains

Luis Martínez Gil
UVA

The permeabilization of the mitochondrial outer membrane (MOM) that leads to the release of apoptogenic factors is primarily regulated by the B-cell lymphoma 2 (Bcl2) protein family. According to their function, preventing or promoting MOM permeabilization, Bcl2 members can be classified into anti-apoptotic and pro-apoptotic proteins. Viral control of programmed cell death relies in part on the expression of viral analogs of the B-cell lymphoma 2 (Bcl2) protein known as viral Bcl2s (vBcl2s). vBcl2s control apoptosis by interacting with host proand anti-apoptotic members of the Bcl2 family. Here, we show that the carboxyl-terminal hydrophobic region of herpesviral and poxviral vBcl2s can operate as transmembrane domains (TMDs) and participate in their homo-oligomerization. Additionally, we show that the viral TMDs mediate interactions with cellular pro- and anti-apoptotic Bcl2 TMDs within the membrane.

Furthermore, these intra-membrane interactions among viral and cellular proteins are necessary to control cell death upon an apoptotic stimulus. Therefore, their inhibition represents a new potential therapy against viral infections, which are characterized by short- and long-term deregulation of programmed cell death.

S2O-02. Effects of Sars-Cov-2 envelope protein on store-operated channels and calcium homeostasis in human lung microvascular endothelial cells

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) triggered the coronavirus diseases 2019 (COVID-19) reaching a global pandemic on 11 March 2020. SARS-CoV-2 contain 29 proteins and three of them are believed to be viroporins, proteins that are capable of forming ion channels in biological membranes of host cells, connecting the ER lumen and cytosol and therefore, being fundamental for viral replication. Among the three SARS-Cov-2 viroporins, the envelope (E) protein forms homopentameric ion channels with poor ion selectivity and has been related with increased pathogenicity and mortality. Here, we report results of calcium imaging, electrophysiological measurements, and proliferation and apoptosis essays, in order to understand the effects of E protein on Ca^{2+} homeostasis in human lung microvascular endothelial cells (HLMVEC). We found that 2 h incubation with the E-protein (1µg/ml) decreases non-selective, store-operated currents (I_{SOC}). However neither voltage operated potassium currents (I_{K+}) or voltage membrane potential (E_m) are altered. Furthermore, after E-protein exposure, showed mixed store-operated currents composed of a reduced inward current (1.8 ± 0.8 pA, at -80 mV) plus a non-selective outward current (5.8 ± 1.2 pA, at + 80 mV). Consistently, HLMVEC cells treated with E protein showed decreased store-operated Ca^{2+} entry and reduced Ca^{2+} stores. Decreased SOCE and depleted Ca^{2+} stores correlate with cell proliferation impairment, however our proliferative and apoptosis data showed conflicting effects. E-protein treatment tend to induce apoptosis and to reduce cell proliferation but differences are not statistically significant. These data suggest that SARS-Cov-2

E-protein may underlie, decreased I_{SOC}, Ca^{2+} store depletion and proliferation in lung endothelial cells. We conclude that SARS-Cov-2 E-protein may contribute to Ca^{2+} remodeling in HLMVEC and could be a target against coronaviruses.

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S2O-03. The viroporin SARS-CoV-2 envelope protein induces calcium release from intracellular stores and apoptosis in rat hippocampal neurons

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COVID-19 pandemic is caused by the infection of SARS-CoV-2 virus, whose effects have not been fully clarified yet. Covid19 effects are deeply influence by age, being aging one of the critical factors for a worst prognosis. Nervous affections like headache, memory loss or epileptic seizures have been reported. In addition, some studies have revealed that SARS-CoV-2 virus could infect neurons as well. Viroporins are viral proteins capable to create pores or assembly oligomeric ion channels in cellular membranes. By similarity to SARS-Cov-1, the SARS-CoV-2 envelope protein (E Protein) is a structural protein identifed as a viroporin. The aim of this investigation is to study the possible effects of the Sars-Cov-2 E Protein on neuron damage and intracellular calcium homeostasis in a model of rat hippocampal neurons aged in vitro. For this end, cultured rat hippocampal neurons were treated with E Protein (0.6 µg/ml, 24 h) and the effects on apoptosis and intracellular calcium were tested using fluorescence microscopy. We found that E protein treatment increased apoptosis of rat hippocampal neurons in primary culture. Neuron cell death was similarly enhanced in both young and aged neurons. Acute treatment of neurons with E Protein (1mg/ml) triggers a transient increase of intracellular calcium levels. This effect is not prevented by removal of external Ca^{2+} in but it is abolished after depletion of intracellular calcium stores using thapsigargin. We conclude that Sars-Cov-2 E Protein acts as a Ca^{2+}-permeable viroporin at the endoplasmic reticulum of rat hippocampal neurons producing a net release of intracellular Ca^{2+} that may lead to neuron apoptosis and brain damage.

This work has been supported by grant RTI2018-099298-B-100 from Ministry of Science and Innovation, Spain and grants CCVC8485 and VA294P18 from Junta de Castilla y León, Spain. SLV is supported by a predoctoral fellowship from Junta de Castilla y León, Spain.

SYMPOSIUM 3. Ca^{2+} IN HEALTH & DISEASE

Chair: Juan Antonio Rosado

INVITED

S3I-01. Orai 1 facilitates post-ischemic angiogenesis after myocardial infarction through Notch1 signaling pathway

Tarik Smani

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Angiogenesis plays important roles in physiological and pathological settings such as heart infarction, cancer, inflammation, and diabetes. It is an essential mechanism in fetal development, reproduction, wound healing, cancer growth and metastasis. During angiogenesis, endothelial cells are rapidly activated, mainly by vascular endothelial growth factor (VEGF), then they migrate toward tissue lacking $O_{2,}$ and later sprout to form new capillaries, which improve blood flow in the ischemic tissue. Our main objective is to study the molecular mechanism involved in the post-ischemic angiogenesis considered very relevant for heart recovery after myocardial infarction, focusing on the role of Orai1, the pore forming subunit of CRAC channels, and Notch signaling pathway. Bioplex analysis of serum collected from patients with myocardial infarction revealed exacerbated concentration of pro-angiogenic factors such as VEGF-A, IL17A, TNFα, and INFα. Using classical assays of angiogenesis, such as wound healing and tube formation, we found that the incubation of Human Umbilical Vein Endothelial Cells (HUVEC) with the ischemic serum promotes vessels-like formation and boosts cells migration. Likewise, HUVEC incubation with the ischemic serum exacerbated thapsigargin-induced Ca^{2+} entry, correlating with significant upregulation of Orai1, and genes related to Notch1 signaling pathway. Interestingly, silencing of Orai1 prevents serum-induced overexpression of Notch1 and its target genes Hes1 and Hey1. Finally, Organ-on-a-chip 3D culturing of HUVEC shows that ischemic serum stimulated *tip*-like endothelial cell migration that was attenuated by Orai1 silencing.

Thereby, herein we show for the first time a new unknown role of Orai1 in post-ischemic angiogenesis involving NOTCH1 signaling pathway.

Knowledge: This research was funded by Agencia Estatal de Investigación [PID2019-104084GB-C22/AEI/10.13039/501100011033].

S3I-02. Store Operated Calcium Entry remodelation in Breast cancer

José J. López, José Sánchez-Collado, Isaac Jardín, Pablo Quintana-Sarti, Tarik Smani, Ginés M. Salido, Juan A. Rosado.

Breast cancer is a heterogeneous disease that is commonly classified by its molecular features and gene expression profile into luminal, HER2 and triple-negative subtypes. This classification displays a high predictive value and reflects the different levels of tumor aggressiveness found in the clinical activity. Recent studies have highlighted the link between Ca2+ signals and some cancer hallmarks in different breast cancer cell subtypes. Store-operated Ca2+ entry (SOCE) is a fine-tuned Ca2+ influx pathway initiated after the depletion of endoplasmic reticulum (ER) Ca2+ stores which mediates a myriad of cellular processes. In breast cancer cells, as in other cancer cell types, SOCE is involved

in the events of epithelial to mesenchymal transition, cell proliferation, angiogenesis, metastasis and resistance to chemotherapy. The molecular toolkit that underpins SOCE mainly includes the Ca2+ release-activated Ca2+ (CRAC) channels, comprised by the heteromeric association of Orai1, Orai2 and Orai3, and the ER Ca2+ sensors STIM1 and STIM2. Furthermore, several regulatory proteins, which modulate SOCE in a cell specific manner, have been described. This talk highlights the strategies adopted by different breast cancer cell subtypes in order to reshape the expression and function of the Ca2+ toolkit involved in SOCE, and thus, promote the development of cancer hallmarks.

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S3I-03. Calcium Channel remodeling in rat hippocampal neurons aged in vitro

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Intracellular Ca^{2+} homeostasis plays an important role in control of neuronal activity including neurotransmitter release, synaptic plasticity and memory storage as well as neuron cell death. Aging is often associated to cognitive decline and it is the most important risk factor for neurodegenerative disorders, particularly Alzheimer's disease (AD). We have recently shown that long-term cultures of rat hippocampal neurons which resemble in many aspects aging neurons, undergo cell death after treatment with different neurotoxins involved in neurodegeneration that increase cytosolic [Ca^{2+}] including glutamate (excitotoxicity), lipopolysaccharide (LPS, neuroinflammation) and oligomers of the amyloid beta peptide (AD), whereas short-term cultures resembling young neurons do not die. In addition, aged neurons display changes in intracellular Ca^{2+} homeostasis that may favour susceptibility to cell death in aging neurons. We have investigated this Ca^{2+} remodelling in aging neurons using both calcium imaging and transcriptomic analysis. We found that aging neurons show increased resting cytosolic [Ca^{2+}], Ca^{2+} store content, Ca^{2+} release from intracellular stores and Ca^{2+} transfer from the endoplasmic reticulum (ER) to mitochondria. Conversely, aged neurons also show decreased store-operated Ca^{2+} entry (SOCE), a Ca^{2+} entry pathway related to memory storage. At the transcriptional level, we found that aged neurons display enhanced expression of some TRP channels, SOCE modulators and Ca^{2+} release channels at the ER. Conversely, aged neurons display decreased expression of genes involved in Ca^{2+} extrusion from cytosol including plasma membrane Ca^{2+} pumps and mitochondrial Ca^{2+} uptake systems. At the protein level, Ca^{2+} remodelling is also associated to changes in NMDA receptor channels, IP_3 receptors, the mitochondrial calcium uniporter (MCU) and molecular players involved in SOCE. These results show a comprehensive view of Ca^{2+} remodelling in aged neurons and its molecular basis that may provide new targets against neuron cell damage associated to age-related neurodegenerative diseases.

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ORAL

S3O-01. Polyamine depletion reverses transcriptomic as well as calcium remodeling in colon cancer cells

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In 2020, colorectal cancer was the third most common cancer worldwide and the second most deadly form of cancer. Alterations in the Wnt/β-catenin pathway are the most frequent cause of colon cancer development. Because of these alterations, the oncogene c-Myc is overexpressed which in turn promotes overexpression of ornithine decarboxylase (ODC) and excess polyamine synthesis, a process that is limited by alpha-Difluoromethylornithine (DFMO), a suicide ODC inhibitor considered a potential treatment against colorectal cancer. Furthermore, it has been suggested that intracellular calcium transport mechanisms are deeply altered in colorectal cancer. Here we asked whether polyamine depletion induced by DFMO may impinge on transcriptional and intracellular calcium remodeling in colorectal cancer. For this end we used calcium imaging, transcriptomic and statistical analysis in HT29 and NCM460 cells used here as models of human colorectal cancer and normal colonic cells, respectively. We found that colon cancer cells display dramatic changes in intracellular Ca^{2+} homeostasis (calcium remodeling) relative to normal cells. Specifically, cancer cells show enhanced store-operated calcium entry (SOCE) and resting cytosolic basal calcium levels, but decreased calcium store content relative to normal cells. On the other hand, cancer cells displayed also differential expression of more than 6500 genes relative to normal cells, 56 among them related to intracellular calcium transport. Polyamine depletion using DFMO reverses largely calcium remodeling in cancer cells. In addition, polyamine depletion changed the expression of 17 genes related to calcium homeostasis in HT29, including 11 of them that were reversed to mimic expression levels in normal cells. Specifically, polyamine depletion increased expression of modulators of store-operated Ca^{2+} entry and the calcium pump PMCA4 along with decreased expression of TRPC1, TRPC5 and TRPV6 channels and the secretory pathway SPCA2 pump. These effects were observed only in cancer cells. We conclude that polyamine depletion reverses calcium remodeling specifically in colon cancer cells acting on a few Ca^{2+} transport systems and modulators.

This work has been supported by grant RTI2018-099298-B-100 from Ministry of Science and Innovation, Spain and grants CCVC8485 and VA294P18 from Junta de Castilla y León, Spain. EPR has been supported by Asociación Española Contra el Cáncer (AECC). EHP is supported by a predoctoral fellowship from Junta de Castilla y León, Spain.

S3O-02. Orai1α controls TRPC1 channel location and function in HeLa cells

José SánchezCollado, José J. López, Isaac Jardín, Alejandro BernaErro, Pedro J. Camello, Diego Mena-Santos, Tarik Smani, Ginés M. Salido, Juan A. Rosado

Store-operated Ca2+ channels mediate a Ca2+ influx activated by the depletion of the intracellular Ca2+ stores. To date, two store-operated currents have been identified, the highly Ca2+-selective *I*crac, which involves the activation of Orai channels by STIM proteins, and *I*soc, which involves the transient receptor potential family member TRPC1, Orai1 and STIM1. After the identification

of two Orai1 variants in mammalian cells, Orai1α and Orai1β, the question of whether they exhibit different functional characteristics arises. Orai1α and Orai1β variants are formed by an alternative translation initiation and differ in the N-terminal 63 amino acids, exclusive of Orai1α. It has been described that Orai1α and Orai1β show different sensitivities to Ca2+-dependent inactivation, as well as distinct ability to form arachidonate-regulated channels. Using a co-expression combination of STIM1, TRPC1, Orai1α and/or Orai1β, we confirmed that both Orai1 isoforms are required for the maintenance of regenerative Ca2+ oscillations, while TRPC1 plays a role in agonist induced Ca2+ influx but is not required for Ca2+ oscillations. By using APEX2 proximity labeling, co-immunoprecipitation and the fluorescence of G-GECO1.2 fused to Orai1α we observed that, after agonist stimulation and Ca2+ store depletion, Orai1α–TRPC1 interaction increases, effect that is not observed in Orai1β. In addition, only Orai1α seemed to be essential for TRPC1 activation and its plasma membrane location, as demonstrated the analysis of Mn2+ influx, as a surrogate of Ca2+ influx, and cell surface biotinylation using a dominant negative Orai1α mutant and the Orai1 inhibitor Synta66. Altogether, our results demonstrate a store depletion dependent co-localization of Orai1α with TRPC1 and the regulation of TRPC1 plasma membrane expression and channel function by Orai1α, and not by Orai1β, in HeLa cells, suggesting that Orai1 variants are non-redundant and might display differential functional roles in calcium signaling.

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S3O-03. Calmodulin is critical for folding of the Kv7.2 calcium responsive domain as the nascent peptide exits the ribosome.

Muguruza-Montero A, M-Alicante S, Nuñez E, Aguado A. Urrutia J, Campos-Zarraga I, Malo C, Villarroel A

Several diseases, such as Alzheimer and Parkinson, are related to protein co-translational missfolding. Protein folding has being historically analysed following *in vitro* unfolding and refolding approaches, that fall short of replicating co-translational folding events occurring in cells. Thus, little is known about co-translational folding of ion channels and other proteins. We have demonstrated that calmodulin (CaM) is essential for vectorial folding of the Calcium Responsive Domain (CRD) of the Kv7.2 channel. The force exerted during the early folding events of the nascent chain with single residue resolution can be assessed using state-of-the-art techniques. We describe here the force profile of the CRD during translation. We find that CaM is required to generate early folding events on this domain at critical places: IQ site, helix TW and helix B. Surprisingly, and that there is not a hierarchical requirement for folding, which is not significantly affected in permuted variants. This investigation provides new insights into how a critical Kv7.2 channel domain acquires its final functional conformation during co-translational synthesis.

S3O-04. The effects of aging on pancreatic β-cell function involve multiple events in the regulation of insulin secretion.

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Aging is associated with a decline in peripheral insulin sensitivity and an increased risk of impaired glucose tolerance and type 2 diabetes. During conditions of reduced insulin sensitivity, pancreatic β-cells undergo adaptive responses to increase insulin secretion and maintain euglycemia. However, the existence and nature of β-cell adaptations and/or alterations during aging are still a matter of debate. In this study, we investigated the effects of aging on β-cell function from control (3-month-old) and aged (20-month-old) mice. Aged animals were further categorized in two groups: high insulin sensitive (aged-HIS) and low insulin sensitive (aged-LIS). Aged-LIS mice were hyperinsulinemic, glucose intolerant and displayed impaired glucose-stimulated insulin and C-peptide secretion, whereas aged-HIS animals showed characteristics in glucose homeostasis similar to controls. In isolated β-cells, we observed that glucose-induced inhibition of K_{ATP} channel activity was reduced with aging, particularly in the aged-LIS group. Glucose-induced islet NAD(P)H production was decreased in aged mice, suggesting impaired mitochondrial function. In contrast, voltage-gated Ca^{2+} currents were higher in aged-LIS β-cells, and pancreatic islets of both aged groups displayed increased glucose-induced Ca^{2+} signaling and augmented insulin secretion compared with controls. Morphological analysis of pancreas sections also revealed augmented β-cell mass with aging, especially in the aged-LIS group, as well as ultrastructural β-cell changes. Altogether, these findings indicate that aged mouse β-cells compensate for the aging-induced alterations in the stimulus-secretion coupling, particularly by adjusting their Ca^{2+} influx to ensure insulin secretion. These results also suggest that decreased peripheral insulin sensitivity exacerbates the effects of aging on β-cells.

SYMPOSIUM 4. ION CHANNELS AND PAIN

Chair: Ana Gomis

INVITED

S4I-01. Piezo2 channels: from structure to nociception

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Piezo channels constitute a unique family of ion channels that transduce mechanical forces, including membrane deformation and stretch. While Piezo1 is broadly expressed, Piezo2 is present in mechanoreceptors and proprioceptors. In these afferent neurons, Piezo2 senses, with remarkable sensitivity, hair movements or the stretch of muscle fibers. However, the molecular substrates for such refined sensitivity and responsiveness to different mechanical stimuli are missing. Recent work from our group has highlighted the intracellular regions in Piezo2 architecture that underlay those fundamental properties. Furthermore, we show that Piezo2 is also present in a particular subtype of pain fibers known as the Silent Nociceptors. Notably, Piezo2 is responsible for the mechanically activated currents induced by inflammation in these fibers.

S4I-02. Clues to understanding TRPC5 as a cold-activated channel and its inhibition by the analgesic duloxetine

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The human transient receptor potential canonical 5 (TRPC5) is a polymodal, calcium-permeable, non-selective cation channel mainly activated by G-proteins and their downstream signal transduction pathways, but also by noxious cold temperatures. It is expressed in the central and peripheral nervous system and in other tissues such as kidney, synovium and odontoblasts [1]. It has recently been shown that pharmacological and genetic inhibition of TRPC5 leads to relief of neuropathic and inflammatory pain, making TRPC5 an attractive target for pain management strategies [2].

We explored the cold sensitivity of human TRPC5 at the single-channel level using transiently transfected HEK293T cells. In the control extracellular solution, as the temperature decreased, the open probability increased robustly between 16 °C and 11 °C and reached saturation below 5-8 °C. Thermodynamic analysis revealed significant changes in enthalpy and entropy suggesting that substantial conformational changes accompany cold-induced gating.

Moreover, we explored the effects of duloxetine on TRPC5. This compound is the only drug that demonstrates efficacy for severe cold–induced pain states associated with diabetic and chemotherapy-induced neuropathy [3] and its analgesic effect has been previously attributed to the inhibition of voltage-gated sodium channels.

The combination of electrophysiological measurements, point mutagenesis, molecular docking and molecular dynamic simulations enabled us to show that duloxetine inhibits TRPC5 in a concentration-dependent manner with a potency within the physiological range. Thus, TRPC5 may

contribute to the efficacy of duloxetine in cold-related pain. The mechanism of duloxetine's effect seems to be direct binding to an inhibitory pocket in the voltage sensor-like domain, where the putative hydrogen bond with Glu418 is of particular importance.

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S4I-03. Skin sensory glia as detector cells for pain and touch

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Mammals are able to sense their external environment through their peripheral sensory neurons which are pseudo-unipolar neurons with their cells bodies in the dorsal root ganglia (DRG) and their terminations innervating the skin. DRG neurons can be broadly classified as mechanoreceptors responsible for touch sensation or nociceptors which detect harmful mechanical, thermal and chemical stimuli. Historically, these neurons were considered the sole detectors and transducers of cutaneous stimuli, but recent discoveries have shown that non-neuronal cells are also critical for those processes[1,2]. We have studied a specialized type of Schwann cell located at the epidermal-dermal boundary which we called nociceptive Schwann cells. Those cells are capable of triggering pain sensation in mice[2]. We detected nocifensive behavior in mice after artificially activating nociceptive Schwann cells in mice hindpaws with optogenetic tools. We also discovered that those cells are inherently mechanosensitive to poking stimuli in culture. Taking advantage of the skin-nerve technique, we found that mechanosensory function of almost all nociceptors, including those signaling fast pain were critically dependent on nociceptive Schwann cells. Regarding specifically polymodal nociceptors, sensory Schwann cells signaled mechanical but not thermal stimuli. Finally, using inhibitory optogenetics we show that Meissner´s corpuscle Schwann cells are necessary for in vivo detection and perception of vibrotactile stimuli in mice.

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ORAL

S4O-01. The endocrine disruptor bisphenol A regulates sodium Nav1.7 ramp currents in mouse dorsal root ganglion neurons and increases nociception

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Estrogens regulate various physiological processes such as cell growth, reproduction, development and differentiation. Furthermore, the predominant estrogen, 17β-estradiol, mediates the sensitivity to pain and is involved in sex differences in nociception. Bisphenol A (BPA) is a widespread environmental endocrine disrupting chemical with estrogenic activity. BPA is produced in the manufacture of plastics, epoxy resins and thermal paper and it is released from common household materials, explaining why 93% of the U.S. population present measurable amounts of BPA in their urine. However, BPA implications in pain are mostly unknown.

Here we show that treatment of male mice with BPA (50 µg/kg/day, the tolerable daily intake recommended by the U.S. Environmental Protection Agency) during 8 days, decreases the latency to pain behaviour in response to heat (hot plate test), suggesting increased pain sensitivity. To understand how BPA exacerbated pain sensation, we isolated dorsal root ganglia (DRG) neurons and studied their electrical activity by patch-clamp electrophysiology. Incubation of DRG nociceptors with 1 nM BPA increased the frequency of action potential firing in response to current injection.

The gene *SCN9A* encodes the voltage-gated sodium channel Nav1.7, which is present in DRG nociceptors and is essential in pain signaling. Nav1.7 and other voltage-gated sodium channels in mouse DRG are considered threshold channels because they produce ramp currents, amplifying small depolarizations and enhancing electrical activity. BPA increased Nav-mediated ramp currents elicited with slow depolarizations. Furthermore, the natural hormone 17β-estradiol (1 nM) increased the magnitude of ramp current to a similar extent. Experiments using pharmacological tools as well as DRG from ERβ−/− mice indicate that this BPA effect involves ERα and phosphoinositide 3-kinase. The mRNA gene expression, measured by qRT-PCR, and biophysical properties other than ramp currents of Nav channels, were unchanged by BPA.

Our data suggest that BPA at environmentally relevant doses affects the ability to detect noxious stimuli and therefore should be considered when studying the etiology of pain conditions.

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S4O-02. Discovery of new TRPM8 modulators and their therapeutic potential

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The Transient Receptor Potential Melastatin type 8 (TRPM8) is a non-selective cationic channel expressed mainly in sensory neurons sensitive to pain and temperature. This channel is activated by innocuous cool to cold temperatures (10-28°C), membrane depolarization, mechanical stimuli, and cooling agents such as menthol and icilin. The functional alteration of TRPM8 has been implicated in numerous physiological and pathological processes, highlighting the hypersensitivity and allodynia to cold that accompanies inflammatory and neuropathic pain. This fact makes the TRPM8 channel a therapeutic target in the treatment of these conditions, being many the pharmaceutical companies and academic institutions seeking new channel modulators. In this respect, our project aims to develop and characterize new TRPM8 modulators and to study its mechanism of action through *in vitro, in vivo* and *in silico* studies. In this work we focus on a series of β-lactam derivatives which have shown TRPM8 antagonistic activity in previous studies. So far, these compounds have been characterized *in vitro* using fluorometric techniques in the stable cell line rTRPM8 HEK293 to study their activity and potency. The same techniques have been employed to study the selectivity towards other TRP channels. Potency and selectivity have been confirmed by electrophysiological techniques towards the rTRPM8 HEK293 line and DRG neurons respectively. Further characterization of the most promising compounds has been carried out to study toxicity by colorimetric techniques and efficacy *in vivo* using cold allodynia animal models. Compounds' skin permeability is also being studied to evaluate topical application. All the experiments carried out to date have exhibited a good pharmacological profile of the compounds regarding potency, selectivity, safety and efficacy *in vivo* (1)(2)(3).

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S4O-03. Light-gated channel Channelrhodopsin-2 improves neuroregenerative potential of Neural Precursors Cells after Spinal Cord Injury (SCI)

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Spinal Cord Injury (SCI) is a severely debilitating condition that causes motor, sensory and autonomic dysfunctions. Currently, SCI remains a worldwide problem due to its complexity, involving diverse biochemical and physiological processes [1]. Nowadays, neural progenitor cells (NPC) transplantation has been shown as a useful tool for treatment of SCI [2], demonstrating potential to recovery complex neurological functions after the injury, however, the limited cell survival rates and host circuit integration limits the extension of their capabilities.

Optogenetic control of light-gated cation channel Channelrhodopsin-2 (ChR2) is a powerful and versatile tool to modulate neural fate and improving therapeutics outcomes in vitro and in vivo [3]. Previous results from our group have shown that blue-light stimulation of NPC engineered to ectopically express the photosensitive membrane protein channelrhodopsin-2 (ChR2-NPC) prompted an influx of cations and a subsequent increase in proliferation and enhanced NPC-differentiation into oligodendrocytes and neurons. Furthermore, light-stimulated ChR2-NPC triggered the polarization of astrocytes from a pro-inflammatory phenotype to a pro-regenerative/ anti-inflammatory phenotype with decreased activation of NF-kB. On the other hand, neurons derived from blue-light-stimulated ChR2-NPC exhibited both, increased branching and axon length and improved axon growth in the presence of axonal inhibitory drugs such as lysophosphatidic acid.

To further study this approach, ChR2-GFP-NPC transplantation was performed in an in vivo subacute rat model of SCI by T8 hemisection. In vivo optogenetic activation was carried out using Neurolux spinal cord device which has tethered a blue μ-LED. Rats received 1h of stimulation every day for a total of 4 weeks at 20 Hz with 5 ms on and 45 ms off. Preliminary data shows that optogenetic stimulation of transplanted ChR2-NPCs enhance endogenous tissue preservation, improving the maintenance of the corticospinal tract fibers, reduce the extension of the injured area, improve the activation of host neurons and grafted cells and modify the identity of transplanted cells.

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S4O-04. Testosterone-TRPM8 interactions drive pain resilience in a mouse model of chronic migraine

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Chronic migraine is a primary headache affliction highly prevalent in women that is also associated with cutaneous sensitization more frequently in females[1,2]. Interestingly, Transient receptor potential Melastatin 8 (TRPM8), the principal receptor of environmental cold, has been related with migraine after the discovery of genetic polymorphisms of the TRPM8 gene tightly associated with chronic migraine. Since TRPM8 was also described as a testosterone receptor[3], we investigated the role of testosterone and TRPM8 in a mouse model of nitroglycerine-induced chronic migraine that displays a sexual dimorphic phenotype. Possible interactions between testosterone and TRPM8 were further analysed through calcium imaging, electrophysiological recordings and computational docking. Thus, repeated nitroglycerine induced a long-lasting sensitization that was persistent in wild-type female mice, while wild-type males completely recovered baseline mechanical thresholds 12 days after ending the nitroglycerine treatment. TRPM8 knockout males developed a persistent sensitization and did not recover baseline mechanical thresholds, similar to wild-type females, whereas TRPM8 deletion did not aggravate sensitization in females. In parallel, orchidectomized wild-type males developed persistent sensitization that was prevented by chronic testosterone administration, and testosterone supplementation in wild-type females reverted nitroglycerine-induced hypersenstivitiy. This protective effect of testosterone was strongly reduced in TRPM8 knockout mice. Accordingly, *in vitro* calcium imaging assays showed that testosterone activates HEK cells and primary trigeminal ganglion neurons expressing TRPM8, and electrophysiological recordings confirmed testosterone-TRPM8 interactions independent of the androgen receptor. These results agree with computational modelling revealing high-affinity testosterone-TRPM8 interactions. In conclusion, TRPM8 exhibits a protective function selectively in male mice, and this protective role requires of the presence of the endogenous TRPM8 agonist testosterone, that is found in much higher levels in males than in females. Thus, selective TRPM8 agonists with low affinity for the androgen receptor represent a potential pain-relieving strategy for women or individuals with low testosterone levels.

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RECI PLENARY

Deadly human coronaviruses: origin, pathology and protection

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La relevancia del mecanismo de replicación de los coronavirus, que facilita la recombinación y la fijación de mutaciones como un generador de variabilidad genética será ilustrada, así como la repercusión de estas características en la emergencia de nuevas epidemias o pandemias. También se describirá la habilidad de los coronavirus para cruzar la barrera de las especies y la necesidad de encontrar el origen del SARS-CoV-2, prácticamente determinado. Así mismo, se revisará una comparación de los tres coronavirus mortales para las personas, junto con las bases moleculares de su patogenicidad, y el desarrollo de vacunas para prevenir la infección por coronavirus, basadas en replicones RNA derivados del genoma de estos coronavirus. Estas vacunas dan una protección esterilizante en el modelo experimental del ratón.

SYMPOSIUM 5. PHOTOPHARMACOLOGY AND DRUG DISCOVERY

Chairs: Rosario González-Muñiz and Francisco Ciruela

INVITED

S5I-01. Enantiopure lactams as NMDA receptor antagonists

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The overactivation of *N*-Methyl-D-aspartate receptors (NMDARs) is associated with several neurodegenerative and psychiatric disorders. Therefore, these receptors are considered relevant targets for many central nervous system disorders. In this talk will be discussed some recent advances from our laboratory on the design and synthesis of novel NMDA receptor antagonists (Figure 1).[1-3] The enantiopure compounds were obtained by chiral pool synthesis using enantiomerically pure aminoalcohols as chiral inductors, and their stereochemistry was proven by X-ray crystallographic analysis. The most active compounds display IC_{50} values in a Ca^{2+} entry-sensitive fluo-4 assay in the same order of magnitude of the clinically approved NMDAR antagonist memantine. In addition, these derivatives are brain permeable and non-toxic.

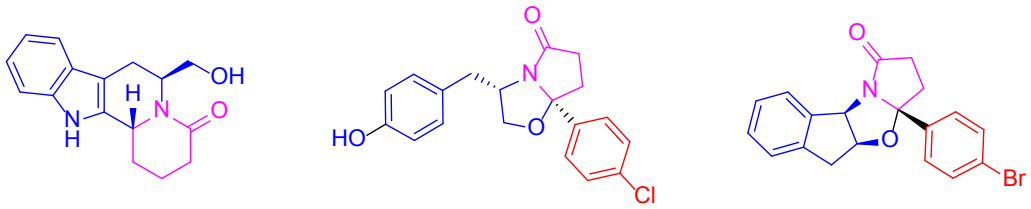

Figure 1. Chemical scaffolds developed in the group with antagonistic activity for NMDAR

Acknowledgements – I would like to thank all co-authors whose contribution was key for the presented results including Dr. C de los Rios, Dr. D.J.V.A. dos Santos, Dr. E. Molins, Dr. J. Bosch, Dr. L. Gonçalves, Dr. M. Amat and Dr. M. I. Rodríguez-Franco, and Fundação para a Ciência e Tecnologia for financial support (IF/00732/2013, CEECIND/01772/2017, PTDC/QUI-QUI/111664/2009, PTDC/QUI-QOR/29664/2017, PTDC/QUI-QOR/1304/2020).

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S5I-02. Controlling receptor activity with photoswitchable drugs: basic research and future therapies

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The large number of photoswitchable biomolecules discovered and developed in recent years covers a great variety of cellular functions like catalysis of metabolic processes, cytoskeletal polymerization and motors, nucleic acids dynamics, intracellular signaling and perhaps most dazzlingly membrane excitability, which has been at the focus of optogenetics and photopharmacology. The dream of precisely and remotely photocontrolling every aspect of the cell's workings in intact tissue appears within reach and offers the promise of interrogating complex cellular processes to discover their molecular mechanisms. Recent and ongoing projects at IBEC focused on photopharmacology will be reviewed, including the development and applications of freely diffusible and tethered photoswitchable ligands of ionotropic and G protein-coupled receptors. These molecular tools allow spatiotemporal control of endogenous proteins in single neurons, and of emerging activity in the brain, including cortical waves.

S5I-03. Purinergic photopharmacology: towards new therapeutic opportunities

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Adenosine, a ubiquitous extracellular signalling molecule, acts through cell surface G protein-coupled receptors. These receptors control many physiological functions, thus becoming promising therapeutic targets in a wide range of pathological conditions. Yet, the ubiquity of adenosine receptors and the eventual lack of selectivity of adenosine-based drugs often reduced the therapeutic expectations. Photopharmacology is a novel approach based on the use of photosensitive drugs allowing spatiotemporal control of receptor function in a light-dependent manner, thus circumventing some of the classical pharmacology limitations. Accordingly, we developed light-sensitive drugs to photocontrol adenosine receptor's function both *in vitro* and *in vivo*. To this end, two types of adenosine-based photosensitive drugs were developed: i) Photocaged; and ii) Photswitchable.

We developed two adenosine-based photocaged derivatives. MRS7145 is a photocaged $A_{2A}R$ antagonist which binds and blocks $A_{2A}R$ in a light-dependent manner. Thus, precise fibre optic brain irradiation allows MRS7145 uncaging and striatal $A_{2A}R$ blockade, thus fine-tuning $A_{2A}R$-dependent spontaneous locomotor activity and reversing pharmacologically induced Parkinsonian-like behaviour [1]including Parkinson's disease (PD. Similarly, the first A_3R photocaged agonist, MRS7344, in photopharmacological experiments prevented the psoriatic-like phenotype in the IL-23 animal model [2]and aberrant immune responses are major factors in its pathogenesis. Available treatments for moderate to severe psoriasis are directed to immune system causing systemic immunosuppression over time, and thus concomitant serious side effects (i.e. infections and cancer. Thus, we have demonstrated the feasibility of using a non-invasive, site-specific, light-directed approach to psoriasis treatment. Conversely, we developed MRS5543, a photoisomerizable nucleoside derivative containing an aryl diazo linkage on the N(6) substituent. Interestingly, while in dark conditions (i.e., relaxed isomer) it behaves as a full A_3R and partial $A_{2A}R$ agonist, but upon photoisomerization with blue light it turns into an $A_{2A}R$ antagonist. Thus, MRS5543 is a photoswitchable purinergic drug that allow a light-dependent control of $A_{2A}R$ intrinsic

activity. Finally, we recently introduced a photoswitchable adenosine derivative, AzoAdenosine-3, that effectively reduced pain perception in a light-dependent manner [3]. Interestingly, by using this compound we were able to map the contribution of ARs mediating analgesia *in vivo*.

Overall, the design and synthesis of light-operated adenosine receptor ligands opens new opportunities to widen the phototherapeutic window of adenosine receptors.

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ORAL

S5O-01. Evaluation of the EAR-20 peptide as a positive allosteric modulation of NMDA receptors

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The amino acid glutamate serves as a chemical signalling molecule in the central nervous system (CNS). It acts through the activation of several glutamate receptors, including N-methyl-D-aspartate receptors (NMDARs), an important ionotropic glutamatergic receptor subtype involved in excitatory synaptic transmission. The stimulation of NMDARs allows a Ca^{2+} influx through the ion channel pore, activating several intracellular pathways that regulate synaptic plasticity, a key cellular mechanism involved in learning and memory. Evidence from genetic studies and basic and clinical research involves NMDAR hypofunction in several CNS conditions as autism, schizophrenia, Alzheimer's disease or intellectual disability among others. This situation has driven interest for finding positive allosteric modulators (PAMs) of NMDARs as a potential therapeutic strategy for treating the associated cognitive deficits of NMDAR dysfunction. Based on structural analysis between the GluN2B subunit and conantokin-G toxin, which interacts selectively with the GluN2B subunit, we have recently designed several peptides (EARs) predicted to act on NMDARs. In this project we have functionally evaluated the EAR-20 peptide as a new type of PAM for NMDARs. We have studied the effect of the EAR-20 on NMDAR currents by means of patch-clamp recordings on HEK293T cells transfected with heteromeric GluN1-GluN2A or GluN1-GluN2B NMDARs. We show that the EAR-20 potentiates whole-cell NMDAR-mediated currents similarly in both, GluN2A and GluN2B heteromeric receptors. We also demonstrate that the EAR-20 peptide is able to partially activate NMDARs even in the total absence of natural co-agonists (glutamate and glycine). These results prove that rational design of peptides is a good strategy to obtain new potential therapeutic agents for loss-of-function related disorders.

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S5O-02. Ion channel based development of biosensors for discovering improved treatments for amyotrophic lateral sclerosis

M-Alicante S, Nuñez E, Campos-Zarraga I, Araujo A, Muguruza-Montero A, Malo C, Urrutia J, Villarroel A

SK channels, widely expressed in excitable cells, contribute to the after-hyperpolarization following an action potential, mediate the intrinsic excitability of neurons, and control resting potential in lymphocytes and erythrocytes. Ca2+ gates their activity via calmodulin, which is bound at the C-terminus. These channels are targets of riluzole, the only known treatment for amyotrophic lateral sclerosis (ALS), which has limited effectiveness. This drug increases the efficacy of Ca2+ to open the channel. Thus, finding new drugs with improved pharmacology with a similar mode of action is of

utmost interest. However, the methodology to assess Ca2+ sensitivity by current electrophysiological methods represents a bottleneck for screening programs. An alternative is creating simplified systems, devoid of most of the protein components, amenable to other techniques. The challenge is to capture the main molecular events that take place during activation yet preserving the same pharmacological profile. The aim of this work is to design biosensors based on the SK4 channel gating that recent cryoEM studies have revealed. Based on these structures, we predicted that upon Ca2+ biding, two regions of the structure should come in close proximity, a movement amenable for detection by FRET. However, this first generation of rationally designed biosensors responded in the opposite direction, with a very small signal to noise ratio. After a fresh new redesign, and several phases of optimization, we have obtained a biosensor template that recapitulates the response to Ca2+ and riluzole observed in the full channel. In addition, we have designed a new library of riluzole analogues oriented by structural bioinformatic approaches. These new biosensors could provide a fast, cheap and effective platform for new therapeutic drug screening.

S5-O03. New chiral heterocyclic compounds with potent TRPM8 antagonist activity

Carolina Izquierdo,[1] Alberto Ponce,[1] Alicia Medina-Peris,[2] Asia Fernández-Carvajal,[2] Antonio Ferrer-Montiel,[2] Mercedes Martín-Martínez,[1] Rosario González-Muñiz[1]

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Transient Receptor Potential Melastatin type 8 (TRPM8) is a non-selective cationic channel, activated by innocuous cool to cold temperatures (15-30 °C), membrane depolarization, cooling agents, such as menthol and icilin, and different synthetic molecules.[1] Moreover, it is known that functional changes or mutations in these channels produce abnormal sensitivity to pain, while overexpression of this channel contributes to the development of various cancer types.[2] TRPM8 channels are also involved in asthma, cardiovascular, gastrointestinal and neurodegenerative diseases.[2] The implication of TRPM8 in all these pathologies makes it a valuable therapeutic target and different pharmaceutical companies and academic research groups focus their efforts in the search for new modulators.[3] Based on previous results obtained by our group,[4] and following the guidelines provided by computational studies, we designed a new family of compounds, having a chiral heterocyclic central scaffold with three elements of diversity. Docking studies suggest appropriate appendages for each substituent, and the designed compounds were synthesized following solution methodologies. In this communication we will talk about the modifications of just one element of diversity. All components of the prepared sublibrary have been evaluated *in vitro* using the HEK cell line stably expressing TRPM8 channels. A fluorimetry assay was used to measure the entrance of Ca^{2+} through the cell membrane. Obtained results indicate that some of the new compounds are potent TRPM8 antagonists, as further confirmed by electrophysiology.

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Key words: TRPM8, antagonist, chiral heterocycles, molecular modeling

Acknowledgments: Supported by the Spanish MINECO (RTI-2018-097189-B-C2) and CSIC (PIE201980E030). We thank Jessy Medina for technical assistance.

S5O-04. Structural and functional insights into TRPM8 modulation mediated by the immunosuppressant macrolide Rapamycin

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Transient Receptor Potential Melastatin 8 (TRPM8), is a polymodal non-selective cation channel activated by cold temperature, cooling compounds (e.g. menthol) and voltage. TRPM8 is expressed in primary sensory neurons and their peripheral nerve terminals. TRPM8 plays crucial physiological roles in environmental cold sensing and thermoregulation. It is also involved in pathophysiological conditions like cold hypersensitivity in painful neuropathies, migraine and dry eye disease. On the other hand, activation of TRPM8-expressing fibers by cold or menthol has analgesic and antipruritic effects. This functional profile makes TRPM8 a prime channel for development of novel pain modulators.

Previously, we identified the immunosuppressant macrolide Tacrolimus (FK506) as a specific activator of TRPM8 channels in recombinant and native systems (Arcas et al., 2019). Here, we explored the actions of Rapamycin (Sirolimus), a structurally analogous macrolide molecule, characterised by mTOR inhibition. mTOR is a kinase which regulates different cellular processes and is widely used clinically as an immunosuppressant to prevent tissue rejection following organ transplantation.

Combining *in vitro* calcium imaging and patch-clamp recordings, we found that Rapamycin activates TRPM8 channels from different species, including humans, expressed heterologously in a HEK293 cell line. Furthermore, Rapamycin activated large whole-cell currents in HEK293 cells expressing mouse TRPM8. The current-voltage relationship of Rapamycin-activated current showed strong outward rectification and a reversal potential close to 0 mV, in line with the previously described characteristics of TRPM8 channels (Voets et al,.2004). Cold-evoked inward and outward currents, measured at -100 mV and +100mV respectively, were strongly potentiated in the presence of Rapamycin. Additionally, Rapamycin caused a strong shift of $V_{1/2}$ for channel activation towards more negative membrane potentials. The selective TRPM8 antagonist AMTB completely supressed Rapamycin-evoked currents whereas it partially inhibits cold response in the presence of Rapamycin. In cold-sensitive TRPM8-expressing DRG neurons, Rapamycin selectively activated inward currents, strongly potentiated their cold evoked response and increased the firing frequency during cold stimulation.

Finally, in order to understand the interactions of Rapamycin with TRPM8, we examined the effects of single point mutations on different segments, including S2, S4 and TRP domains.

In conclusion, our results identified TRPM8 as a novel molecular target of the clinically approved drug Rapamycin.

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Keywords: thermotransduction, cold, noxious cold, ion channel, inflammation

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CLOSING PLENARY

Understanding TRPC1/4/5 channels through chemical/structural biology

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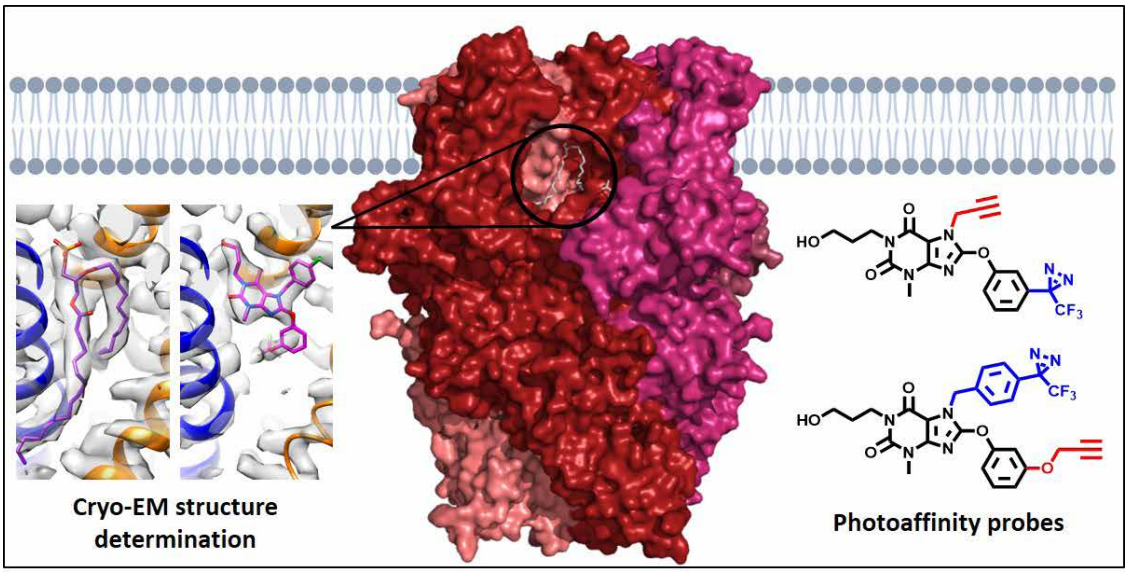
TRPC proteins form tetrameric, non-selective cation channels permeable by Na+ and Ca2+. These channels may consist of homomers or heteromers of subunits, each with their own characteristics and functions. Our research focuses on channels formed by the closely related TRPC4 and TRPC5 proteins – including heteromeric TRPC1/C4 and TRPC1/C5 channels. These channels are receiving increasing attention from both academia and industry as potential drug targets for the treatment of, for example, cancer, renal disease, cardiovascular remodelling and inflammation, complications of diabetes, and disorders of the central nervous system.

Currently, the best TRPC1/4/5 activator is the natural product (-)-englerin A. The most potent and selective inhibitors are xanthine derivatives such as Pico145, which can be used in cells, tissues and animals, and which can distinguish between different TRPC1/4/5 tetramers.



We use an integrated chemical/structural biology approach to study TRPC1/4/5 channel pharmacology, including: 1) detailed functional profiling of TRPC1/4/5 modulators; 2) determination of structure-activity relationships; 3) determination of high-resolution (2.7-3.0 Å) cryo-EM structures; 4) development of photoaffinity probes and covalent inhibitors; 5) development of channel variants (point mutants, chimaeras, reactive tags). Through these combined efforts, we provide detailed insight into the modulation of TRPC1/4/5 channels by small molecules and endogenous factors such as lipids and metal ions, allowing structure-guided design of new chemical probes.

Acknowledgements: The work I will present is the result of the efforts of a large interdisciplinary team of students, postdocs, facility staff and collaborators, all of whom will be duly acknowledged during the talk.

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POSTERS

P01. Direct conversion of human foetal lung fibroblasts to sensory neurons

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Chronic pain affects ~20% of the European population and drugs currently used to treat this condition present limiting side effects, which alter the compliance to the treatment. Therefore, there is a need to develop translational *in-vitro* sensory neurons models to study pain transduction in humans [1,2]. For these reasons, we propose an *in-vitro* human-based nociceptor model for investigating peripheral pain signalling and its modulation by anti-nociceptive molecules.

Lentiviral vectors expressing Brn3a, Ngn1 and rtTA were produced in HEK 293LTV through lipofectamine transfection and titred by qPCR. Afterwards, human foetal lung fibroblasts were transduced with Brn3a, Ngn1 (BN1) and rtTA and treated with doxycycline to induce BN1 transcription [3]. Fibroblasts were then treated with differentiation medium with small molecules and neurotrophic factors for 7 days. Differentiated cells were characterized by qPCR, immunocytochemistry, calcium imaging and electrophysiological assays.

Lentiviral vector titres were stable and reproducible at 10^8-10^9 IU/mL. Differentiated cells expressed pan-neuronal markers such as Map2 and Tuj1 and sensory neurons markers such as CGRP, Nav 1.7, Nav 1.8, Brn3a and Isl1. KCl and TRPs agonists elicited responses in functional assays. Culturing human sensory neurons in microfluidic chambers is being optimized at the moment. This culturing method would open the possibility to study peripheral pain signalling in an *in-vivo*-like fashion, having terminals separated from somata.

In conclusion, we optimized a protocol to directly convert human foetal lung fibroblasts to sensory neurons, however further optimization may be needed. This opens new venues for studying nociceptive signalling and for testing anti-nociceptive drugs. Furthermore, we aim to expand these cultures to human sensory neurons obtained from cutaneous fibroblasts.

Keywords: Direct conversion, Sensory neurons, Pain, In-vitro model

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P02. Role of estrogen receptor ERβ on K+ channel regulation in the human beta cell line EndoC-βH1

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Three different estrogen receptors, ERα, ERβ and GPER, make up the estrogen response pathways. Endocrine disruptors bind to these receptors and alter estrogen responses.

In mouse pancreatic β-cells, the estrogen receptor ERβ is involved in the regulation of ion channel function and expression, hence affecting insulin release. E2 decreases Na+ and Ca2+ currents as does the ERβ agonist DPN and the xenoestrogen Bisphenol-A (Villar-Pazos et al, 2017; Martinez-Pinna et al, 2019). The action of E2, DPN and BPA is different on K+ currents; while DPN and BPA decreased K+ currents, E2 had no effect.

Here we investigate the role that ligands of the three estrogen receptors have on K+ channels in the human pancreatic beta cell line EndoC-βH1 using the patch-clamp technique. Treatment with 1nM E2 during 48 hours had no effect on whole K+ currents, yet 1nM BPA partially inhibited voltage-gated K+ currents. We measured K+ currents in the absence or presence of iberiotoxin (IbTx), a KCa1.1 channel blocker, or stromatoxin-1 (ScTx1), an inhibitor of Kv2.1/2.2 channels. BPA decreased K+ current density in the presence of 100nM IbTx and did not alter K+ currents in the presence of 100nM ScTx1. This indicates that BPA reduces K+ currents through Kv2.1/2.2 rather than KCa1.1. To investigate a role of estrogen receptors in the effect of BPA, we measured K+ currents after treatment with estrogen receptors agonists. The GPER agonist, G1, and the ERα agonist, PPT, had no effect, whereas the ERβ agonist, DPN, decreased K+ current density. This indicates a role of ERβ in the regulation of K+ currents through Kv2.1/2.2. ERα (MPP) and ERβ (PHTPP) antagonists did not modify the density of K+ currents. A possible effect of these antagonists on BPA action is ongoing. We treated EndoC-βH1 cells during 48h with E2 and BPA and measured the mRNA expression of Kcnb1, Kcnb2, Kcnma1, Kcnip, by qRT-PCR. We observed a downregulation of Kcnb1 and Kcnb2 genes encoding for Kv2.1 and Kv2.2 channels, respectively. Our preliminary results indicate that ERβ activation could play a role in the inhibition of Kv2.1/2.2. channels.

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P03. The prokaryotic ligand-gated ion channel GLIC, homologous to nicotinic receptors, is blocked by the anti-inflammatory compound Peimine

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The alkaloid Peimine (Pm) is contained in extracts of bulbs from *Fritillaria* plants, which have been used for millennia as anti-inflammatory and analgesic therapy in Traditional Chinese Medicine. This

fact constitutes a strong evidence of the beneficial effects of these extracts and their low toxicity. The main therapeutic actions described for Pm include anti-inflammatory actions mediated by activation of MAPKs kinases [1] or by blockade of Kv1.3 channels [2] at concentrations above 20-140 μM. Despite this, Pm also blocks K^+ hERG channels (IC_{50} around 40 μM [1]), suggesting that the true therapeutic targets must have higher affinity for Pm than those so far reported to explain its effects.

Recently, we have reported that Pm inhibits muscle-type nicotinic acetylcholine receptors (nAChRms) with very high potency (IC_{50} circa 1 μM) and selectivity, since Pm does not affect other members of the Cys-loop family, as $GABA_A$ receptors [3]. Considering that Pm plasma levels after oral administration of *Fritillaria* extracts are, at most, in the low micromolar range and that nAChRs constitute a high affinity target for Pm, it turns out that its therapeutic effects could be mediated through this interaction. Furthermore, nAChRs are expressed in non-neuronal cells, including macrophages, and different subtypes of nAChRs are involved in inflammation. The molecular structure of the proton (H^+)-gated cation channel from the cyanobacterium *Gloeobacter violaceous* (GLIC) constitutes a useful model to explore structural properties, function and modulation of vertebrate Cys-loop receptors, since GLIC and nAChR pores share high sequence similarities.

We have now studied, by electrophysiological methods, the effects of Pm on GLIC receptors expressed in *Xenopus* oocytes. The aim of this work was twice: ***i)*** to determine whether GLIC is, or not, modulated by Pm; ***ii)*** if so, to decipher the mechanisms by which Pm modulates GLIC activity, comparing these results with those reported for nAChRm modulation. Our results showed that GLIC was reversibly blocked by Pm in a dose-dependent (IC_{50}≈9 μM, n_H≈1), non-competitive and voltage-dependence manner. Besides, there were prominent hump-currents elicited by Pm washout. Altogether, these results strongly suggest that GLIC inhibition by Pm was mostly due to an open-channel blockade.

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P04. S-palmitoylation-dependent spatial localization of the regulatory Kvβ2.1 subunit

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The voltage-dependent potassium (Kv) channel Kvβ family was the first identified group of modulators of Kv channels. Kvβ regulation of the α-subunits has been under extensive study. However, scarce information about their specific α-subunit-independent biology is available. Kvβs expression is ubiquitous and, similar to Kv channels, tightly regulated in leukocytes. Although Kvβ subunits exhibit cytosolic distribution, spatial localization, in close contact with plasma membrane

Kv channels, is crucial for a proper immune response. Therefore, Kvβ2 is located near cell surface Kv1.3 channels within the immunological synapse during lymphocyte activation. The objective of this study was to analyze the structural elements that participate in the cellular distribution of Kvβs. Kvβ peptides, in addition to the cytoplasmic pattern, targeted the cell surface in the absence of Kv channels. Furthermore, Kvβ2.1, but not Kvβ1.1, targeted lipid raft microdomains in an S-acylation-dependent manner, which was concomitant with peptide localization within the immunological synapse. A pair of C-terminal cysteines (C301/C311) was mostly responsible for the specific palmitoylation of Kvβ2.1. Several insults altered Kvβ2.1 membrane localization. Thus, growth factor-dependent proliferation enhanced surface targeting, whereas PKC activation impaired lipid raft localization. However, PSD95 stabilized Kvβ2.1 in these domains. This data shed light in the molecular mechanisms by which Kvβ2 clusters into immunological synapses during leukocyte activation.

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P05. KCNE4-dependent modulation of Kv1.3-related leukocyte physiology

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The voltage-gated potassium channel Kv1.3 plays an essential role in the leukocyte physiology. The channel forms heterooligomeric complexes by associating to several modulatory subunits, which fine-tune Kv1.3 function. The ancillary peptide KCNE4 regulates the surface abundance of Kv1.3, reducing the currents and enhancing the C-type inactivation of the channel. Because the therapeutic relevance of Kv1.3, a role for KCNE4 emerges as a modulator of the immune system physiology. In this work, we manipulated KCNE4 expression in various leukocyte cell lines. While Jurkat T lymphocytes exhibit low KCNE4 levels, CY15 dendritic cells, a model of professional antigen-presenting cells (APC), robustly express KCNE4. The increase of KCNE4 abundance affected important T cell physiological features, such as Kv1.3 rearrangement at the immunological synapse (IS) and IL-2 production. Conversely, the LPS-dependent activation of CY15 cells increased Kv1.3/KCNE4 ratio and the amount of free Kv1.3 with no KCNE4 interaction. The stoichiometry of the Kv1.3/KCNE4 complex, which relies on the differential regulation of Kv1.3 and KCNE4 expression, fine-tunes the architecture of the complex. Single fluorescence bleaching steps demonstrated an open Kv1.3/KCNE4 stoichiometry with up to four KCNE4 subunits per Kv1.3 channel. In addition, several engineering constructs determined that increasing the number of KCNE4 subunits steadily decreased the abundance of Kv1.3 at the cell surface, whereas the presence of a single KCNE4 peptide was sufficient to enhance the C-type inactivation of the channel. Overall, our results demonstrate that KCNE4 regulates the immune system physiology via the modulation of the Kv1.3 channelosome composition. The variable architecture of the Kv1.3/KCNE4 complex, which depends on KCNE4 availability, differentially modulates Kv1.3 function. Thus, the expression remodelling of KCNE4 triggers functional consequences in the immune physiology by the modulation of the Kv1.3-dependent physiological functions.

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P06. Mitochondrial Kv1.3 routing and dynamics control during cell cycle progression

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The voltage-gated potassium channel Kv1.3 is a potential therapeutic target for obesity and diabetes. The mechanism of regulation of body weight and energy homeostasis involves Kv1.3 expression in different organs, including white and brown adipose tissues. Kv1.3 is functional both at the plasma membrane and at the inner mitochondrial membrane. Plasma membrane Kv1.3 mediates cell activation and proliferation. Several forward-traffic motifs target the channel to the plasma membrane in a COPII-dependent manner. However, the mitochondrial import pathway for Kv1.3 is largely unknown. Here, we deciphered the mitochondrial routing of the mitoKv1.3 channel. Kv1.3 used the TIM23 complex to translocate into the inner mitochondrial membrane. This mechanism is unconventional because the channel is a multispanning protein without a defined N-terminal pre-sequence. We found that transmembrane domains cooperatively mediate Kv1.3 mitochondrial targeting identifying the Hsp70/Hsp90 chaperon complex as key regulator of the process. We show that Kv1.3 promoted the proliferation of preadipocytes through the control of mitochondrial dynamics. Kv1.3 is expressed in mitochondria exhibiting high affinity for the perinuclear population. The mitochondrial network is highly dynamic during the cell cycle, showing continuous fusion-fission events. The formation of a hyperfused mitochondrial network at the G1/S phase of the cell cycle was dependent on Kv1.3 expression. Our results shed light into the mechanisms mediating localization of Kv1.3 into mitochondrial membranes, where promotes preadipocyte proliferation and differentiation by controlling mitochondrial membrane potential and mitochondrial dynamics at the G1 phase of the cell cycle.

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P07. SARAF is responsible of the inhibition in the Thr-evoked Ca^{2+} entry found in neonatal platelets.

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Neonatal platelets present a reduced response to physiological agonists, like thrombin (Thr) or adenosin-3',5'-bisphosphate (ADP), as well as, an impaired regulation of the intracellular calcium homeostasis[1,2], being this later barely investigated. Here, we have firstly corroborated the alterations in the Ca^{2+}-mobilization from the intracellular stores and the extracellular Ca^{2+}-entry in response to Thr in platelets isolated from umbilical cord blood (neonatal platelets) or adult blood (control women and mothers). Thus, our data reveal differences in the Ca^{2+}-mobilization patterns, which agree with previous investigations found in the literature[1]. Neonatal platelets present a reduced Ca^{2+}-entry in response to Thr, as compared to control women and their own mothers. WB analysis of the main proteins involved in Ca^{2+}-entry revealed an altered STIM1 expression, but also

an enhanced SARAF expression, which has been reported to be responsible of the slow calcium dependent inactivation (SCID)[3]. Additionally, we observed an enhanced STIM1/SARAF coupling in neonatal platelets under resting conditions, which does not change upon Thr stimulation. In line with this observation, overexpression of SARAF in the platelet precursor cells (MEG01 and DAMI cells) resulted in a reduction in the Thr-evoked Ca^{2+}-entry. Therefore, this manoeuvre reproduced the inhibitory pattern observed in neonatal platelets, suggesting that SARAF overexpression could be the main responsible for the phenotypes observed in these cells. Finally, we have found that during Thr stimulation in MEG01 cells, SARAF was ubiquitinated upon its dissociation of PDCD61/ALG, which does not occur in MEG01 cells overexpressing SARAF. Altogether, our data point to an altered regulatory mechanism of SARAF due to its overexpression in neonatal platelets that would be responsible of the reduced STIM1-dependent Ca^{2+} entry in response to Thr.

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P08. Spatial memory training reverses GirK channels modulation in the transgenic $APP_{Sw,Ind}$ Alzheimer's disease model

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Alzheimer's disease (AD) is a dementia characterized by progressive memory decline and neurodegeneration by the accumulation of amyloid-β (Aβ) and the hyperphosphorylation of *tau*. The hippocampus, responsible for learning and memory processes, is one of the most susceptible brain areas to Aβ. Last findings point to an imbalance between excitatory and inhibitory neurotransmission as the initial impairment in early stages (Jeremic et al., 2021).

The G-protein-gated inwardly rectifying potassium (GirK) channel decreases excitability and contributes to inhibitory neurotransmission. Therefore, it has been proposed as a potential target to restore excitatory/inhibitory balance in amyloidosis models. Indeed, GirK channel has been related to AD since its modulation could reverse LTP and memory deficits induces by Aβ (Sánchez-Rodríguez et al., 2017). Furthermore, it is well stablished that periodic cognitive training on a hippocampal-dependent memory task mitigates early AD memory deficits (Parra-Damas et al., 2014), although its effect on GirK channels remain unknown.

Here, the effects of genotype, age and training in a hippocampal-dependent memory task on the protein expression of GirK subunits and modulators were studied using male $APP_{Sw/Ind}$ mice. Results showed that Aβ caused a reduction of GirK2 expression as well as an increase of GIRK-modulator SNX27 expression in 6-months-old transgenic mice. Additionally, there was a developmental effect, exhibiting a down-regulation of GirK2 and an up-regulation of Girk3 in older mice compared to young ones, regardless the genotype. Finally, training in a hippocampal-dependent memory task, such as the Morris Water Maze, restored GirK2 and SNX27 levels in 6-month-old $APP_{Sw/Ind}$ mice. Thus, the effect of Aβ on GirK2, a subunit essential for the proper inhibitory function of GirK channels, could partially account for the excitatory/inhibitory unbalance transmission found in AD models, and training in a cognitive hippocampal-dependent task reverses this effect and lessens early Aβ dependent-AD's deficits.

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P09. Poster: Role of Store Operated Ca^{2+} entry (SOCE) in post-ischemic angiogenesis

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Background:

Post-ischemic angiogenesis contributes to tissue repair and heart recovery after myocardial infarction (MI). This process is controlled by pro-angiogenic factors, mainly vascular endothelial growth factor (VEGF), which triggers signaling pathways involving the increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Recently, we demonstrated that store-operated calcium entry (SOCE) plays a key role in angiogenesis. However, the function of SOCE in post-ischemic angiogenesis remains unknown.

Methods:

We examined serum VEGF-A and IL-17A levels using ELISA in healthy volunteers and patients with ST-segment elevation MI (STEMI) who underwent primary percutaneous coronary intervention (pPCI). In vitro angiogenesis was analyzed using Human Umbilical Vein Endothelial Cells (HUVEC) tube formation assay on Matrigel® and wound healing assays. Organ-on-a-chip 3D culture was also performed to mimic the physiological formation of vessel and to assess endothelial cells activation by human serum. Changes in $[Ca^{2+}]_i$ were measured using FURA-2 AM in HUVEC. Moreover, genes and protein expression were determined by RT-qPCR and western blot, respectively.

Results:

Our findings revealed that STEMI patients have higher serum levels of VEGF-A and IL-17A than healthy controls. Moreover, the incubation of HUVEC seeded on Matrigel® with 5% serum of STEMI patients enhanced vessels-like formation, as assessed by the increase in the number of meshes and junctions. Likewise, wound healing assay demonstrated that ischemic serum promoted faster HUVEC migration. Furthermore, long-term incubation of HUVEC with ischemic serum stimulated an exacerbated SOCE induced by thapsigargin, which correlated with Orai1 overexpression. Interestingly, using Organ-on-a-chip 3D culture of HUVEC we demonstrated that ischemic serum stimulated *tip* endothelial cells migration that was attenuated by Orai1 silencing. Finally, we found that knockdown of Orai1 inhibited significantly *Notch1*, *Hes1* and *Hey1* expression induced by STEMI patients' serum.

Conclusion:

Our data show for the first time that serum from STEMI patients promotes angiogenesis, involving Orai1 upregulation and the activation of Notch1 signaling pathways.

P10. Bacterial sodium channel NaChBac and dissociated dorsal root ganglia neurons for an improved cell therapy for spinal cord injury

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Spinal cord injury (SCI) is a devastating and debilitating injury that hampers the life of a patient characterized by partial or complete loss of muscle function. A lot has been studied about the regeneration, increase in functional recovery and mechanisms behind this loss to improve the current therapies and/or strategies used to treat SCI without success [1]. Current consensus defends two major impediments limiting neuronal regeneration; the inhibitory extrinsic signal generated by the hostile microenvironment after injury and the limited intrinsic capacity of adult central neurons to regrow and/or survive. Therefore, the combination of strategies targeting extrinsic and intrinsic limitations have been accepted to show better results [2].

Peripheral nerve grafts transplantation creates permissive substrate and allow central axons to regenerate in a complete lesion.

However, the ephemeral life cell engraftment and the axonal growth inhibitory signals results in a non-functional regeneration.

In this regard, there are several studies suggesting that neurons with greater excitability, hyper excited neurons are more receptive to **integration in circuits** and **can escape apoptosis.** It has been published that the expression of a bacterial voltage gated sodium channel, known as NaChBac (NC), which, increases the excitability of neurons by decreasing the firing threshold, increases neuronal survival as well as their integration in circuits [3].

Our findings suggest that peripheral neurons are able to survive and grow axons near around the lesion site in mice after SCI, with a greater survival tendency in NC expressing neurons.

Recently, the modification of the neuronal cytoskeleton, which drives the axon growth, has also been pointed as a regenerating alternative by targeting dystrophic growth cones [1,2].

Exploring this area of increased excitability in neurons and its effects on survival and integration, our working **hypothesis** consists in the **combination of two strategies to improve axon regeneration and further functional neuronal activity after a spinal cord injury**.

P11. Electrophysiological and computational characterization of membrane pores formed by the neuropeptide Dynorphin A

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Dynorphins are endogenous neuropeptides that function as ligands for the k-opioid receptor. In addition to opioid activity, dynorphins can induce several pathological effects such as neurological dysfunctions and cell death. Previous studies have suggested that Dynorphin A (DynA) mediates

some pathogenic actions through formation of transient pores in lipid domains of the plasma membrane. Here, we use planar bilayer electrophysiology to show that DynA induces pore formation in negatively charged membranes. We find a large variability in pore conformations showing equilibrium conductance fluctuations, what disregards electroporation as the dominant mechanism of pore formation. Ion selectivity measurements showing cationic selectivity indicate that positive protein charges of DynA are stabilized by phosphatidyl serine negative charges in the formation of combined structures. We complement our study with computational simulations that assess the stability of diverse peptide arrangements in the hydrophobic core of the bilayer. We show that DynA is capable of assembling in charged membranes to form water-filled pores that conductions.

P12. Role of TRPM8 and Kv1 channels in orofacial cold allodynia

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Cold allodynia is a frequent symptom of orofacial neuropathic pain resulting from trigeminal nerve damage. The molecular and neural bases underlying this sensory alteration are still poorly understood. In this study, we explored the mechanisms underlying the altered orofacial cold-sensitivity resulting from axonal damage of the infraorbital branch of the trigeminal nerve using chronic constriction injury (CCI) in mice, combined with behavioral analysis, Ca^{2+} imaging, patch-clamp recordings of retrogradely labeled infraorbital nerve (ION) neurons in culture, immunohistochemistry, and AAV vector-based gene delivery *in vivo*. We found that cold allodynia induced by ION-CCI is linked to an increase in the proportion of cold-sensitive neurons (CSNs) contributing to this branch and a shift in their thermal thresholds to higher temperatures. These changes are related to a reduction of the Kv1.1-1.2-dependent brake current I_{KD} and an augmented TRPM8 channel expression in ION-CSNs. The electrophysiological properties of CSNs contributing to the ION revealed an increase in a nociceptive-like phenotype among these TG neurons from injured animals compared to sham mice, suggesting that painful cold hypersensitivity is linked to recruiting silent afferents that became cold-sensitive in response to nerve damage. Thus, these silent nociceptors, which are normally insensitive to moderate cooling, became sensitive to mild cold temperatures by a combined effect of TRPM8 upregulation and a functional reduction of I_{KD}. Notably, AAV transduction with Kv1.1 channels effectively reverted the nociceptive phenotype of injured mice. Our results unveil a key role of TRPM8 and Kv1 channels in damage-triggered orofacial cold allodynia, suggesting that the overexpression of potassium channels underlying I_{KD} can be an effective tool to revert this sensory alteration.

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P13. Constitutive phosphorylation of serine 29 as a critical regulator of TRPM8 channel function

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The main molecular entity involved in innocuous cold detection in mammals is TRPM8. This polymodal TRP channel is activated by cold, cooling compounds such as menthol, voltage, and rises in osmolality. Basal kinase activity phosphorylates TRPM8 and modulates its function under resting conditions. However, which specific residues, how this post-translational modification modulates TRPM8 activity, and its influence on cold sensing are still poorly understood. We identified four serine residues within the N-terminal domain constitutively phosphorylated in the mouse ortholog by mass spectrometry. TRPM8 function was assessed by Ca^{2+}-imaging and patch-clamp recordings, revealing that treatment with staurosporine, a kinase inhibitor, increased cold- and menthol-evoked responses of the channel. S29A mutation is sufficient to enhance TRPM8 activity, suggesting that phosphorylation of this residue is a critical molecular determinant of this negative regulation. Biophysical and TIRF-based analysis revealed a dual mechanism in the potentiated responses of unphosphorylated TRPM8: an increase in the number of active channels at the plasma membrane and a shift in the voltage activation curve towards more negative potentials. Notably, basal kinase activity downregulates TRPM8 function at cold thermoreceptor neurons, an observation accounted for by mathematical modeling. Overall, our findings suggest that cold temperature detection could be rapidly and reversibly fine-tuned by controlling the TRPM8 basal phosphorylation state, a mechanism that acts as a dynamic molecular brake of this thermo-TRP channel function in primary sensory neurons.

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P14. Development of a compound as neurocosmetic ingredient to alleviate allodynia symptoms induced by chemotherapy

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Chemotherapy-Induced Peripheral Neuropathy (CIPN) is a nerve dysfrunction in the peripheral system, which is developed in most patients receiving chemotherapy in cancer. To date, there are no effective therapeutic strategies to combat this pathology, which causes pain, cold and mechanical sensitivity, paresthesia, inflammation and muscle weakness, normally starting by hands

and feet.[1] Several experimental evidences implicate the TRPM8 channel in cold hypersensitivity triggered by chemotherapy. Therefore, the modulation of these channels could be an alternative therapeutic approach to treat CIPN.[2] A few years ago, we start working with Alodia Farmacéutica SL in the development of a series of monocyclic β-lactam TRPM8 antagonists.[3] Since drug development is a long way, we started by progressing one of these β-lactams as a neurocosmetic ingredient to alleviate cold allodynia within CIPN. First studies include: scale-up synthesis, *in vitro* characterization, selectivity against other pain-related channels, dermal irritancy and mutagenicity. The advancement in the cosmetic preparations will also be reported.

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P15. Biphasic concentration pattern in the kinetic and thermodynamic analysis of ionic transport in nanochannels

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The understanding of ion transport through membrane nanochannels is based on the conductivity of the electrolytes flowing through them. Indeed, most simple models would describe ionic conductance of a cylindrical pore of radius R and length L as $G = kpR^2/L$ where k is the solution conductivity. Considerable effort has been made to elucidate how nanoscale confinement affects ionic transport. Questions about the molecular nature of the fluid, especially as regards the finite size of ions and the shielding effects, the importance of interfacial effects (access resistance) or entropic effects due to obstacles and irregularities of the boundaries remain under discussion.

Here, we aim to explore how electrolyte concentration affects ion transport features of membrane nanochannels and to what extent nanoscale confinement contributes to it. To do so, we measure current-voltage relationships, conductance, ionic selectivity and perform noise analysis (current fluctuations) and thermodynamic analysis (activation enthalpy) both in a biological channel of nanometric dimensions (bacterial porin OmpF 1-2 nm wide) and a larger synthetic nanopore (conical polyimide nanopore of 20-200 nm entrances).

Overall, we find biphasic concentration patterns in steady-state voltage-driven experiments and in quasi-equilibrium experiments with net zero-current, both in the biological channel OmpF and synthetic conical nanopores. We conclude that there are two separate concentration regimes regulating ion transport, one ruled by coulombic screening in dilute solutions and another in concentrated solutions where ion-ion repulsion, steric hindrance and ion trapping at the pore surface become dominant.

P16. Transcriptomic remodeling of intracellular calcium homeostasis in hippocampal neurons during *in vitro* aging

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Neurodegeneration associated to aging may involve changes in intracellular calcium homeostasis. Accordingly, we have investigated calcium remodeling in aging neurons from a transcriptomic point of view. For this end, we used microarrays analysis in neonate rat hippocampal neurons cultured for 7 days *in vitro* (DIV) that resemble young neurons and long-term cultures (21 DIV) resembling aged neurons. Since the process of neuron isolation in long-term cultures is extremely difficult, we have carried out transcriptomic analysis from both mixed cultures (neurons and glia) and cultures devoid of neurons. Accordingly, we have four different experimental conditions: young mixed cultures, aged mixed cultures, young isolated glia and aged isolated glia. Then, since the number of neurons before and after "glial isolation" is known, it is possible to quantify differential expression through hypothesis contrast. Our results show that a few transcripts coding for plasma membrane calcium channels are overexpressed in aging neurons including Kainate receptor 4, the molecular players involved in store-operated Ca^{2+} entry Orai2 and Stim1, and the modulators SARAF and Septin4, as well as the members of the superfamily of TRP channels TRPM2 and 3. In addition, intracellular Ca^{2+} release channels IP_3R 1 and 2 and ryanodine receptor 3 are also overexpressed in aging neurons whereas only the ryanodine receptor 2 is downregulated. In contrast, plasma membrane Ca^{2+} pumps PMCA1 and 3 are downregulated in aging neurons along with mitochondrial Ca^{2+} transport systems MCU, MICU2 and VDAC1,2 and 3. These results indicate that neuron aging is associated to a transcriptional remodeling of molecular players consistent with enhanced Ca^{2+} entry into the cytosol and decreased Ca^{2+} exit from the cytosol which is according to the "calcium hypothesis of brain aging".

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P17. Role of Orai1 variants in spheroid generation and self-renewal in cancer stem cells derived from triple negative breast cancer cells

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Breast cancer is the most common cancer in women worldwide. Among its different types, triple negative breast cancer is the deathliest due to its ability to invade different tissues and metastasize. Recent reports support the hypothesis that exclusively a sub-population of cancer cells within a tumor, termed cancer stem cells (CSC) have the capability for tumor initiation. Breast CSC, with low proliferative rates and self-renewing capacity, form discrete cluster of cells denominated mammospheres. Aberrant Ca^{2+} homeostasis has been proposed as a hallmark for cancer initiation and progression. Store operated calcium entry (SOCE), a major mechanism for Ca^{2+} influx from

the extracellular medium in not excitable cells, and the expression of its key mediators, the members of STIM, Orai and TRPC families, are altered in several cancers, including breast cancer. By a combination of molecular biology, biochemistry, and microscopy techniques we have studied the relationship between Orai1 and the ability for CSC to form spheroids and self-renew. Our results show that Orai1 is over-expressed in mammospheres derived from MDA-MB-231, both at the mRNA and proteins levels. MDA-MB-231-derived CSC where Orai1 was knocked-down by a shRNA showed a significant impairment in sphere forming efficiency. Moreover, the self-renewal ability of those cells was inhibited. Similar results were obtained in CSC derived from MDA-MB-231 cells with CRISPR-mediated knockout of Orai1 (Orai1-KO). Furthermore, over-expression of Orai1α and Orai1β in CSC derived from MDA-MB-231 Orai1-KO significantly restored their ability to form spheroids and self-renew, with similar efficiency. Altogether, our results support the involvement of Orai1α and Orai1β in the formation of mammospheres in CSC derived from the triple negative cell line MDA-MB-231, thus in their ability for tumor initiation, pointing out Orai1 proteins as a suitable candidate for new therapies against breast cancer metastasis.

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P18. Trafficking-deficient Kir2.1 mutation in mice disrupt a new Kir2.1 function at the SR leading to both Ca^{2+}-mediated and reentrant arrhythmias

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Background: Andersen-Tawil syndrome type 1 (ATS1), caused by trafficking-deficient mutations in the gene *KCNJ2* coding the inward rectifier K^+ channel Kir2.1, is associated with life-threatening arrhythmias, which in some patients resemble catecholaminergic polymorphic ventricular tachycardia (CPVT), but the mechanisms are poorly understood. We tested the **hypothesis** that dysfunction of two different populations of mutant Kir2.1 channels, one at the sarcolemma, and the other at the sarcoplasmic reticulum (SR) membrane, directly alters conduction and intracellular calcium dynamics, respectively, to promote the ATS1 phenotype and CPVT-type arrhythmias.

Material and Methods: We generated a new mouse model of ATS1 by a single i.v. injection of cardiac specific adeno-associated viral (AAV) transduction with the mutant Kir2.1$^{\Delta 314\text{-}315}$ protein. We investigated the arrhythmogenic mechanisms of the mouse model using *in-vivo*, cellular, structural and functional analyses of the model were carried out by electrocardiogram (ECG), optical mapping, patch-clamping, immunolocalization and live calcium imaging.

Results: Kir2.1$^{\Delta 314\text{-}315}$ mice had abnormal ECGs with prolonged QT interval and frequent extrasystoles in the form of bigeminy. In addition, they were highly inducible of reentrant tachycardia/fibrillation. Cardiomyocytes from Kir2.1$^{\Delta 314\text{-}315}$ mice had significantly reduced inward rectifier K^+ and Na^+ inward currents, depolarized resting membrane potential, and prolonged action potential duration. Immunolocalization in wildtype cardiomyocytes and skeletal muscle cells revealed a novel SR microdomain of functional Kir2.1 channels contributing to intracellular Ca^{2+} homeostasis. Kir2.1$^{\Delta 314\text{-}315}$ cardiomyocytes showed defects in SR Kir2.1 localization and function, which contributed to abnormal spontaneous Ca^{2+} release events.

Conclusions: Cardiac-specific AAV transduction with Kir2.1$^{\Delta 314\text{-}315}$ in mice recapitulates the ATS1 phenotype by disrupting localization and function of Kir2.1 channels at the SR, and the Kir2.1-$Na_V1.5$

channelosome at the sarcolemma. These results reveal a novel dual mechanism of arrhythmogenesis in ATS1 involving defects in Kir2.1 channel trafficking and function at two different microdomains. They also provide the first demonstration at the molecular level of the mechanism underlying the overlap between ATS1 and CPVT associated with defects in intracellular calcium homeostasis.

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P19. IQM-22110, a new selective inhibitor of KChIP3 and its pharmacological effects on Kv4.3, Kv4.3/KChIP2 and Kv4.3/KChIP3 currents

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Kv4 channels generate outward currents activated at subthreshold membrane potentials and are responsible for the repolarization of both cardiac and neuronal action potentials through *I*TO (transient outward current) and *I*A (A-type K+ current), respectively. Kv4 dysfunctions have been identified in cardiac diseases (i.e. Brugada syndrome or atrial fibrillation) as well as in neuronal diseases (i.e. schizophrenia, epilepsy or Alzheimer's disease). However, Kv4 channels need to assemble with regulatory or β subunits to fully reproduce *I*TO and *I*A currents. Among them, we will focus on KChIPs (potassium channel interacting proteins), being KChIP3 predominant in the brain and KChIP2 in both brain and heart. The assembly of these β subunits alters not only the biophysical properties of the channel, but also its pharmacology. For this reason, we have analysed the electrophysiological effects of IQM-22110 (a new compound designed to bind KChIP3) on the currents generated by Kv4.3, Kv4.3/KChIP2 and Kv4.3/KChIP3 channels. CHO cells were transiently transfected (Kv4.3, Kv4.3/KChIP2 or Kv4.3/KChIP3), and the potassium currents were recorded using the whole-cell patch-clamp technique. Our results indicate that IQM-22110 exerts differential effects on Kv4.3, Kv4.3/KChIP2 and Kv4.3/KChIP3 currents. First of all, the concentration-dependence of inhibition was modified, being the IC50 = 5.5 μM for Kv4.3 channels, 10.4 μM for Kv4.3/KChIP2 and exhibited a biphasic curve when KChIP3 is present (IC50(1) = 0.03 μM and IC50(2) = 26.3 μM), suggesting two binding sites for IQM-22110 in this complex. With molecular dynamics and site-directed mutagenesis, we have identified two aminoacids (Y130 and K166) in KChIP3 critical to the binding of IQM-22110 to its high affinity site. Also, we selected equipotent concentrations of IQM-22110 to further study the biophysical effects of this compound on each situation, demonstrating that IQM-22110 binds to the closed-active state of the channel. In this study we concluded that: i) at low concentrations, IQM-22110 is a selective inhibitor of Kv4.3/KChIP3, ii) Y130 and K166 in KChIP3 are critical to the binding of IQM-22110 to the high affinity site in Kv4.3/KChIP3, and iii) IQM-22110 binds to the closed-active state of Kv4.3, Kv4.3/KChIP2 and Kv4.3/KChIP3.

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P20. Mitoception, the transfer of mitochondria from colon cancer cells to normal colonic cells, reverses remodeling of store-operated channels

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During cancer process there is a metabolic reprograming which provides survival advantages to tumor cells. The cornerstone of this reprograming is the Warburg effect consisting in the rewiring of aerobic metabolism to glycolisis due to defective mitochondrial synthesis of ATP. Due to this effect, most tumor cells display enhanced mitochondrial potential (ΔΨ), the driving force for mitochondrial Ca^{2+} uptake. Mitochondria are critical players in intracellular Ca^{2+} homeostasis. For instance, they control the Ca^{2+}-dependent inactivation of store-operated channels involved in cell proliferation and other cancer hallmarks. In addition to metabolic reprogramming, cancer cells undergo a deep remodeling of intracellular Ca^{2+} homeostasis. To learn about the contribution of cancer mitochondria to this remodeling we asked whether transfer of mitochondria from normal cells may influence Ca^{2+} remodeling in cancer cells. For this end we isolated mitochondria from normal, human colonic NCM460 cells and labelled them with a fluorescent marker. Then we adapted a protocol of mitoception and transfer of exogenous, normal mitochondria to human colon cancer HT29 cells before investigating intracellular Ca^{2+} homeostasis in mitocepted cells. Our preliminary results showed that HT29 cells with normal mitochondria (HT29 mitocepted) show a lower store-operated Ca^{2+} entry (SOCE) than control HT29. In contrast, when colon cancer cells are self-mitocepted with mitochondria isolated from colon cancer cells, SOCE is enhanced. These results suggest that transformed mitochondria may modulate dramatically CRAC channels involved in store-operated Ca^{2+} entry likely actin on the slow Ca^{2+}-dependent inactivation of these channels.

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P21. New approaches for the identification of KChIP2 ligands to study the K_V4.3 channelosome in atrial fibrillation

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Ion channels are macromolecular complexes present in the plasma membrane and in intracellular organelles of the cells, where they play important functions. The dysfunction of these channels

results in several disorders named channelopathies, which represent a challenge for study and treatment.[1]

We are focused on voltage-gated potassium channels, specifically on K_V4.3. Kv4.3 is expressed in smooth muscle, heart and brain. Within the heart, Kv4.3 channels generate the transient outward potassium current (I_{TO}). However, I_{TO} characteristics are only observed when Kv4.3 assemble with accessory subunits as KChIP2 and DPP6.

K_V4.3 *channelosome* play a key role in atrial fibrillation (AF), the most common cardiac arrhythmia, with an estimated prevalence in the general population of 1.5–2%. However, current antiarrhythmic drugs for AF prevention have limited efficacy and considerable potential for adverse effects.[2]

KChIP2 (Potassium Channel Interacting Protein 2) belongs to the calcium binding protein superfamily. It is the KChIP member predominantly expressed in heart and a key regulator of cardiac action potential duration.

The identification of novel KChIP2 ligands could be useful to understand the role of K_V4.3 channelosome in AF and it could help to discover new treatments for AF. [3]

In this regard, structure-based virtual screening could be an important tool to accelerate the identification of novel KChIP2 ligands.

In this communication, we will describe a multidisciplinary approach that, starting with a structure-based virtual screening, followed by an iterative process of synthesis/biological evaluation/docking studies, has led to the identification of new KChIP2 ligands.

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P22. Different modes of synaptic and extrasynaptic NMDA receptor alteration in the hippocampus of P301S tau transgenic mice

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N-methyl-*D*-aspartate receptors (NMDARs) are pivotal players in the synaptic transmission and synaptic plasticity underlying learning and memory. Accordingly, dysfunction of NMDARs has been implicated in the pathophysiology of Alzheimer's disease (AD).

Aims: to investigate the expression and subcellular localization of GluN1, the obligatory subunit of NMDARs, in the hippocampus of P301S mice.

Methods: We used histoblot and SDS-digested freeze-fracture replica labelling (SDS-FRL) techniques.

Results: Histoblots showed that GluN1 expression was significantly reduced in the hippocampus of P301S mice in a laminar-specific manner at 10 months of age but was unaltered at 3 months. By SDS-FRL, excitatory synapses and extrasynaptic sites on pyramidal cells and interneuron were analysed throughout the CA1 field. Density of GluN1 was high at synaptic sites and substantially lower at extrasynaptic sites. Labelling density for GluN1 in excitatory synapses on spines was significantly reduced in P301S mice, compared to age-matched wild type mice, in the three strata. Density for synaptic GluN1 on interneuron dendrites was significantly reduced in P301S mice in the strata oriens and radiatum, but unaltered in the stratum lacunosum-moleculare. Labelling density for GluN1 at extrasynaptic sites showed no significant differences in pyramidal cells, and only increased density in the interneuron dendrites of the stratum radiatum.

Conclusions: This differential alteration of synaptic versus extrasynaptic NMDARs supports the notion that the progressive accumulation of phospho-tau is associated with changes in NMDARs, in the absence of Aβ pathology.

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P23. Electrophysiological characterization of the macrophage phenotype associated to metabolic syndrome.

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Atherothrombotic cardiovascular disease is the leading cause of death worldwide despite significant progress in risk factors prevention and in medical management. A major reason for this trend is the ongoing epidemic of obesity-induced insulin resistance and T2DM. T2DM not only increases the prevalence and the morbidity of coronary artery disease, but also worsens the outcomes after revascularization. Under T2DM conditions, chronic inflammatory signaling in the vasculature sustains endothelial dysfunction, leukocyte infiltration, and a pro-thrombotic environment. This low grade metabolic inflammation appears to be triggered by the recruitment and activation of macrophages, which upon metabolic dysfunction switch to a metabolic-disease-specific phenotype (MMe) different from the classical M1 phenotype observed during infection (Kartz et al 2014).

We have previously found that Kv1.3 blockers inhibit smooth muscle cell proliferation, representing a novel target against restenosis. However, systemic application of Kv1.3 blockers also ameliorate metabolic dysfunction in a T2DM mice model, acting on other yet unidentified cells different from vascular smooth muscle. In light of the importance of the macrophage in the pathogenesis of atherosclerosis in diabetes, we propose to explore the functional changes in MMe phenotype, which could contribute to the increasing risk of vascular complications in T2DM, with a focus on ion channel remodeling upon metabolic dysfunction.

Hypertensive (BPH) mice fed on a high fat diet (HFD) develop metabolic syndrome and T2DM. Peritoneal and bone marrow derived macrophages (BMDM) were characterized by their expression profile of specific markers (by flow cytometry and qPCR), both in basal conditions and upon LPS activation. In parallel studies, we carried out an electrophysiological characterization of macrophages (in basal and after LPS treatment) from control and HFD mice. We measured resting membrane potential and we explored the functional expression of several K currents (Kv1.3, Kir2.1, KCa3.1, other Kv currents) as well as purinergic P2X1 and P2X7 currents using kinetic and pharmacological parameters. While M0 and M1 phenotypes associate with a specific channel fingerprint, we found differences in macrophages from HFD mice, which could be associated to the MMe phenotype. We propose that the correction of these differences could revert some of the T2DM associated vascular risk factors.

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P24. Physiopathological effects of CPT1C deficiency: from synapses to behavior

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CPT1c (Carnitine Palmitoyltransferase 1C) is a neuron-specific enzyme widely distributed throughout the entire central nervous system (CNS) and densely expressed in discrete brain areas, including the hypothalamus, hippocampus and amygdala. It is located in the endoplasmic reticulum, where it interacts with malonyl-CoA, a key indicator of the energy status in hypothalamic neurons, and regulates ceramide metabolism and triacylglicerol synthesis. More recently, it has also been demonstrated its involvement in dendritic spine maturation and GluA1-containing AMPA receptor synthesis and trafficking. Consistent with its widespread distribution in the CNS and its molecular functions, CPT1C plays a crucial role in hypothalamic control of food intake, energy homeostasis, motor function and hippocampal-dependent spatial memory. However, CPT1c might have additional functions that remain unexplored. Here, we carried out a systematic characterization of the role of CPT1C at different levels of complexity -molecular, synapses, neural networks, behavior and cognition- by comparing CPT1C knock-out (KO) mice and wild-type littermates. First, CPT1C expression pattern in the CNS was studied by immunohistochemistry. Then, we assessed the impact of CPT1c deficiency on locomotor activity, energy state and mood. Additionally, we investigated CPT1C involvement in motor learning, hippocampal-dependent non-associative spatial memory and associative instrumental learning. Finally, to correlate neural activity with hippocampal-dependent memory processes, we analyzed dendritic spine maturation in the hippocampus, synaptic plasticity in hippocampal slices *ex vivo* and electrocorticographic recordings obtained in freely-moving animals.

Our data confirmed the presence of CPT1C across almost all brain regions, with strong expression in the hippocampus and amygdala. CPT1C-deficient animals exhibited energy deficits and impaired locomotor activity, but no anxiety-related or depression-like behaviors were detected. These animals also showed deficits in motor and instrumental learning, as well as nonassociative contextual memory, these latter effects being explained by the long-term plasticity impairments observed at the CA3-CA1 hippocampal synapse, inefficient dendritic spine maturation and abnormal cortical oscillatory activity. Together, our results not only confirm the role of CPT1C in energy homeostasis and motor function, but also support the notion that CPT1C is required for learning and memory processes taking place in brain areas underlying motor, associative, and non-associative learning.

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We propose the combination of a transplant of excited peripheral neurons which express NC channel and local pharmacological treatment for axon microtubules and actin-myosin remodelling, tested in a preclinical model of SCI could serve as a therapy for SCI in rodent models.

***Keywords**: Spinal cord injury, cytoskeleton, drug, neuron, genetic modification, sodium channel, regeneration*

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P25. Loss of Kv7.2 channel function is associated with the severity of the clinical phenotype observed in children with KCQ2-related epilepsy

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Kv7.2 (KCNQ2) and Kv7.3 (KCNQ3) channels underlie the neuronal M current that stabilizes the resting potential and controls excitability. Therefore, alterations in these channels lead to neuronal pathologies with different severity in children. Whereas some newborns show epileptic seizures that resolve before the age of 2 years, others suffer from a global developmental delay (GDD) with greater or lesser loss of gross motor function. At the molecular level, several mutations have been found in the KCNQ2 of these patients.

The aim of this study was to analyze whether severe loss of channel function was related to the severe phenotype. We focused on six KCNQ2 variants: A178V, R553W, C544G, D282GfsTer49,

Q778RfsTer87 y V320_K331dup. Variants and wild type channels were transfected in HEK293 cells and ion current was recorded using whole-cell patch-clamp technique.

When expressed in heterozygosis with the wild type, the A178V and R553W variants (and to a lesser extent Q778RfsTer87) had current densities similar to that of the wild type KCNQ2 (22.22±6.7 pA/pF). However, current density was significantly lower in the C544G and D282GfsTer49 mutants (8.8±3.8 pA/pF and 13.49±2.5 pA/pF respectively, $p<0.05$). Last, V320_K331dup variant yielded very little current (1.35±0.75 pA/pF).

Children with A178V and R553W variants do not suffer from GDD and their clinical phenotype is considered as Benign (Familiar) Neonatal Epilepsy. In the patch-clamp experiments, these variants have normal current. On the other hand, C544G, D282GfsTer49 and Q778RfsTer87 variants are found in newborns with non-motor developmental epileptic encephalopathy. The current density of these variants is less than 50% smaller than that of the wild type KCNQ2. Last, V320_K331dup mutation was found in one patient with developmental epileptic encephalopathy and significant loss of gross motor function. Interestingly, in the electrophysiological characterization this variant displayed massive current loss of current amplitude compared to the wild type channel. Our results show an association between loss of KCNQ2 channel function and the severity of the clinical phenotype observed in patients.

P26. Synaptic plasticity regulation exerted by G-protein-gated inwardly rectifying potassium (GirK/Kir3) channels in the dorsal hippocampus supports cognitive processes

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G-protein-gated inwardly rectifying potassium (GirK) channels are the effectors of numerous G-protein-coupled receptors that are activated in response to different neurotransmitters, and they can be constitutively active due to ambient adenosine. Hence, hiperpolarizing currents mediated by GirK channels crucially contribute to the resting membrane potential, inhibitory neurotransmission and cell excitability in many brain regions.

In this work, we aimed to investigate the contribution of GirK channels expressed in the mouse dorsal hippocampus to cognitive functions and the underlying synaptic plasticity phenomena. For this purpose, GirK channel activity in this brain region was pharmacologically modulated with ML297, a GirK channel opener, or Tertiapin-Q, a GirK channel blocker. Excitability and long-term synaptic plasticity processes at the dorsal CA3-CA1 hippocampal synapse were assessed *in vivo* after intracerebroventricular (*icv*.) administration of the drugs and also in hippocampal slices *ex vivo*. Learning and memory capabilities were analyzed in freely-moving mice; namely, non-associative habituation and recognition memory as well as associative instrumental learning. Additionally, we examined potential side effects on motor function and emotional state through a battery of behavioral tests. In order to check whether behavioral and synaptic plasticity alterations induced by the drugs were associated to changes in GirK channel expression, GIRK1 and GIRK2 protein levels was quantified through immunohistochemistry.

Our data showed that both gain and loss of GirK channel function at the CA3-CA1 synapse modify its excitability and transform HFSinduced long-term potentiation (LTP) into long-term depression (LTD). These changes correlate with hippocampal-dependent cognitive deficits affecting both

associative and non-associative learning and memory, and were not accompanied by motor function impairments, anxiety-related nor depression-like behaviors. Changes in GIRK1 and GIRK2 expression were exclusively detected after multiple *icv.* injections, but our electrophysiological and behavioral evidence suggests that the impact of the drugs on GirK channel conductance is responsible for the "net" effects on synaptic plasticity and cognition. Together, our results indicate that GirK channels play a pivotal role in metaplastic regulation of the induction threshold for LTP/LTD and reveal their involvement on cognitive processes that rely on synaptic plasticity processes taking place in the dorsal hippocampal CA3-CA1 synapse.

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P27. TRPM3 role in renal management of hypertension

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Essential hypertension is a highly prevalent cardiovascular disease of unknown etiology and unclear physiopathology. TRPM3 is a Ca^{2+}-permeable non-selective cation channel activated by the pregnenolone sulfate (PS). PS contracts mouse aorta by activating TRPM3 in vascular smooth muscle cells (VSMCs). However, TRPM3 channels are absent in VSMCs from mesenteric arteries, where PS induced vasodilation by activating channels present in the sensory nerve endings. Due to these opposing effects, the role of TRPM3 channels controlling blood pressure remains unknown.

Using TRPM3-KO mice and hypertensive mice strains we studied the role of TRPM3 channels in integrated cardiovascular responses. TRPM3-KO mice are hypotensive and resistant to Angiotensin II (AngII)-induced hypertension, suggesting a possible hypertensive role of TRPM3 channels modulating the Renin/AngII/aldosterone axis.

As the kidney is a key organ in the control of the Renin system, we studied TRPM3 mRNA expression by qPCR in kidneys from normotensive (BPN) and hypertensive mice (BPH), observing a 2 fold higher expression in BPH. This difference is mainly due to changes in the expression in renal cortex, although a significative increase was also found in renal medulla from female mice. To explore the role of TRMP3 channels in the kidney vasculature, we studied the effect of PS 10 mM on vascular flow measured in isolated kidneys perfused at constant pressure, obtaining similar responses in BPN and BPH when phenylephrine was used as stimulus, but opposite results when the stimulus was AngII (vasoconstriction in BPN and vasodilation in BPH). These findings led us to explore TRPM3 expression throughout renal tissue using RNAScope, finding clear expression in the distal convoluted tubule and in some yet undefined glomerular cells. Since these data suggested a possible role of TRPM3 modulating renin secretion, we measured the plasma concentrations of the renin, angiotensin II and aldosterone. Although renin was elevated and angiotensin diminished in BPH mice, unexpectedly there were not significant differences between WT and TRPM3-KO mice.

Altogether, these data suggest the involvement of renal TRPM3 channels in the regulation of blood pressure, although further experiments are needed to define the actual mechanisms involved.

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P28. Functional implication of P2Y6 and AT1 heterodimers in the pathogenesis of essential hypertension

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Hypertension is a major risk factor of various diseases including stroke, heart failure, vascular disease, and kidney disease. Changes in calcium handling by vascular smooth muscle cells (VSMCs) together with changes in the contribution of membrane transporters and G-protein coupled receptors (GPCRs) contribute to the increased vascular tone characteristic of essential hypertension (Nishimura et al., 2016). Here we explored if changes in the functional expression or the association of these GPCRs participate in the vessel remodeling associated to essential hypertension.

We use third order mesenteric arteries from a hypertensive mouse strain (BPH) and its corresponding normotensive control (BPN). Mice were anesthetized by isoflurane inhalation (5%O2 at 2.5 L/min) and sacrificed by decapitation. Wire and pressure myography techniques were used. The mRNA expression levels were determined with Taqman® low density arrays and the protein expression and location was explored with immunocytochemistry and proximity ligation assays (PLA).

Dose response curve for phenylephrine (Phe), UTP and Angiotensin II (ATII)-induced contraction showed in all cases increased maximal contraction in BPH vessels, suggesting an enhanced efficiency of the contractile machinery in these arteries. UTP dose-response curves in BPH also showed a significant decrease of the EC50, due to the appearance of a second high-affinity binding site. Changes in mRNA expression of P2Y receptors together with their pharmacological characterization point to an increased functional expression of P2Y6R in BPH mesenteric arteries. ATII dose response curve in BPH vessels exhibited an inverted U-shape with a peak around 100nM, together with a slower desensitization of the contractile response compared to BPN. As it has been suggested that P2Y6 abundance can contribute to ATII–induced vasoconstriction via AT1R-P2Y6R heterodimers, we explored if this mechanism could explain BPH responses. In BPH vessels, desensitization of the ATII-induced contraction was accelerated in the presence of the P2Y6R blocker MRS2578 and slowed down upon preincubation with UTP. PLA suggested increased P2Y6R-AT1R interaction in VSMCs from BPH. We conclude that the formation of AT1R-P2Y6R heterodimers due to the increased P2Y6 abundance could represent an important mechanism contributing to the natural history of essential hypertension.

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P29. Probing the dynamics of the selectivity filter of the NaK channel: consequences on ion selectivity and inactivation.

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The prokaryotic NaK channel has been used as a model to study the origins of selectivity of ion channels, mainly because just introducing two mutations it goes from a two sites non-selective to

a four sites selectivity filter (SF), termed NaK2K, which is identical in sequence to the prototypical potassium channel KcsA. We have added a tryptophan to both channels at the pore helix adjacent to the SF, to probe the structural dynamics of this region through fluorescence studies.

Patch-clamp experiments reveal a modest gain of selectivity and conductance for K^+ in the case of NaK2K relative to NaK, probably due to the presence of four sites at the SF. However, both channels are able to conduct Na^+ and do not show inactivation, opposite to KcsA.

Our studies on ion binding to both channels show a loss of the micromolar event for K^+ found in KcsA, associated to a strong preference for this ion, maintaining the second submilimolar event, associated to ion conduction, and the absence of the low affinity binding to Na^+, linked to SF collapse in KcsA. Through homofret measurements, we have found that the SF of these two channels are able to widen when in the presence of Na^+, or to narrow when in K^+, an effect that is more pronounced in NaK2K, again because of the presence of four binding sites. Thus, we propose that Na^+ would pass partially or fully hydrated through the SF similar to what happens in Na^+ channels, but K^+ would induce a fit of the SF, binding it in a dehydrated form, as in K^+ channels.

Therefore, the identical four binding sites found in the SF of NaK2K and KcsA, is not suffice to acquire the strong selectivity of the latter. This reveals the importance of the scaffold behind the SF, which in the case of NaK and NaK2K would endow this domain with a higher flexibility as to adopt very different conformations, allowing the flux of Na^+ or K^+. This flexibility would also impede the loss of affinity for K^+ and the SF collapse, associated to the inactivating process.

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P30. Hypoglycosylation of Piezo1: pathophysiological relevance for Stroke-Like Episodes in Phosphomannomutase-2 deficiency?

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Congenital Disorders of Glycosylation (CDG) is a disease family caused by mutations in genes involved in the glycosylation pathways. Phosphomannomutase-2 (PMM2) catalyzes an early step in N-glycosylation, and its mutation is associated with PMM2-CDG [1]. Stroke-like episodes (SLEs) are among the acute neurological complications that may occur in PMM2-CDG. Although their pathomechanisms are not fully understood, head trauma has been recently described as a potential trigger [2]. Mechanosensitive ion channels have been suggested to underlie the transduction of mechanical forces into neurological responses in the brain. Although the molecular nature of these channels remains unknown, Piezo family are well positioned candidates. Interestingly, Piezo1 has been identified as an N-glycosylated protein [3].

We studied the effects of Piezo1 hypoglycosylation on its activity, biophysical properties and localization in heterologous expression system and/or primary cultures of murine cortical neurons. Hypoglycosylation of wild-type (WT) human Piezo1 (hPiezo1) by using the glycosylation blocker kifunensine induced an increase in the mechanical sensitivity in response to increasing pulses of negative pressure on cell-attached patches of HEK293 cells. Additionally, hypoglycosylated WT hPiezo1 showed slower inactivation kinetics. Interestingly, asparagine-to-glutamine hPiezo1 mutants N2293Q and N2330Q mimicked the effect of kifunensine on channel mechanical sensitivity. The increased mechanical sensitivity of N2293Q hPiezo1 mutant was lost when changing the surface coating from poly-L-lysine to collagen. In cortical neurons from mice, kifunensine- and swainsonine-induced hypoglycosylation resulted in an increased calcium influx in response to the Piezo1 specific agonist Yoda1. Immunofluorescent staining revealed that treatment with either inhibitor increased Piezo1 levels at the soma, but we did not observe changes in its synaptic localization pattern.

Based on these results, we suggest that hypoglycosylation of Piezo1 might contribute to the neurological symptoms found in PMM2-CDG patients, by favoring neuronal excitability in response to mechanical stimulation.

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P31. Effects of IQM-110 on Kv1.5/Kvβ2.1 channels

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The outward potassium current I_{Kur} is the main responsible of the atrial repolarization process and it is generated by the activation of $K_V1.5$ channels, widely expressed in human atria. It is known that mutations in *KCNA5* gene, which induce both gain- and loss-of-function in $K_V1.5$ channel, enhance atrial fibrillation susceptibility. Thus, these channels represent a pharmacological target for the development of antiarrhythmic drugs useful in the treatment of supraventricular arrhythmias. $K_V1.5$ channels assembly with several regulatory subunits such as $K_V\beta$. It has been described that $K_V\beta2.1$ interacts with $K_V1.5$. Our research group has demonstrated that the molecule IQM-110 produces electrophysiological effects on the $K_V1.5$. The aim of the present study is to analyze the electrophysiological effects of IQM-110 on $K_V1.5$ channels when it was expressed together with the regulatory subunit $K_V\beta2.1$. In order to achieve this objective, *Ltk*$^-$ cell line constitutively expressing $K_V\beta2.1$ and with an induced expression of $K_V1.5$ were used. Currents were recorded using the whole-cell configuration of the patch-clamp technique.

The effects of IQM-110 on $K_V1.5$/Kvβ2.1 current were concentration-dependent with an IC_{50} of 166μM (n=64). This compound at 100μM produced a time-dependent block, inducing a: 1) faster activation (τ=2.8±0.3 vs. 4.1±0.3 ms, n=10, p<0.01), 2) faster inactivation reducing the slow time constant, (τ_{slow}=3172.6±113.8 vs. 629.1±43.8ms, n=5, p<0.001), and 3) slower deactivation kinetics, (τ_{slow}= 73.5±3.5 vs. 115.2±12.1ms and τ_{fast}=19.5±1.3 vs. 27.9±1.9ms, n=10, p<0.01 and p<0.5 respectively). These results are consistent with an open channel block mechanism. Finally, IQM-110 (100 μM) enhanced the degree of use-dependent block of the current (1.7±0.4 vs. 47.7±2.4%, n=5, p<0.001). This phenomenon was explained by a slowing of the recovery process in the presence of IQM-110 ($\tau_{r=}$462.6±94.9 vs. 3545.0±254.1ms, n=5, p<0.001).

In summary, IQM-110 modulates $K_V1.5$/$K_V\beta2.1$ channels consistent with an open channel block mechanism.

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P32. The anti-restenosis effect of Kv1.3 channel blockers is increased in type 2 diabetic vessels

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Vascular occlusive disease is a leading cause of death and disability in the western world. The development of bypass surgery and coronary stents have represented a huge therapeutically improvement, but there are still unsolved problems associated with these therapies, such as restenosis of the vessel. The mechanical injury generated by surgery promotes proliferation of the vascular smooth muscle cells (VSMCs) of the vessel wall, triggering the development of intimal hyperplasia and leading to vessel occlusion. In the clinics, restenosis will be happening in pathological vessels and the underlying disease will determine prognosis. In particular, type 2 diabetes (T2DM) represents the dark side of restenosis. T2DM patients have more aggressive forms of vascular disease and worse outcomes, with exacerbated restenosis after vascular surgery.

In previous work, we showed that the potassium channel Kv1.3 is a critical player in VSMCs proliferation, demonstrating that pharmacological blockade of Kv1.3 channels inhibits VSMCs proliferation and migration and prevents restenosis of human vessels. We also found that Kv1.3 signaling to proliferation is mediated through the MER/ERK signaling pathway. Here we want to explore if Kv1.3 blockers are also efficient anti-restenosis agents in T2DM vessels

Using control and T2DM vessels from donors and VSMC in culture derived from these vessels, we found that T2DM vessels showed increasing remodeling in organ culture. These changes persisted in cultured VSMCs from T2DM patients, which showed augmented migration and proliferation, together with a downregulation of PI3K/AKT and upregulation of MER/ERK pathways. Kv1.3 blockade was more efficient preventing migration and proliferation in these cells.

Analysis of miRNAs involved in T2DM VSMCs epigenetic signature showed a significant upregulation of miR-126, associated with T2DM. miR-126 overexpression increased migration and proliferation in non-T2DM VSMCs, associated with Kv1.3 pathway (MER/ERK activation) but had no effect in T2DM VSMCs.

We conclude that Kv1.3 blockers are more efficient preventing remodeling of T2DM VSMCs. miR-126 plays crucial roles in T2DM VSMC metabolic memory through activation of the Kv1.3-dependent pathway.

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P33. Role of sorting motifs in polarized trafficking of the voltage-gated sodium channel β2 subunit

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The voltage-gated sodium (Na_V) channel complex is critical for cardiomyocyte function as it triggers cellular contraction in response to electrical stimulation. It consists of a pore-forming α subunit and associated β subunits. β subunits regulate function and subcellular localization of the α subunit. We previously demonstrated that *N*-linked glycosylation of β2 – encoded by *SCN2B* – is required for its trafficking to the plasma membrane, which promotes surface localization of $Na_V1.5$, the major cardiac α isoform (1). In polarized Madin-Darby canine kidney (MDCK) cells, we then provided evidence that β2 is *S*-acylated and that this modification partitions the protein into lipid rafts. We also showed that the polarized apical trafficking of β2 depends on plasma membrane cholesterol content (2). Here, we present evidence of β2 dimerization and the implication of its transmembrane domain and cytoplasmic tail on proper β2 folding and export to the apical surface in MDCK cells. Moreover, we explore – by acyl-biotin exchange assay – palmitoylation of β3, which we hypothesize occurs on this subunit in agreement with *in silico* predictions and homology with the related β1 subunit (3). In MDCK cells, however, and unlike β2, both β1 and β3 distribute mostly intracellular, largely within the endoplasmic reticulum network. This is likely because of differences in their sorting motifs, suggesting that these subunits also perform differentiated roles within and outside the Na_V channel complex. Altogether, our data couple the architecture of β subunits with their subcellular localization and function, which we analyze in immortalized MCDK cells growing polarized, and suggest that the findings in this model cell system provide valuable insights to get a deeper comprehension of the elusive biology of these subunits in excitable cells, such as neurons and cardiomyocytes.

Keywords: *SCN2B*; $Na_V1.5$; voltage-gated sodium (Na_V) channel; posttranslational modifications; polarized trafficking; MDCK cells.

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P34. K_V1.3 channel inhibition by a family of indolic compounds

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in western countries, it has no cure and pharmacological refractoriness to current treatments are common. Indole-3-carbinol (I3C) presents pharmacological activity against CLL. K_V1.3 potassium channels are involved in B-cell function, cell cycle regulation and proliferation, being considered a new therapeutic target. We aim to determine if I3C can act, in part, by modulating K_V1.3 function and the molecular requirements of I3C and derivatives for channel binding.

The inhibitory effect of I3C on Kv1.3 currents was assessed in I3C sensitive (I3CS-CLL) or resistant (I3CR-CLL) cells. For structure-function analysis, K_V1.3-pEYFP-C1 was transfected in HEK-293 cells. I3C derivatives with different B-cell cytotoxicity were tested. Currents were recorded by whole-cell patch-clamp. Statistical analysis was performed by ANOVA or t-Student tests.

K_V1.3 current was ≈3-fold higher in I3CR- than in I3CS-CLL cells, whereas Kv1.3 expression was similar. I3C inhibitory potency was similar in both groups (IC_{50}=7.43±2.17 μM vs 4.57±0.9 μM, for I3CS- and I3CR-CLL cells). Cytotoxic compounds (50 μM) inhibited K_V1.3 current by 41.18±7.66% and 29.11±3.70% (n=15, p>0.05). Non-cytotoxic derivatives did not inhibit the current.

Thus, Kv1.3 current magnitude in CLL cells inversely correlates with their pharmacological sensitivity to I3C. Moreover, I3C is an inhibitor of Kv1.3 current in these cells. Therefore, K_V1.3 channel could be involved in CLL pharmacological resistance mechanisms and its inhibition could be part of the cytotoxic effect of I3C. In addition, it seems to be a relationship between Kv1.3 inhibitory potency and B-cell cytotoxic activity. Only the inhibitory compounds present an ethyl group in position 3 of the indolic ring, that allows their dimerization. Thus, this group seems to be necessary to inhibit the Kv1.3 current; either for binding to the protein or for their dimerization before binding to the channel.

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P35. Characterization of novel endogenous Kv1.3 channel isoforms in T cells

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Kv1.3 is a voltage-dependent potassium channel expressed in normal and cancer cells, where it contributes to cell proliferation and apoptosis. In T cells, Kv1.3 is the only Kv channel expressed; however, lack of reliable antibodies has prevented its accurate detection under endogenous circumstances. To overcome this limitation, we designed a T cell derived Jurkat cell line in which the

endogenous channel was tagged to determine expression, location and changes upon activation of the native Kv1.3 channels. The CRISPR-Cas9 technique was used to generate Jurkat clones with the KCNA3 gene tagged at the C-terminus.

We identified two isoforms of Kv1.3 different from the canonical channel (60KDa) that differ on their N-terminus: A longer isoform of 70KDa and a truncated isoform of 43KDa.

Canonical Kv1.3 and both isoforms are upregulated after T cell activation.

To study protein localization, we generated constructs for heterologous expression. In HEK cells, canonical Kv1.3 and the longer isoform localize mainly at the plasma membrane while the 43KDa isoform accumulates in endoplasmic reticulum and in the nuclear membrane. As the 43KDa truncated isoform is expressed in Kv1.3-/-mice models, we focused on its functional characterization. To this end, we generated new Jurkat clones: a total KO for all isoforms and a clone expressing only the 43KDa isoform. 43KDa Jurkat cells did not show Kv1.3 mediated currents, and resting membrane potential was not different from KO cells. However, 43 KDa Jurkat cells showed differences in ERK phosphorylation and in capacitive calcium entry when compared to KO Jurkat cells, suggesting a role of the 43KDa isoform in T cell activation. HEK293 cells overexpressing this isoform exhibit some membrane expression of the construct together with small amplitude Kv1.3 currents, which could be attributed to heteromultimers of 43KDa with endogenous Kv channels. Contrary to canonical Kv1.3, HEK proliferation rate was unaffected upon 43KDa expression.

Altogether, we develop a preparation to explore expression and function of endogenous channels and we demonstrated that T cells express at least three forms of Kv1.3 channels. High homology between others voltage dependent potassium channels suggests that this could be also the case in other Kv channels.

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P36. Pre-clinical model for the study of gender dimorphism in inflammatory processes during a migraine attack

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Chronic pain is one of the most prevalent pathologies in society and, therefore, one of the main causes for which we go to medical consultation. Despite this, existing treatments are usually not entirely effective and are accompanied by adverse effects that limit their use. This type of pain can be found in different pathologies, among which, migraine and other diseases that occur with inflammation should be highlighted. This work will focus on the study of the molecular mechanisms that contribute to establish migraine attacks, as well as on revealing the pathogenic bases of the marked sexual dimorphism that this pathology presents. To address this study, an in vitro preclinical model is established using rat nociceptors in which the pathophysiological conditions that occur during a migraine attack are simulated, sensitizing these cells with pro-inflammatory molecular mediators such as histamine, bradykinin, prostaglandin E2 and serotonin. The validation of this model, as well as the evaluation of functional changes induced by inflammatory substances, is carried out using the electrophysiological technique of microelectrode array (MEA). As a preliminary results, the differences obtained between males and females for TRPA1 are shown, observing a lower TRPA1 desensitization after repeated stimuli, as well as a lower recovery, not being able to recover completely the activity.

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P37. The role of TRPM8 and TRPA1 channels in cold sensitivity of trigeminal and vagal sensory neurons

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Thermal perception is essential for thermally-driven reflexes, energy homeostasis and conscious behaviors (1). Specific molecular mechanisms are responsible for detecting cold temperatures (2). Although somatic cold sensitive neurons have been widely studied, very little is known about the mechanisms underlying cold sensitivity of visceral vagal neurons (3). Here, we used pharmacological and genetic tools to characterize cold-sensitive (CS) neurons in adult mouse trigeminal (TG) and vagal (VG) ganglia. We found that there are more CS neurons in VG compared to TG. In VG, most of the CS neurons are responding to TRPA1 agonists and cold responses are severely blunted with TRPA1 deletion or pharmacological TRPA1 blockade, suggesting TRPA1 as the predominant mechanism for cold transduction in this neurons. By in vivo retrograde labelling of airway-innervating vagal neurons we demonstrate their enhanced cold sensitivity and higher TRPA1 expression compared to vagal neurons innervating the stomach wall. On the other hand, CS neurons from TG co-express TRPM8 markers and cold responses are reduced the presence of TRPM8 antagonists and in TRPM8 KO mice, pointing to this channel as the main mechanism for cold transduction in this ganglia. Nevertheless, deletion or blockage of TRPA1 decreases cold sensitivity, demonstrating its contribution to this process. In both ganglia, a fraction of CS neurons responds to cooling by a mechanism independent of TRPA1 or TRPM8 yet to be characterized.

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P38. Structural consequences on the selectivity filter of the potassium channel KcsA by anionic lipids.

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Membrane lipids modulate the structure and function of many integral membrane proteins, including ion channels. Frequently, specific binding sites for lipids at transmembrane segments of the proteins are classified as annular or non-annular, although the exact mechanisms through which the bound lipids exerts such modulatory function remains elusive. Ion channels are of the essence to many physiological and pathological processes and constitute an important pharmacological target. Among them, KcsA is a prokaryotic potassium channel that serves as a model for the potassium ion channel superfamily due to the high homology with its eukaryotic counterparts. Anionic lipids have been shown to modulate KcsA in different manners and are required for channel function. For instance, anionic lipids increase ion channel conductance and open probability, provide stability to the tetrameric channel against thermal or chemical denaturation and enable its *in vitro* proper folding, although it is still unclear how and where these lipids exert such effects.

The selectivity filter of KcsA is the main determinant of the channel conductance, selectivity and inactivation. Thus, in this work, we have used the heat-induced denaturalization of the protein to characterize the binding of K^+ and Na^+ to this protein domain, to probe its conformation when phospholipids are added to the detergent-solubilized protein. With this mixed micelle system, we just observe the specific effects of phospholipids, since the nonspecific interactions through which the general physical properties of the membrane influence the protein structure and function are avoided. Finally, the R64 residue, located at the non-annular site of the channel, and related to the electrostatic attraction of anionic phospholipids to it, was mutated to alanine to evaluate the role of this site in this process.

P39. Structural and functional insights into TRPM8 modulation by the immunosuppressant macrolide Rapamycin

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TRPM8 is a polymodal non-selective cation channel activated by cold temperature, cooling compounds and voltage. TRPM8 is expressed in primary sensory neurons and their peripheral endings. TRPM8 plays crucial physiological roles in environmental cold sensing and thermoregulation. It is also involved in pathophysiological conditions like cold hypersensitivity in painful neuropathies, migraine and dry-eye disease. On the other hand, activation of TRPM8-expressing fibers by cold or menthol has analgesic and antipruritic effects. This functional profile makes TRPM8 a prime channel for development of novel pain modulators.

Previously, we identified the immunosuppressant macrolide Tacrolimus (FK506) as a specific activator of TRPM8 in recombinant and native systems. Here, we explored the actions of Rapamycin (Sirolimus), a structurally analogous macrolide molecule, characterised by mTOR inhibition. mTOR is a kinase which regulates different cellular processes and is widely used clinically as an immunosuppressant to prevent tissue rejection following organ transplantation.

Combining *in vitro* calcium imaging and patch-clamp recordings, we found that Rapamycin activates TRPM8 channels from different species, including humans, expressed heterologously in HEK293 cells. Furthermore, Rapamycin activated large whole-cell currents in HEK293 cells

expressing TRPM8. The current-voltage relationship of Rapamycin-activated current showed strong outward rectification and a reversal potential close to 0 mV, in line with the previously described characteristics of TRPM8 channels. Cold-evoked inward and outward currents, measured at -100 mV and +100mV respectively, were strongly potentiated in the presence of Rapamycin. Additionally, Rapamycin caused a strong shift of $V_{1/2}$ for channel activation towards more negative membrane potentials. The selective TRPM8 antagonist AMTB completely supressed Rapamycin-evoked currents whereas it partially inhibits cold response in the presence of Rapamycin. In cold-sensitive TRPM8-expressing DRG neurons, Rapamycin selectively activated inward currents, strongly potentiated their cold evoked response and increased the firing frequency during cold stimulation.

In order to understand the interactions of Rapamycin with TRPM8, we examined the effects of single point mutations on different transmembrane segments. The results revealed unexpected differences in the activation profile compared with canonical TRPM8 agonists.

Our results identified TRPM8 as a novel molecular target of the clinically approved drug Rapamycin, generalizing the agonist effect of different macrolides on this channel.

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P40. TARP subfamilies differ in their stoichiometry-dependent functional modulation of AMPARs

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AMPA receptors (AMPARs) are fundamental components of fast excitatory neurotransmission and are necessary for neuronal plasticity. In neurons, the trafficking behaviour and the functional properties of AMPARs are crucially controlled by their auxiliary subunits TARPs (Transmembrane AMPAR Regulatory Proteins), which are highly abundant in the central nervous system (CNS). TARPs are divided in three subfamilies: type Ia (g-2 and g-3), type Ib (g -4 and g-8) and type II (g -5 and g-7). All TARPs importantly affect biophysical properties such as gating and pharmacology of AMPARs. However, they show differences in the fine AMPAR modulation. All these differences in AMPAR fine tuning are important in defining the precise behaviour of the postsynaptic neuron in the process of neuronal communication. In our group we have previously reported how the prototypical and most profuse TARP g-2 differently modulates biophysical properties of the channel depending on the given AMPAR:TARP stoichiometry. However, whether this modulation is shared by other members of the family is an open question. Thus, we have tested the stoichiometrically-dependent modulatory effect on representative members for each subfamily: g-2, g-4 and g-5. We have studied the effect of a variable number of TARPs in the AMPAR structure by means of electrophysiological recordings in transiently transfected HEK293T cells. Our data revealed important differences between subfamilies in terms of AMPAR modulation depending on the number of TARP molecules attached to the complex. In contrast to g-2, g-4 and g-5 enhance single-channel conductance with less TARPs. Other modulatory effects (desensitization kinetics or recovery from desensitization) also differ between subfamilies. Interestingly, some fully TARPed conditions are not favoured as we observed very small currents indicating that some 4-TARPed AMPARs might not be present in neurons. Since TARP and AMPAR subunits are differentially expressed throughout the brain, these results will help us to better understand

the complexity in the regulation of AMPARs. Importantly, it has been described that some newly designed drugs selectively affect certain AMPARs depending on the TARP accompanying the receptor. Thus, a better knowledge of the AMPAR:TARP stoichiometry will avoid side effects derived from drugs targeting AMPARs.

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P41. Identification of the native AMPAR:TARP stoichiometry in cerebellar granule cells

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Glutamate is the most important and widespread neurotransmitter in the brain, being crutial for the communication process between neurons that occurs at the synapse. AMPA receptors (AMPARs), a subclass of glutamate-activated receptors, mediate roughly the 90% of fast-excitatory glutamatergic transmission. The trafficking and biophysical properties of these ionotropic receptors are modulated by several membrane-spanning proteins. One of the most important, abundant and studied families of modullating proteins of AMPARs are the so-called Transmembrane AMPAR Regulatory Proteins (TARPs). Indeed, AMPARs at synapses are always together with TARPs since the absence of these auxiliary subunits translate into the lack of synaptic AMPARs. Despite the extensive work describing whether TARPs modulate many gating and pharmacological properties of AMPARs, it is still undetermined how many TARP molecules are needed to induce significant changes in AMPAR intrinsic behaviour. This work depicts how the AMPA receptor behave differentally depending on the number of auxiliary subunits attached to it. Moreover, we describe a functional stoichiometry in cerebellar granule cells (CGCs), which express a limited variety of AMPAR subunits and TARPs.

AMPARs can importantly differ in their functional effects depending on the composition of the complex (pore forming subunits and auxiliary subunits as TARPs). Given the high number of possible combinations between AMPAR subunits and TARP molecules – besides the existence of other auxiliary subunits – these results will contribute to a better understanding of the complexity of this key player in the synaptic transmission. This work is specially relevant since newly described drugs can selectively affect certain AMPAR subtypes depending on the TARP present into the AMPAR complex. Our work adds valuable information for future therapies focused on the targeting of specific AMPAR subtypes, which are differentially expressed along the brain.

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P42. Potentiation of NMDA receptor currents by a novel nutraceutical compound

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Glutamatergic neurotransmission plays a central role in the nervous system networks and is key for processes such as learning and memory. In this context, the N-methyl-D-aspartate receptor (NMDAR) is an essential ion channel in glutamatergic synapses. Recent developments have led to the identification of "de novo" genetic variants affecting GRIN genes – which encode for NMDAR subunits – associated with neurodevelopmental disorders termed GRIN-related disorders (GRDs). These abnormal pathogenic variants can translate into different functional phenotypes in the receptor producing either a loss- or gain-of-function (LOF and GOF, respectively). Several evidences have demonstrated that NMDAR LOF can be pharmacologically rescued. For instance, a recent publication by our group showed that L-serine dietary supplementation results on a clinical improvement in a pediatric patient carrying a LOF GRIN variant (Soto et al, 2019). However, new therapeutic compounds with higher potency and specificity are still needed. Thus, we aimed to evaluate a novel positive allosteric modulator (PAM) of NMDARs, towards the functional rescue of LOF GRIN variants associated with GRDs. In this work, based on computational studies, we have identified a natural compound with a potential PAM effect on GluN2B subunit-containing NMDARs. In order to functionally test this hypothesis, we have conducted electrophysiological whole-cell recordings in NMDAR-expressing heterologous cell lines. Our functional studies showed that the candidate molecule potentiates roughly by 2-fold the peak current amplitude in (GluN1)2-(GluN2B)2-expressing cells. This potentiating effect was not detected in GluN2A-containing NMDARs. In conclusion, our data suggest that this novel compound can be used as a specific potentiator for the treatment of GRDs that comprise GluN2B subunit.

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P43. Loss of sodium current caused by a Brugada syndrome-associated variant is determined by patient-specific genetic background

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Background: Brugada syndrome (BrS) is an inherited cardiac arrhythmogenic disease that predisposes patients to sudden cardiac death. It is associated with variants in *SCN5A*, encoding the cardiac sodium channel alpha subunit ($Na_V1.5$). BrS-related variants have incomplete penetrance and variable expressivity within families.

Objective: To determine the role of patient-specific genetic background on the cellular and clinical phenotype among carriers of the $Na_V1.5$_p.V1525M channel.

Methods: We studied sodium currents from patient-specific induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) and heterologously transfected HEK tsA201 cells using the whole-cell patch clamp technique. We determined gene and protein expression by qPCR and western blot, and performed a genetic panel for arrhythmogenic diseases.

Results: Our results showed a large reduction in I_{Na} density in hiPSC-CM derived from two V1525M carriers compared with hiPSC-CM derived from a non-carrier, suggesting a dominant negative (DN) effect of the $Na_V1.5$_p.V1525M channel. I_{Na} was not affected in hiPSC-CM derived from a $Na_V1.5$_V1525M carrier who also carried the $Na_V1.5$_p.H558R polymorphism. Heterologous expression experiments showed that heterozygous expression of V1525M produced a loss of I_{Na} function, not observed when it was expressed together with $Na_V1.5$_p.H558R. In addition, antiarrhythmic drug mexiletine rescued I_{Na} function in hiPSC-CM.

Conclusion: Our results in patient-specific hiPSC-CM point to a DN effect of $Na_V1.5$_p.V1525M, which can be reverted by the presence of $Na_V1.5$_p.H558R. Overall, our data points to a role of patient-specific genetic background as a determinant for incomplete penetrance in BrS.

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P44. Functional annotation of de novo GRIN2A variants of the NMDAR

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Glutamate is the most common neurotransmitter along the central nervous system (CNS), driving the majority of excitatory pathways in the brain. Several ionotropic receptors with distinct roles (AMPA, NMDA and KA receptors) are expressed at the glutamatergic synapses. NMDARs are the primary inducers of synaptic plasticity processes. Upon their activation, NMDARs allow Ca^{2+} influx that allows the synaptic plasticity changes that constitute the cellular mechanism of learning and memory, to occur. When NMDARs do not gate properly, Ca^{2+} signaling is altered and plasticity might be compromised. One of the primary causes for NMDAR dysregulation is the presence of a de novo mutation in the GRIN genes, which encode GluN subunits. During the last years, the increasing number of reported GRIN variants in patients with neurodevelopmental disorders, has led to the categorization of these alterations under the term GRINpathies. The determination of the functional impact of these alterations whitnin the context of GRINpathies is important for future personalized treatments and/or predictions based on the annotation outcome. In this framework, we have examined the effect of GRIN2A(p.V563L) and GRIN2A(p.G664S) on NMDAR-mediated currents by means of electrophysiological recordings on cells transfected with heteromeric GluN1-GluN2A, GluN1-GluN2A(V563L) or GluN1-GluN2A(G664S) NMDARs. While the V563L change translates into a minor hypofunction of NMDARs, the impact of G664S in the GluN2A subunit correlates with a significant decrease of NMDAR-mediated currents. Furthermore, G664S mutation elicits significant alterations in the normal gating of the receptor, which aggravates the loss-of-function displayed by the NMDARs harbouring this mutation. Our results are in accordance with modelling predictions: the more severe mutation G664S is located at the linker between the ligand binding domain and the pore entrance while the milder V563L mutation is located at the external part of transmembrane 1 domain, which does not constitute part of the pore.

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P45. Thermo TRP ion channels as a key molecular and functional landmark for neuropathic pain transduction in subsets of somatosensory neurons

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Peripheral neuropathy is a challenging complication arising from treatment with many anti-cancer agents. Chemotherapy-induced peripheral neuropathy (CIPN) constitutes a major problem, especially because there is no single effective method of prevention [1]. My study is focused on Paclitaxel, a

taxane commonly used to treat cancers, which binds and stabilizes microtubules, but the cellular mechanisms that underlie paclitaxel's neurotoxic effects are not well understood [2]. Many studies have shown the involvement of the thermos TRP ion channels in several neuropathies [3].

The aim of this work will be to elucidate the role of three members of the TRP ion channel family - TRPA1, TRPM8, and TRPV1 in the development of CIPN produced by Paclitaxel. To achieve this goal, after extracting the Dorsal Root Ganglia from adult mice, sensory neurons are treated with two consecutive administrations of Paclitaxel in order to emulate a situation similar to a multi-cycle chemotherapy treatment.

Preliminary results from calcium imaging suggest that two consecutive administrations of Paclitaxel induce an increase in the percentage of neurons responding to AITC and capsaicin, TRPA1 and TRPV1 agonists, respectively. The percentage of neurons responding to menthol, the TRPM8 agonist remains almost unchanged. To confirm the increase in neuronal excitability in Paclitaxel treated DRGs, by a rise in TRPA1 and TRPV1 channel activity, electrophysiological studies will be performed using patch clamp and microelectrode array techniques.

In conclusion, once the involvement of these ThermoTRPs in the induction of CINP by Paclitaxel has been characterized, our goal will be to implement an in vitro model system more similar to the human one using sensory neurons from transdifferentiated human fibroblasts that could constitute a screening platform for the identification of new compounds able to reduce CIPN symptoms through the modulation of thermo TRP ion channels.

Keywords: TRP channels, Sensory neurons, CIPN, In-vitro model

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P46. K^+ channels are responsible for alterations in intrinsic electrical properties in a Shank3 mouse model of autism spectrum disorder

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Autism Spectrum Disorders (ASD) are a group of neurodevelopmental alterations arising from a combination of multiple environmental and genetic factors. In the past few years, a strong relation between Shank3 protein mutations and alterations linked to ASD has been established. Patients with alterations in these proteins usually present neural excitability abnormalities. In fact, some studies have found a link between Shank3 mutations and potassium channels dysfunction, but the role of this association on neuronal electrical properties remains unclear.

The aim of this work was to study the alterations of the resting membrane potential and the excitability in sympathetic neurons obtained from a Shank3-mutant mouse model of autism. We also investigated the role of background KCNK, KCNQ and HCN channels in those alterations.

Using perforated-patch whole-cell patch clamp techniques we recorded sympathetic neurons in culture both in current- and voltage-clamp. When compared with wild type, neurons obtained from Shank3 mutant mice showed a more positive resting membrane potential and a clear increase in their excitability. Our results also showed that significant alterations in TREK, m and h-currents are, at least in part, responsible for these variations in neuronal excitability.

P47. TRPV2: a key player in myelination disorders of the Central Nervous System

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Transient potential receptor vanilloid 2 (TRPV2) is widely expressed through the nervous system and specifically found in neuronal subpopulations and some glial cells. TRPV2 is known to be sensitized by methionine oxidation, which results from inflammation. Here we aim to characterize the expression and regulation of TRPV2 in myelination pathologies, such as hypomyelination and demyelination. We validated the interaction between TRPV2 and its putative interactor Opalin, an oligodendrocyte marker, in mixed glial cultures under pro- and anti-inflammatory conditions. Then, we characterized TRPV2 time-course expression in experimental animal models of hypomyelination (jimpy mice) and de-/remyelination (cuprizone intoxication and experimental autoimmune encephalomyelitis, EAE). TRPV2 showed upregulation associated to remyelination and inflammation in cuprizone and EAE models, and TRPV2 downregulation in hypomyelinated jimpy mice. TRPV2 expression was altered in human samples of multiple sclerosis (MS) patients. Additionally we analyzed methionine sulfoxide reductase A (MSRA) expression, an enzyme that reduces oxidated methionines in TRPV2, that we found increased in inflammatory conditions. These results suggest that TRPV2 may be a key player in the myelination, in accordance with the recapitulation hypothesis, and becomes an interesting clinical target in the treatment of demyelination disorders.

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P48. Characterization of spinal cord circuits involved in cold/burning-pain sensory crosstalk

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Pain is a complex experience consisting of a physiological and psychological response to noxious stimuli. While nociceptive signals from the afferent sensory fibers trigger pain, the central processing at the spinal cord and higher brain regions generate the actual percept. In this process, the dorsal horn of the spinal cord plays a fundamental role. At this level, different sensory modalities can interact and modify the flow of information transmitted to higher brain regions. A case in point is the interaction between cold and heat sensory pathways, which lies at the core of the pain relief we experience when applying cold to a burn or inflamed tissue.

In this research, we are interested in figuring out how cooling modulates burning pain integration to produce analgesia. To achieve this goal, we are developing and characterizing a novel mouse model of burn injury that allows for a consistent marking of activated spinal cord burning pain circuits. Combined with the TRAP2 mouse, which expresses the tamoxifen-inducible Cre recombinase only inactive neurons, this model will allow us to visualize spinal ensembles integrating sensory information behind burning pain and cold analgesia.

We will present the mouse model of Burn Injury by Repeated Heat Exposure (BIRHE) in the poster. Next, we will show the initial molecular characterization of the captured spinal cord circuits engaged in heat and cold processing.

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