X MEETING OF THE SPANISH ION CHANNEL NETWORK

SAN LORENZO DE EL ESCORIAL
25-27 MAY 2026

ABSTRACTS

WELCOME

We welcome you to the X RECI (X Meeting of the Spanish Ion Channel Network), which will take place in San Lorenzo de El Escorial (Spain), on May 25–27, 2026, at the Aula Magna of the Real Centro Universitario María Cristina (P.º de los Alamillos, 2, 28200 San Lorenzo de El Escorial, Madrid).

Since the first RECI Meeting, more than two decades ago, and now entering its 10th edition, the RECI series has become a well-established biennial gathering for specialists from Spain and across Europe who share a common interest in the world of ion channels. The meeting will focus on a wide range of topics related to ion channels, including their involvement in signaling, their structure, their roles in physiological and pathological processes, and their pharmacology and medicinal chemistry.

We expect the X RECI will be an excellent opportunity to learn from highly experienced senior scientists, as those for plenary lectures (N. Voigt, J. Jalife, T. Voets, and A. Ferrer-Montiel) and invited lectures (S. Tajada, M. Navarro-Pérez, J.A. Rosado, X. Altafaj, R. Estévez, A. Villarroel, L.O. Romero, B. Herguedas, G. Sandoz, N. Comes, J. Fernández-Trillo, M. Gutiérrez, R. Caballero, V. Moreno). Three talks from some of our Sponsors (3Brain, Sophion and Nanion) will keep us updated with latest technology in ion channel research. Postdoctoral researchers and post graduate students will also have the opportunity to present their work as short communications (20 selected from abstracts) and posters during the meeting.

We would like to deeply acknowledge the Institutions (MICIU, RECI, UCM and CSIC) and the X RECI sponsors (3 Brain, Sophion, Nanion, SEF and bioNova) for their financial support.

We hope you will have time to enjoy San Lorenzo de El Escorial, a lively and attractive World Heritage Site near Madrid. A guided visit has been programmed on Tuesday after the sessions and before the Gala Dinner.

We look forward to welcoming you in person at El Escorial, 25th-27th of May 2026 for the X RECI.

The Organizing Committee

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Organizing Committee

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X RECI FINAL PROGRAM

Monday - May 25, 2026

15:00-16:00 **REGISTRATION**

16:00-16:15 **OPENING**

16:15-17:00 **PLENARY LECTURE** **Niels Voigt** (University of Göttingen, Germany)

Buffer Breackdown: When Ca^{2+} Shocks Absorbers Fail in Atrial Fibrillation

SYMPOSIUM 1 **Ion Channels and Calcium Homeostasis**

Chairs: Juan Antonio Rosado & Antonio Felipe

INVITED LECTURES

17:00-17:25 **Sendoa Tajada.** *University of Valladolid*
Ion Channel Remodeling in Colon Cancer *via* Mitochondrial Transfer and Inhibition of Polyamine Synthesis.

17:25-17:50 **María Navarro-Pérez.** *University of Oxford*
Calcium-Mediated Modulation of Kv1.3 at the Immunological Synapse

17:50-18:15 **Juan Antonio Rosado.** *University of Extremadura*
Analysis of the Heteromerization of Orai1 Variants

SHORT COMMUNICATIONS

18:15-18:25 **Javier Picañol.** *Barcelona University*
Heterogeneous AMPA Receptor Populations Regulate Calcium Signaling in Hippocampal Astrocytes.

18:25-18:35 **Isabel Rodríguez-Valle.** *Institute of Biomedicine and Molecular Genetics of Valladolid*
Functional and Transcriptomic Analysis of Intracelular Calcium Remodeling in Pancreatic Cancer.

18:35-18:45 **Celia Cordero-Sanchez.** *University of Sevilla*
ORAI1-Dependent Regulation of Macrophage Adhesion and Polarization.

18:45-18:55 **Isaac Jardin.** *University of Extremadura*
Orai1 Flips the SOX2-Nanog Switch to Restrain Cancer Stemness in Luminal A Breast Cancer.

19:45-21:00 **WELCOME RECEPTION** Real Centro Universitario María Cristina

Tuesday - May 26, 2026

SYMPOSIUM 2 **Channelopathies and Rare Diseases**

Chairs: Eva Delpón & Álvaro Villarroel

INVITED LECTURES

9:00-9:25 **Xavier Altafaj.** University of Barcelona
Comprehensive Delineation and Precision Medicine of NMDAR-Related Rare Neurodevelopmental Disorders: From Biophysics to Clinical Trials.

9:25-9:50 **Raul Estevez.** University of Barcelona
From Astrocyte Signaling to Therapy: Unravelling MLC Pathophysiology through GPRC5B, MLC1 and VRAC.

9:50-10:15 **Alvaro Villaroel.** *Instituto Biofisika, CSIC-UPV/EHU, Leioa*
Folding Defects During Translation in Rare Diseases.

SHORT COMMUNICATIONS

10:15-10:25 **Manel C. Hadid.** Instituto de Investigación en Biotecnología y Salud (IDiBE), UMH Elche
Developmental and Epileptic Encephalopathy Associated with KCNB1: Toward Personalized Therapies

10:25-10:35 **Gorane Rodríguez Urquirizar,** *Universitat Pompeu Fabra, Barcelona*
Inhibition as a Strategy to Counteract Pathogenic $Ca_V2.1$ Gain-of-Function in CACNA1A Disorders

10:35-10:45 **Lucía Núñez**, University of A Coruña
Functional Effects of KCNA5 *p.G183R Mutation May Be Related to the Development of Ventricular Fibrillation in Acute Myocardial Infarction*

10:45-10:55 **Gillermo Javier Pérez**, *Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta*
AE3 p.G1070d Reveals a Chansporter Interaction in Short QT Syndrome

11:00-12:00 **COFFE BREACK & POSTERS**

SYMPOSIUM 3 **Structure and Function of Ion Channels**

Chairs: Mercedes Martín & Carmen Valenzuela

INVITED LECTURES

12:00-12:25 **Luis O. Romero**, Universidad de la Laguna
Functional and Structural Basis of TRPC3-Mediated Cerebellar Ataxia

12:25-12:50 **Beatriz Herguedas**, Instituto BIFI, Universidad de Zaragoza
Reshaping the Desensitized State: Subunit- and Editing-Dependent Dynamics in AMPA Receptors

SHORT COMMUNICATIONS

12:50-13:00 **María Redondo-Moya,** *Instituto de Investigaciones Biomédicas Sols-Morreale IIBM -UAM*
New Insights in the Modulation of the Electrophysiology of Kv1.5/Kvβ2.1 Channels by Lgi4

13:00-13:10 **Ana Gómez Del Campo,** *Instituto de Neurociencias UMH-CSIC, San Juan De Alicante*
Evolutionary Tuning of TRPM8 Cold Sensitivity: Identification of Functionally Relevant Residues across Vertebrate Classes

13:10-13:20 **Victoria Del Pilar Ribón**, *Institute for Biocomputation and Physics of Complex Systems, Zaragoza*
Sybody-Based Screening for State-and Subunit-Specific Recognition in AMPA Receptors

13:20-13:30 **Alex Perálvarez-Marín.** Universitat Autonoma de Barcelona, Cerdanyola Del Vallés, Spain
Integrated Computational and Experimental Strategies for TRPV2 Pharmacology and Vascular Therapeutics

SPONSORSHIPS' PRESENTATIONS

13:30-13:45 **3Brain** **Sercan Deniz**
Label-Free Functional Imaging Platforms for Organoids, Cell Cultures and Brain Slices: 3Brain CorePlate™ Technology

13:45:14:00 **Sophion** **Damian Colin Bell**
From Patch Pipettes to Planar Patch: Accelerated Electrophysiology via Automated Patch Clamp

14:00-15:00 **LUNCH**

15:05-15:20 **Nanion** **Nicoletta Murciano**
High-Throughput Automated Patch Clamp of Native Lysosomes for Early Drug Discovery

SYMPOSIUM 4 **Neuronal ion Channels**

Chairs: Xavier Gasull & Rosa Señarís

INVITED LECTURES

15:20-15:45 **Guillaume Sandoz**. Institut de Biologie Valrose (iBV). CNRS UMR7277 - Inserm U1091 - Université Côte d'Azur, France.
Switching On and Off Pain with Light

15:45-16:10 **Nuria Comes,** Institute of Neurosciences, Universitat de Barcelona
Novel TRP and TRESK Variants Linked to Neuropathic Pain

16:10-16:35 **Jorge Fernández-Trillo,** Instituto de Neurociencias UMH-CSIC, Sant Joan D´Alacant
Piezo2 in Mechanical Pain: Insights from In Vivo Recordings of Nociceptors

SHORT COMMUNICATIONS

16:35-16:45 **Helena Lluís**, Institute of Neurosciences, Universitat de Barcelona
Loss of TRESK Potassium Channel Alters Hippocampal Neuronal Excitability, Synaptic Plasticity, and Shapes Behavior in a Sex-Specific Manner

16:45-16:55 **Felipe Ortega,** Universidad Complutense de Madrid
P2X Purinergic Receptors Are Required for Correct Cortical Development in Human Brain Organoids

16:55-17:05 **Sara López-Vázquez,** IBGM, UVA-CSIC, Valladolid
Mechanisms Involved in SARS-CoV 2 E Protein-Induced Calcium Release from Intracellular Stores in Rat Hippocampal Neurons Aged In Vitro

17:05-17:15 **Victor Moreno Lozano,** Instituto De Investigación en Biotecnología y Salud (IDiBE), UMH, Elche
Persistent Nociceptor Hyperexcitability and Axonal Retraction Induced by Repeated Paclitaxel Exposure

17:15-18:00 **PLENARY LECTURE** **José Jalife** (CNIC, Madrid)

Rescuing the Heart from the Tornadoes of Sudden Cardiac Death

18:30-20:00 **GUIDED VISIT TO SAN LORENZO DE EL ESCORIAL**

20:30-22:30 **GALA DINNER** Restaurante La Herrería

Wednesday – May 27, 2026

9:30-10:15 **PLENARY LECTURE** **Thomas Voets** (University of Leuven, Leuven, Belgium)

Small Molecular Modulators of TRPM3 for the Treatment of Pain and Neurological Disorders

SYMPOSIUM 5 **Ion Channel Pharmacology and Therapeutics**

Chairs: Antonio R. Artalejo & Victoria Moreno

INVITED LECTURES

10:15-10:40 **Marta Gutierrez-Rodriguez,** *Instituto de Química Médica, IQM-CSIC, Madrid*
Tailoring Pharmacological and Fluorescent Probes to Study Kv Channelosomes

10:40-11:05 **Ricardo Caballero,** *Universidad Complutense de Madrid*
New Mechanisms of Drug-Induced Modulation of Cardiac Ion Currents: Changing Old Paradigms

11:05-11:30 **Victoria Moreno Manzano,** *Centro de Investigación Príncipe Felipe, Valencia*
Ion Channels for Neuronal Repair

11:30-12:00 **COFFEE BREACK & POSTERS**

SHORT COMMUNICATIONS

12:00-12:10 **Angel Cogolludo,** Complutense University of Madrid
Rescuing Kv1.5 Channels In Pulmonary Arterial Hypertension By Modulating Sigma 1 Receptors With Repurposed Drugs

12:10-12:20 **Pilar Cidad,** Universidad de Valladolid
TRPA1 and TRPM8 Mediate Intrinsic Cold Sensitivity of Cutaneous Arteries

12:20-12:30 **Francisco Rafael Nieto,** University of Granada
Tetrodotoxin (TTX) Attenuates Post-Operative Pain in Mice: Involvement of the Endogenous Opioid System

12:30-12:40 **Sara Pérez-Martín,** Universidad Complutense de Madrid
DECA-11 Selectively Restores Nav1.5 and Kir2.1 Channel Expression in the Membrane

12:40-13:25 **PLENARY LECTURE** **Antonio Ferrer-Montiel** (IDiBE-UMH, Elche)
TRP Channels Involved in Migraine Sex Dimorphism

13:30-14:00 **CLOSING CEREMONY & AWARDS**

14:00-15:00 **LUNCH & FAREWELL**

PLENARY LECTURES

PL1

Buffer Breakdown: When Ca^{2+} Shock Absorbers Fail in Atrial Fibrillation

Niels Voigt

University of Göttingen, Germany

Atrial fibrillation (AF) is the most prevalent sustained cardiac arrhythmia and a major contributor to stroke and heart failure. While abnormal calcium (Ca^{2+}) handling is a recognized driver of AF, the role of altered intracellular Ca^{2+} buffers, acting as a cellular "shock absorber" to stabilize Ca^{2+} fluctuations, has remained poorly understood.

In this talk, Dr. Niels Voigt will present recent findings from his team demonstrating that impaired Ca^{2+} buffering destabilizes atrial cardiomyocytes and promotes proarrhythmic activity. In particular, loss of buffering capacity increases susceptibility to spontaneous Ca^{2+} release events thereby facilitating AF initiation and maintenance.

These insights identify intracellular Ca^{2+} buffering as a central determinant of atrial rhythm stability and a promising therapeutic target. Strategies aimed at restoring buffering capacity may represent a novel approach for rhythm control.

Interestingly, in contrast to AF, the team of Dr. Niels Voigt observed increased atrial Ca^{2+} buffering in heart failure, suggesting a context-dependent remodeling of Ca^{2+} handling. This unexpected finding highlights the complexity of Ca^{2+} homeostasis across disease states and underscores the need for mechanism-based, patient-tailored therapeutic strategies.

PL2

Rescuing the Heart from the Tornadoes of Sudden Cardiac Death

José Jalife

Depatment of Cardiac Arrythmias. Centro Nacional de Investigaciones Cardiovasculares, Madrid, Spain

Sudden cardiac death (SCD) causes 4 to 5 million deaths each year globally. Electrical vortices (tornadoes or rotors) are the origin of ventricular fibrillation (VF), which often causes SCD. Cardiac electrical vortices have complex dynamics and have been shown in many mammalian species. During VF, the heart fails to contract suitably and is unable to pump blood. Once VF is initiated, drug treatments are ineffective and even make things worse. The only effective treatment is electrical shock to the ventricles. Our current understanding of VF mechanisms is fragmentary, hindering the development of personalized therapies. Yet recent insights into the roles of the most critical sarcolemmal ion channels in VF in controlling the excitation-recovery process provide hope. Substantial evidence indicates that the molecular interplay between the main cardiac sodium channel ($Na_v1.5$) and the strong inward-rectifier potassium current (Kir2.1) controls cardiac excitability, wave propagation velocity, and rotor formation, as well as rotor stability and frequency during VF. Studies at the cellular, molecular, and ion channel levels are helping us understand how rotors generate the turbulence that characterizes VF, providing insights into how to prevent their initiation and identifying new therapeutic targets to avert premature death.

PL3

Small Molecular Modulators of TRPM3 for the Treatment of Pain and Neurological Disorders

Thomas Voets

Laboratory of Ion Channel Research, VIB-KU Leuven Center for Neuroscience & Dept. of Cellular and Molecular Medicine, KU Leuven, B-3000 Leuven, Belgium

The transient receptor potential (TRP) channel TRPM3 is a calcium-permeable cation channel activated by heat and by the neurosteroid pregnenolone sulfate (PregS). TRPM3 is highly expressed in sensory neurons, where it plays a key role noxious heat sensing. Upregulation of TRPM3 expression and function contributes to hypersensitivity and ongoing pain in a variety of pain models, and genetic elimination of TRPM3 reduces evoked and ongoing pain in these models. We have developed potent, peripherally restricted TRPM3 antagonists, which bind to a specific ligand-binding site in the channel's transmembrane domain. These peripheral TRPM3 antagonists show potent analgesic effects in rodent models of inflammatory and neuropathic pain, without noticeable side effects. The most advanced compound was found safe in phase I clinical trials, and is currently being tested in Phase 2 trials for various pain indications. TRPM3 is also highly expressed in the brain, and *de novo* TRPM3 variants were described in patients with neurodevelopmental delay, intellectual disability and epilepsy. These variants lead to a dominant gain of channel function, including increased sensitivity to heat and neurosteroid, and altered pharmacology. These findings indicate that TRPM3 in brain regulates neuronal excitability, and suggest that TRPM3 antagonists may be developed as a possible therapy for brain diseases. Based thereon, we developed a class of selective, brain-permeant TRPM3 antagonists, which show potent efficacy in rodent epilepsy models.

PL4

TRP Channels Involved in Migraine Sex Dimorphism

Antonio Ferrer-Montiel

IDIBE-UMH, Avda. de la Universidad s/n, 03200 Elche, Spain

TRPA1 and TRPM8 are transient receptor potential channels present in trigeminal neurons, which have been associated with migraine pathophysiology. Using a mouse model for nitroglycerine-induced chronic migraine, we observed a sexually dimorphic phenotype. Both male and female mice developed mechanical hypersensitivity; however, females exhibited this condition until day 20, whereas it resolved by day 18 in males. The onset of hypersensitivity was dependent on TRPA1 in both sexes, while TRPM8 contributed to more rapid recovery in males. Notably, the antinociceptive activity mediated by TRPM8 was reliant on endogenous testosterone in male subjects. Exogenous testosterone administration to females and orchiectomized males yielded a reduction in hypersensitivity. Importantly, testosterone was found to ionotropically activate both murine and human TRPM8. Collectively, these results indicate that testosterone-mediated activation of TRPM8 may serve a protective function by decreasing the duration of hypersensitivity in migraine.

Acknowledgements
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INVITED LECTURES

IL1

Ion Channel Remodeling in Colon Cancer *via* Mitochondrial Transfer and Inhibition of Polyamine Synthesis

Sendoa Tajada, Verónica Feijóo, Elena Hernando-Pérez, Alejandra Méndez, Enrique Pérez-Riesgo, Lucía Núñez, Carlos Villalobos

Instituto de Biomedicina y Genética Molecular de Valladolid (IBGM), Universidad de Valladolid and Consejo Superior de Investigaciones Científicas (CSIC). 47003 Valladolid, Valladolid, Spain

Colorectal cancer represents a major public health burden and a leading cause of cancer-related death in Spain. A key feature of this disease is the loss of the APC tumor suppressor, leading to c-Myc activation and enhanced polyamine biosynthesis, which contribute to extensive cellular remodeling. Among these alterations, Ca^{2+} signaling and ion channel function are critically affected. Cancer cells commonly exhibit the Warburg effect, characterized by a metabolic shift toward glycolysis and impaired mitochondrial ATP production. This leads to an increased mitochondrial membrane potential ($\Delta\Psi$), enhancing mitochondrial Ca^{2+} uptake and modulating store-operated Ca^{2+} entry (SOCE), a pathway involved in multiple cancer hallmarks. In parallel, K^+ channel remodeling has emerged as an important feature of tumor progression.

Here, we investigated the interplay between mitochondrial function, Ca^{2+} homeostasis, and K^+ channel remodeling in colorectal cancer using HT29 tumor cells and NCM460 normal colonic cells. HT29 cells exhibited reduced total K^+ currents compared to normal cells, alongside increased activity of cancer-associated Kv10.1 and Kv11.1 channels. Gene expression analysis confirmed differential expression of several K^+ channel subtypes between normal and tumor cells. We then assessed whether targeting metabolic and mitochondrial alterations could reverse these phenotypes. Polyamine depletion using the ornithine decarboxylase inhibitor DFMO increased total K^+ currents in HT29 cells and modulated the expression of specific K^+ channel genes. Mitochondrial transfer (mitoception) of normal mitochondria into HT29 cells reduced $\Delta\Psi$ and normalized SOCE, whereas transfer of tumor-derived mitochondria further increased both parameters. No significant effects were observed in normal cells.

Overall, our findings reveal coordinated remodeling of mitochondrial function, Ca^{2+} signaling, and K^+ channel activity in colorectal cancer. Targeting these alterations partially restores normal cellular function, highlighting potential therapeutic strategies.

Acknowledgements

This work has been supported by grants from the Ministry of Science and Innovation (ref. PID2024-1592380B-100 and ref. PID2021-125909OB-100), the Junta de Castilla y León excellence program (Ref. CA9751), and two predoctoral fellowships from the Spanish association against cancer (AECC, to EPR) and Junta de Castilla y León (to VF and EHP).

IL2

Calcium-Mediated Modulation of Kv1.3 at the Immunological Synapse

María Navarro-Pérez[1,2], Jesusa Capera[1,2], Michael L Dustin[1], Antonio Felipe[2]

1. *The Kennedy Institute of Rheumatology, Nuffield Department of Orthopaedics Rheumatology &Musculoskeletal Sciences, University of Oxford, Oxford, United Kingdom*
2. *Molecular Physiology Laboratory, Departament de Bioquímica i Biomedicina Molecular, Institut de Biomedicina (IBUB), Universitat de Barcelona, Barcelona, Spain*

Effective immune responses depend on T lymphocytes forming a stable immune synapse (IS) that sustains calcium (Ca^{2+}) influx. Upon T cell receptor (TCR) engagement, depletion of endoplasmic reticulum Ca^{2+} stores activate CRAC channels, enabling continuous Ca^{2+} entry. This influx is supported by the electrochemical gradient generated through K^+ efflux *via* Kv1.3 and KCa3.1 channels. Thus, K^+ channels play a critical role in regulating Ca^{2+} signalling. However, unlike Ca^{2+} channels, their organisation and function remain poorly understood. Here, we investigated the role of Kv1.3 in regulating Ca^{2+} influx at the immunological synapse (IS), as well as the reciprocal modulation of channel function by Ca^{2+}. We found that Ca^{2+} fluxes generated during IS formation regulate Kv1.3 palmitoylation and its localisation within cholesterol-enriched microdomains. Furthermore, we identified Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) as the key effector mediating Ca^{2+}-dependent regulation of Kv1.3. CaMKII-dependent palmitoylation controls Kv1.3 distribution at IS, thereby influencing channel dynamics. Disruption of the CaMKII phosphorylation sites in Kv1.3 (CaMKless Kv1.3) resulted in reduced channel currents and consequently diminished Ca^{2+} influx. In addition, T cells expressing CaMKless Kv1.3 exhibited decreased expression of both early (CD69) and late (CD25) activation markers, underscoring the critical role of CaMKII-dependent Kv1.3 regulation in T cell activation. Overall, these findings reveal a tightly coordinated interplay between Ca^{2+} and K^+ channels during the immune response, which is essential for maintaining T cell activation within a physiological range.

Acknowledgements
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IL3

Analysis of the Heteromerization of Orai1 Variants

Juan Antonio Rosado[1], Jose J López[1], Isaac Jardín[1], Vanesa Jiménez-Velarde[1], Sandra Alvarado[1], Álvaro Macías-Díaz[1], Joel Nieto-Felipe[1], Francisco J Martín-Romero[2], Tarik Smani[3]

1. *University of Extremadura, Caceres, Spain*
2. *University of Extremadura, Badajoz, Spain*
3. *University of Seville, Seville, Spain*

Calcium release-activated Ca^{2+} (CRAC) channels are highly Ca^{2+} selective plasma membrane pathways formed by the hexameric assembly of Orai subunits, with Orai1 playing a predominant role [1]. In mammalian cells, Orai1 exists as two variants: the full-length Orai1α and a shorter version, Orai1β, which lacks the N-terminal 63–71 amino acids [2]. While these variants exhibit different biophysical properties and membrane mobilities, their potential heteromerization remains poorly understood. Using a combination of lipid raft isolation, advanced imaging techniques and analysis of protein-protein interaction our results demonstrate that when expressed individually, Orai1α is predominantly located in lipid raft domains due to a caveolin-binding domain, whereas Orai1β shows a more uniform membrane distribution and higher mobility. However, co-expression leads to a redistribution of Orai1β into lipid rafts. The altered lipid raft partitioning of Orai1β in the presence of Orai1α suggests an Orai1α-dependent recruitment. Functional assays using the pore-dead Orai1βE43Q mutant and the Ca^{2+} sensor G-GECO1.2 fused to Orai1α revealed that Orai1β interference significantly attenuates Orai1α-mediated Ca^{2+} influx. Furthermore, evidence from co-immunoprecipitation, APEX2 proximity labeling, FRET, and super-resolution microscopy (SIM) indicates that while both variants form mostly independent channels, a small but significant subset of Orai1α and Orai1β physically interacts to form heteromeric CRAC channels. These interactions appear to occur both at the plasma membrane and in intracellular localizations. Altogether, these findings provide new insights into the structural diversity of CRAC channels and explain the altered mobility and spatial distribution observed when both Orai1 variants are co-expressed.

Acknowledgements
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References
[1] Emrich SM, Yoast RE, Xin P, Arige V, Wagner LE, Hempel N, *et al.* Omnitemporal choreographies of all five Stim/Orai and IP3Rs underlie the complexity of mammalian Ca^{2+} signaling. Cell Rep. 2021;34(9):108760.
[2] Fukushima M, Tomita T, Janoshazi A, Putney JW. Alternative translation initiation gives rise to two isoforms of Orai1 with distinct plasma membrane mobilities. J Cell Sci. 2012;125(Pt 18):4354–61.

IL4

Comprehensive Delineation and Precision Medicine of NMDAR-Related Rare Neurodevelopmental Disorders: From Biophysics to Clinical Trials

Xavier Altafaj[1,2]

1. *Universitat de Barcelona, Barcelona, Spain*
2. *IDIBAPS, Barcelona, Spain*

Recent genetic advances showed that glutamatergic neurotransmission disturbance can result from primary de novo mutations of GRIN genes, encoding for the N-methyl-D-Aspartate receptor (NMDAR) subunits. These rare autosomal dominant conditions cause GRIN-related disorders (GRD), a group of severe developmental encephalopathies. GRD display a clinical spectrum including intellectual disability, hypotonia, ASD traits, motor impairment, epilepsy, and gastro-intestinal distress, in a gene and residue-dependent manners. Accordingly, as for other channelopathies, the functional annotation of GRIN de novo variants is critical i) to understand GRD pathophysiology, ii) to evaluate potential therapeutic strategies and iii) to define personalised therapeutic approaches. To address these issues, we have created a multi-angled GRIN cluster initiative, merging computational, experimental, translational, and clinical neuroscience approaches. Bioinformatic analysis was used to build-up a comprehensive and specific GRIN variants database compiling genetic, structural, functional, and clinical annotations. This database allowed to define a superimposition structural algorithm drastically increasing GRIN variants annotations with a high predictive likelihood ultimately accelerating GRIN variants functional annotations. Further, an experimental pipeline has been developed for the annotation of GRIN-orphan variants and their functional stratification. Importantly, we evaluated and experimentally demonstrated the potential therapeutic benefit of safe therapeutic interventions towards the rescue of dysfunctional NMDAR encompassing loss-of-function GRIN variants, both in preclinical cellular and animal models, in proof-of-concept GRD cases and currently in the first reported GRD clinical trial. Beyond GRD personalised therapies, our findings open the avenue for future treatments of genetic and/or environmental conditions perturbing the glutamatergic synapse.

Acknowledgements
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IL5

From Astrocyte Signaling to Therapy: Unravelling MLC Pathophysiology through GPRC5B, MLC1 and VRAC

Raúl Estévez, Guillem Pont-Espinòs, Marc González-Subías, Hector Gaitan-Peñas

University of Barcelona, L'Hospitalet De Llobregat, Spain

Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare leukodystrophy characterized by chronic white matter edema caused by defective ion and water homeostasis in astrocytes. Although mutations in MLC1 and GLIALCAM are the main genetic causes of the disease, the molecular mechanisms linking these proteins to altered channel activity and astrocytic volume regulation have remained elusive.
In this talk, I will present our work identifying the orphan G protein–coupled receptor GPRC5B as a central signaling hub connecting astrocytic signaling to volume-regulated anion channel (VRAC) dysfunction in MLC. Using complementary proteomic, biochemical and functional approaches, we show that MLC1, GlialCAM and GPRC5B form a signaling complex at astrocytic membranes. Loss of MLC1 or GlialCAM leads to excessive GPRC5B activity, contributing to pathological signaling and white matter vacuolization.
Recent work from our group reveals a previously unrecognized GPCR–kinase–channel axis in which GPRC5B directly interacts with the essential VRAC subunit LRRC8A and recruits the Src-family kinase Fyn. This signaling module suppresses hypotonicity-induced VRAC activation by preventing the conformational rearrangements required for channel opening, through phosphorylation of a single regulatory residue on LRRC8A. Importantly, MLC1 weakens the GPRC5B–LRRC8A interaction, providing a direct mechanistic link between MLC protein loss and impaired VRAC function in astrocytes.
Finally, genetic evidence from mouse models supports a causal role of GPRC5B hyperactivity in MLC pathophysiology, highlighting modulation of this pathway as a conceptual framework to understand astrocyte-driven brain edema and as a potential entry point for future therapeutic strategies.

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IL6

Folding Defects During Translation in Rare Diseases

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Voltage-gated potassium channels are regulated by lipids, calcium, and redox signals. Neuronal Kv7 (KCNQ) channels are potentiated by oxidative stress, reducing excitability and contributing to neuroprotection, yet how these inputs are integrated at the level of electromechanical coupling remains poorly understood. Kv7.4 voltage-sensor activation proceeds through two sequential transitions, only the second of which efficiently drives pore opening. Here, combining voltage-clamp fluorometry with manipulation of membrane PIP_2 and targeted mutagenesis, we show that oxidation preserves the intrinsic voltage dependence of voltage-sensor activation while redistributing gating such that pore opening occurs from both the early and late activated states. In this way, redox modification reprograms the electromechanical coupling landscape rather than altering voltage sensing itself. This remodeling requires both the third EF hand (EF3) of calmodulin and the channel S2S3 linker, and is accompanied by reduced functional dependence on PIP_2 availability. These findings identify the EF3-S2S3 interface as a redox-sensitive energetic checkpoint that integrates lipid signaling, calcium sensing, and oxidative state to dynamically tune voltage-sensor-pore coupling. More broadly, our findings reveal how metabolic signals can reshape the gating landscape of voltage-gated ion channels without altering the intrinsic energetics of voltage-sensor activation.

IL7

Functional and Structural Basis of TRPC3-Mediated Cerebellar Ataxia

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Cerebellar ataxias are characterized by impaired motor coordination resulting from neuronal dysfunction within the cerebellum. The mechanisms underlying this pathology and its cerebellar-specific neurodegeneration remain unknown. We uncover how a gain-of-function canonical transient receptor potential member 3 (TRPC3) mutation, coupled with a cerebellum-specific isoform, stabilizes the channel's open state, resists the leading inhibitor Pyr3, and drives calcium-dependent cell death. Restoring calcium homeostasis by expressing a Purkinje cell calcium pump improves cell viability. Transgenic expression of the TRPC3 hypermorphic variant in Caenorhabditis elegans induces neurodegeneration, confirming its pathogenicity across species. Cryo–electron microscopy and molecular simulations reveal the structural basis for the stabilization of the cerebellar-specific TRPC3 variant in its open state and uncover a druggable allosteric inhibitory binding site. These findings provide an explanation for the vulnerability of cerebellar neurons in TRPC3-associated ataxias and highlight a site for therapeutic intervention.

IL8

Reshaping the Desensitized State: Subunit- and Editing-Dependent Dynamics in AMPA Receptors

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AMPA receptors (AMPARs) are tetrameric glutamate-gated ion channels involved in fast excitatory neurotransmission and synaptic plasticity. Their molecular composition is highly diverse: four subunits (GluA1-A4) assemble as homomers or heteromers, generating the GluA tetrameric core. Each of these subunits presents different isoforms resulting from RNA splicing and editing; additionally, more than 30 protein partners interact with and modulate AMPAR properties. Subunit composition is spatially and developmentally regulated and determines receptor functional properties, from calcium permeability to kinetics and pharmacology.

Here, we present our recent structural data on the calcium-permeable GluA4 subunit, focusing on the architecture of the glutamate-bound desensizited states. We reveal how distinct levels of diversity (GluA subunits, auxiliary proteins, and RNA editing) impact the dynamics of the desensitized state, which can be populated even in the absence of agonists. These novel desensitized arrangements do not occur in the brain-dominant GluA2 subunit, which exhibits reduced dynamics during desensitization. The diverse quaternary organizations of AMPARs in resting and desensitized states open new avenues for potential subunit-specific modulation.

Acknowledgements

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IL9

Switching On and Off Pain with Light

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Pain research requires tools that enable high temporal precision, reversibility, and spatial control over nociceptive signaling, while avoiding systemic drug effects and invasive genetic manipulations. Here, we present two complementary, non-invasive optical strategies that exploit peripheral K2P potassium channels as bidirectional switches to modulate pain in intact wild-type animals. Using LAKI (Light-Activated K^+ Channel Inhibitor), a photoswitchable small molecule targeting the K2P channels TREK and TRESK, we achieve wavelength-dependent and reversible modulation of channel function through the skin. LAKI is functionally silent in the dark and under ambient light. However, alternating transdermal illumination at 365 nm and 480 nm reversibly blocks and unblocks TREK/TRESK. Because TREK and TRESK normally limit excitability at sensory endings, their light-driven inhibition through transdermal illumination enables reversible optical facilitation of pain. Conversely, Light-Induced Analgesia (LIA) enables drug-free pain suppression by directly activating the endogenous K2P channel TRAAK with brief 365 nm illumination. This activation is mediated by oxidation of a single native methionine, which is both necessary and sufficient to confer light sensitivity. Gentle skin illumination is sufficient to activate TRAAK channels, leading to nociceptor inhibition, and robust analgesia without compound administration. In this context, light functions as an "analgesia ON" command. Together, LAKI and LIA constitute a bidirectional optical toolkit for switching pain states: LAKI promotes nociception by inhibiting inhibitory K2P channels, whereas LIA suppresses nociception by activating TRAAK. This approach enables mechanistic dissection of peripheral pain pathways and offers a practical route to improve experimental control, reproducibility, and animal welfare in preclinical pain research.

IL10

Novel TRP and TRESK Variants Linked to Neuropathic Pain

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Peripheral neuronal hyperexcitability in Small Fiber Neuropathy (SFN) remains a complex driven by the biophysical dysregulation in the biophysical dysfunction of key sensory transducers and background leak channels. In this study, we report the functional characterization of novel genetic variants in TRPA1 and TRESK (*KCNK18*) identified SFN patients. Whole-cell patch-clamp and calcium imaging recordings performed in HEK293T cells revealed that the two TRPA1 missense variants p.Thr311Asn and p.Lys1046Glu constitute gain-of-function (GoF) mutations, exhibiting significantly enhanced current densities and sensitized responses to the electrophilic agonist allyl isothiocyanate (AITC). Single-channel analysis for p.Lys1046Glu showed a substantial increase in unitary conductance (234 pS versus 169 pS for WT) and a markedly elevated open probability (NPo of 0.285 versus 0.076), pointing to a pore-level mechanism for enhanced nociceptive signaling. In parallel we investigated heterozygous frameshift mutation (c.1107del) in the K2P channel TRESK, which is predicted to generate an elongated C-terminal domain with a novel extension spanning residues 384 to 397.

Electrophysiology demonstrated a severe reduction in K^+ current density through a mechanism of haploinsufficiency, while confocal microscopy confirmed impaired plasma membrane trafficking despite maintained oligomerization ability with WT subunits. Bioinformatic modeling suggests that altered C-terminal hydrophobicity and phosphorylation patterns underlie this loss-of-function (LoF). Collectively, these data provide a mechanistic framework for SFN pathophysiology and the transition to a chronic neuropathic pain state.

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IL11

Piezo2 in Mechanical Pain: Insights from *In Vivo* Recordings of Nociceptors

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Piezo2 is a mechanotransducer involved in low-threshold touch and proprioception[1], and has also been implicated in pain processes such as mechanical allodynia[2]. Transcriptomic studies show that Piezo2 is expressed in MRGPRD neurons[3], a population of non-peptidergic nociceptors mediating mechanical pain under physiological and pathological conditions. However, its specific role in these neurons remains unclear. This study aimed to determine the functional contribution of Piezo2 in MRGPRD nociceptors and its involvement in nerve injury-induced mechanical hyperalgesia.

Calcium imaging of MRGPRD neurons isolated from mouse dorsal root ganglia (DRG) cultures revealed two subpopulations: β-alanine-responsive neurons, likely polymodal nociceptors, and β-alanine-unresponsive neurons, previously classified as pure mechanonociceptors. Whole-cell patch-clamp recordings showed that approximately 50% of neurons in both groups exhibited mechanically activated currents with distinct inactivation kinetics. These currents were largely absent in DRG neurons from conditional knockout mice lacking Piezo2 specifically in MRGPRD neurons.

Behavioural experiments using the chronic constriction injury (CCI) model showed reduced mechanical hypersensitivity in mice lacking Piezo2 in MRGPRD neurons, particularly in response to high-intensity mechanical stimuli.

In vivo calcium imaging in the CCI model revealed increased activity of MRGPRD neurons innervating the sciatic nerve following noxious mechanical stimulation of the hind paw. This increase was abolished in conditional knockout mice, along with a significant reduction in the number of responsive neurons.

Overall, these results support a key role for Piezo2 in the mechanical sensitivity of MRGPRD neurons in both physiological and neuropathic conditions.

Finally, we observed Piezo2 loss alters the morphology of dermal MrgprD$^+$ nerve endings without eliminating them, likely reducing responses to noxious mechanical stimuli. Subepidermal Piezo2$^+$ fibers may require stronger mechanical activation, while epidermal fibers remain polymodal and respond to diverse stimuli.

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IL12

Tailoring Pharmacological and Fluorescent Probes to Study Kv Channelosomes

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Ion channels form signaling complexes or channelosomes, which are essential for optimal, fast and efficient signal transmission from the extracellular or intracellular medium.

In the human heart, Kv1.5 and Kv4.3 channels deserve special mention. Kv1.5 activation generates an atria-selective current that plays a crucial role in the human cardiac atrial repolarization (I_{Kur}). The Kv4.3 channels generate the fast transient outward potassium current (I_{to}) and its activity is crucial in the human cardiac action potential duration both in atrial and in ventricle.

Dr. Valenzuela's laboratory has demonstrated that the proteins associated with the Kv1.5 and Kv4.3 channelosomes closely modulate the activity of these two channels. In this regard, KChIP2, Lgi3, and Lgi4 have been identified in human atrial samples, but only KChIP2 and Lgi4 are downregulated in human cardiac samples from patients with persistent atrial fibrillation (AF). The limited efficacy of existing AF therapies highlights the necessity of exploring novel targets and therapeutic strategies. The progress in the search of new strategies for AF involves the combination of solid knowledge about its molecular basis together with the discovery and validation of innovative therapeutic approaches.

Therefore, it is of great interest to develop new chemical tools capable of modulating the interactions between proteins and channels as well as fluorescent probes to visualize them.

In this communication, we will present our latest results in the identification of KChIP ligands as modulators of Kv channelosomes and in the development of novel fluorescent probes.

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IL13

New Mechanisms of Drug-Induced Modulation of Cardiac Ion Currents: Changing Old Paradigms.

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Cardiac ion channels play fundamental roles in cardiac physiology and their acquired or inherited derangements can lead to life-threatening cardiac arrhythmias, such as ventricular arrhythmias (VA) including ventricular tachycardia and fibrillation. Sudden cardiac death (SCD) due to VA accounts for 50% deaths among patients with heart failure with reduced ejection fraction (HFrEF) despite the use of guideline-recommended therapies, posing a major unmet medical need. HFrEF produces electrical remodelling mainly characterized by the downregulation of Nav1.5 and Kir2.1 channel expression at the cardiomyocyte sarcolemma and of their currents, the sodium (I_{Na}) and inward rectifier K (I_{K1}), predisposing to malignant VA by decreasing ventricular excitability and prolonging repolarization. Current antiarrhythmic drugs (AAD), most of which are Na^+, Ca^{2+}, and/or K^+ channel blockers, have limited efficacy and safety (they can produce severe proarrhythmic effects). For instance, they are no longer the treatment of choice for VA associated to HFrEF as they increase mortality because they further decrease cardiac excitability and lengthen repolarization. Our group coordinates the ARCADIA Consortium devoted to the design and development of advanced therapies potentially useful for the prevention and treatment of arrhythmias associated to HFrEF. The overarching hypothesis was that the traditional AAD strategy based on ion channel blockers is no longer useful and, thus, novel mechanisms of channel modulation are warranted. We pursue the development of next-generation AAD based on small molecules (e.g. sodium-glucose cotransporter 2-SGLT2 inhibitors) and peptide-based therapies to simultaneously increase I_{Na} and I_{K1} acting at different levels: i.e., protein transcription and trafficking, ion channel biophysics, etc. The success of these novel therapies would produce a paradigm shift in the field allowing the discovery of novel and long-pursued antiarrhythmic mechanism of actions.

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IL14

Ion Channels for Neuronal Repair

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During development, central nervous system (CNS) axons display a high intrinsic ability to grow and innervate their target. However, as they mature, CNS neurons lose this capacity, including after injury. This limitation is attributed to several factors, including the lack of appropriate regenerative machinery governed by growth-associated genes. Increasing the intrinsic neuronal activity by the ectopic expression of ion channels and additional uptake of cations demonstrated to support higher neuronal circuit integration, prevent activity-dependent death. In the adult spinal cord, after traumatic injury, a significant reduction of the excitatory supraspinal input, induces a neurotransmitter remodeling phenotype from excitatory to inhibitory, especially in the premotor neurons. This switch in the excitatory phenotype promotes inhibition at synapses contacting motor neurons, which is accompanied by neuronal inactivation and locomotor deficits, even the neuronal circuit remains alive. Therefore, preserving or even enhancing an excitatory phenotype in the interneuron circuits could boost their contribution for adaptive motor control. Different strategies can be applied for that purpose, even providing additional excitatory cells into the damaged circuits, as we recently described by two different cell transplantation strategies, by using modified sensory neurons constitutively expressing a small sodium ion channel (NaChBac) or by the transplantation of neural precursor cells expressing a photosensitive channel (channelrhodopsin-2). In both cases, transplanted animals exerted an increased neuronal excitatory input, with an increased number of VGlut2 contacts, immediately caudal to the injuries, providing neurotrophic support and neuron preservation, allowing for adaptive remodeling within the propriospinal circuits for detour pathways through the supraspinal descending tracts. Overall, these findings underscore that additional benefits of a selective modulation of intraspinal neuronal activity through the manipulation of ion channels, being for instance a crucial strategy for successfully reactivating dormant connections and/or promoting the rewiring of canonical and newly adaptive motor circuits to achieve functional recovery after CNS injuries.

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COMPANY TALKS

CT1

Label-Free Functional Imaging Platforms for Organoids, Cell Cultures and Brain Slices: 3Brain CorePlate™ Technology

Sercan Deniz, PhD

3Brain AG, Switzerland

3Brain is a deep-tech life science / biotech company developing microchip-based technology for advancing life science research. Our goal is to provide researchers with the most advanced tools to study electrophysiological activity at a cellular and network level in brain organoids, slices, and cultures.
We are the first in the industry to produce intelligent plates (CorePlate™) capable of detecting electrical signals from cells. When combined with one of our plate readers, CorePlate™ is capable of sensing and processing data in situ, providing exceptional spatiotemporal resolution and allowing for real-time analysis. This technology is the result of 20 years of continuous research and development, in order to provide the most advanced platform for conducting scientific research in neurons, cardiomyocytes and to further drug discovery.

CT2

From Patch Pipettes to Planar Patch: Accelerated Electrophysiology *via* Automated Patch Clamp

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Since the early 2000s, automated patch clamp (APC) has reshaped electrophysiology from a specialist, low-throughput technique into a scalable and reproducible platform. Sophion Bioscience has been at the forefront of this evolution, advancing planar-patch APC technologies that preserve data quality while markedly improving ease and efficiency.

Sophion's APC systems enable parallel recordings across tens to hundreds of cells simultaneously, supporting high-throughput workflows with reduced operator variability. Complex voltage and pharmacology protocols can be executed consistently, enabling robust dataset generation for both mechanistic studies and screening.

Key technological developments underpin this progress. Microfluidic architectures (see below) allow rapid, complete solution exchange with minimal dead volume, using only a few microlitres per addition. Integrated temperature control enhances physiological relevance and experimental stability. Together, these features streamline assay development and improve experimental reproducibility.

Recent innovations further extend APC capability. Advances in low-noise amplifier design now permit resolution of smaller currents, enabling applications such as single-channel and transporter recordings. In parallel, high-resistance (HiR) recording plates support stable measurements from 'small' cells and subcellular structures, including red blood cells and organelle-derived vesicles.

APC has therefore evolved beyond a high-throughput alternative to manual patch clamp, becoming a versatile platform for increasingly precise and physiologically relevant interrogation of ion channels and transporters.

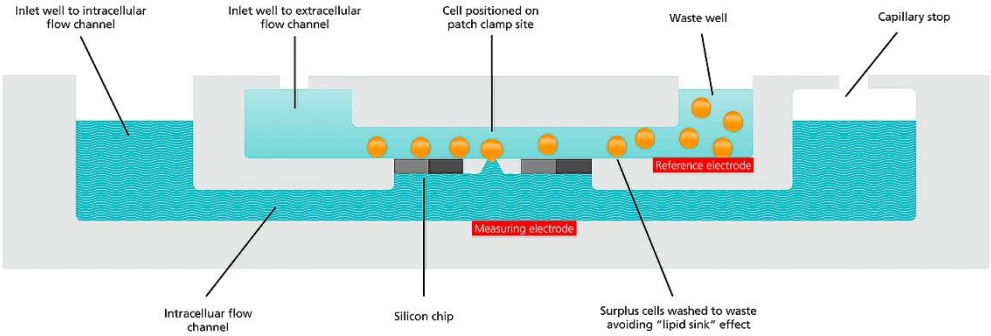

Figure. Sophion planar patch recording site in cross section, highlighting the low volume microfluidics architecture giving fast, efficient test solution exchange around the recording site microenvironment.

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CT3

High-Throughput Automated Patch Clamp of Native Lysosomes for Early Drug Discovery

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Lysosomal ion channels and transporters are increasingly recognized as key regulators of cellular homeostasis and intracellular signaling, with important implications for diseases such as Parkinson's disease and cancer[1]. As a result, they have attracted growing interest as potential therapeutic targets, driving the need for high-throughput electrophysiological approaches. Despite this, direct functional characterization of these channels in native lysosomes remains technically challenging, largely due to the low throughput and labour-intensive nature of conventional manual patch-clamp techniques.

Although automated patch-clamp platforms have long been established for whole-cell recordings, we have adapted a high-throughput automated system (Nanion's SyncroPatch 384) to investigate ion channel activity in native lysosomal preparations. These preparations were obtained either from using a novel organelle purification method by ORIA Bioscience[2] or through a modified differential ultracentrifugation protocol.

Using lysosomes overexpressing TMEM175, TRPML1, and TPC2 channels, we characterized their activity using reference compounds, demonstrating the feasibility of pharmacological profiling in a high-throughput patch-clamp format. Interestingly, we found that current responses to pharmacological activation of TMEM175 by TUG-891 and DCPIB are significantly higher at elevated temperatures. In contrast, activation of TRPML1 by MLSA1 and TPC2 by PIP2 shows similar current levels at 21°C and 35°C. These findings suggest that experimental temperature may represent a crucial factor for lysosomal ion channels with respect to compound affinity and/or gating mechanisms, consistent with observations for plasma membrane ion channels.

This approach enables fast, reproducible, and scalable measurements of lysosomal ion channel activity, facilitating more comprehensive studies of lysosomal function in both physiological and pathological contexts, and supporting advances in drug discovery.

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SHORT TALKS

ST1

Heterogeneous AMPA Receptor Populations Regulate Calcium Signaling in Hippocampal Astrocytes

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Glutamate is the primary excitatory neurotransmitter in the central nervous system and exerts its effects through ionotropic receptors such as AMPA receptors (AMPARs). While their neuronal roles are well established, their function in astrocytes—particularly within the hippocampus—remains insufficiently characterized. In this study, we examined the expression, functional properties, and contribution of astrocytic AMPARs to intracellular calcium signaling and glial communication.

Biochemical and immunodetection approaches confirmed the presence of GluA1 and GluA2 subunits, along with lower levels of GluA4, in primary hippocampal astrocytes in culture. Functional analyses, including electrophysiology and calcium imaging, revealed that AMPAR presence and their activation induces intracellular Ca^{2+} elevations in a subset of astrocytes, displaying heterogeneous response profiles.

Our findings indicate that part of these Ca^{2+} responses arise from direct calcium influx through calcium-permeable AMPARs (CP-AMPARs), while another component depends on sodium entry, suggesting a cooperative interaction between Na^{+} and Ca^{2+} dynamics. Indeed, pharmacological inhibition supports the coexistence of CP- and CI-AMPAR populations, which differ in their Ca^{2+}permeability properties.

Finally, sustained AMPAR activation was found to promote ATP release, contributing to intercellular signaling and the propagation of calcium responses through gliotransmission mechanisms. Altogether, these results demonstrate that hippocampal astrocytes express functional AMPARs that actively participate in calcium signaling and neuroglial communication.

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ST2

Functional and Transcriptomic Analysis of Intracelular Calcium Remodeling in Pancreatic Cancer

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Pancreatic cancer is one of the most aggressive and lethal forms of cancer, characterized by a devastating prognosis, largely due to its resistance to conventional therapies. Intracellular calcium signaling plays a critical role in controlling key cellular processes that become dysregulated in cancer. In pancreatic ductal adenocarcinoma (PDAC) specifically, altered Ca^{2+} homeostasis is increasingly recognized as a driver of tumor progression, although its molecular basis remains incompletely understood. This study aimed to perform a comprehensive analysis of intracellular Ca^{2+} dynamics and their underlying transcriptomic regulators in pancreatic cancer cells. Using single-live-cell Ca^{2+} imaging and RNA sequencing (RNA-Seq) in human PDAC cells (CaPan) and non-malignant pancreatic ductal epithelial cells (hTERT-HPNE), we detected alterations in Ca^{2+} handling, including increased store-operated Ca^{2+} entry (SOCE) and changes in Ca^{2+} mobilization. Transcriptomic analysis identified differentially expressed genes (DEGs) related to the Ca^{2+} toolkit. Notably, the plasma membrane Ca^{2+} ATPase 4 pump (PMCA4) was strongly downregulated, suggesting impaired Ca^{2+} extrusion and a potential link to poor prognosis, consistent with previous findings in colon cancer. In contrast, transient receptor operated channels TRPV6 and TRPM7 were significantly upregulated, consistent with enhanced Ca^{2+} influx and a more aggressive, invasive phenotype, supported by alterations in epithelial-mesenchymal transition (EMT) -related pathways. Key changes in Ca^{2+} entry mechanisms included a shift towards increased molecular players involved in SOCE including ORAI1 and STIM2, consistently with enhanced SOCE activity. Additionally, altered mitochondrial Ca^{2+} handling was suggested by decreased expression of the mitochondrial Ca^{2+} uniporter (MCU) and increased modulator MCUb, pointing to reduced mitochondrial Ca^{2+} uptake, perhaps related to cell death resistance. Ongoing work aims to validate these findings using publicly available gene expression datasets and further explore their clinical relevance. Overall, these results highlight a functional rewiring of Ca^{2+} signaling that may contribute to tumor progression and help identify potential prognostic and therapeutic targets.

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ST3

ORAI1-Dependent Regulation of Macrophage Adhesion and Polarization

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Macrophages play essential roles in physiological and pathological processes, including tissue homeostasis, inflammation, and cardiac remodeling after injury. Calcium signalling is a key regulator of macrophage activation, with store-operated calcium entry (SOCE) representing a central pathway in immune responses. Among its components, ORAI1-mediated Ca^{2+} entry has been implicated in multiple cellular functions, although its role in macrophage adhesion and polarization remains unclear.

In this study, THP-1-derived macrophages were stimulated with PMA. Cell adhesion was evaluated using a calcein-based fluorescence assay, quantifying both adherent cell number and spreading area. Cell viability was assessed by flow cytometry. For polarization studies, THP-1 cells were stimulated with LPS and IFN-γ to induce an M1 phenotype, IL-4 for M2a, and IL-10 for M2c polarization. Gene expression analysis was performed by quantitative PCR following RNA extraction using the NucleoSpin kit and SYBR Green chemistry. Correlation analyses were conducted to examine the relationship between ORAI1, STIM1, and polarization-associated genes. ORAI1 function was selectively inhibited using CM4620.

Results show that CM4620 inhibits macrophage adhesion in a dose-dependent manner (IC50 = 14.42 +/- 4.01 μM) without affecting cell viability. Inhibition of ORAI1 also induces notable morphological changes, suggesting the involvement of Ca^{2+}-dependent mechanisms in cytoskeletal regulation. Furthermore, macrophage polarization was associated with increased mRNA expression of ORAI1 and STIM1, which correlated with markers such as TNFα, SOCS3, PPAR-γ, MRC1, SOCS1, CCL22, CCL18, and IL10.

Overall, these findings indicate that ORAI1-mediated Ca^{2+} entry regulates macrophage adhesion and may contribute to macrophage polarization, highlighting ORAI1 as a potential target for modulating immune responses in pathological conditions.

Acknowledgements

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ST4

Orai1 Flips the SOX2-Nanog Switch to Restrain Cancer Stemness in Luminal A Breast Cancer

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Store-operated Ca^{2+} entry mediated by Orai channels is increasingly recognized as a central driver of cancer stem cell (CSC) phenotypes, yet how individual Orai1, Orai2 and Orai3 isoforms shape luminal A breast cancer biology is still poorly defined [1]. In this work, we integrate CRISPR engineered MCF7 models, targeted silencing of each Orai isoform and CSC immunophenotyping with in silico exploration of the TCGA BRCA luminal A cohort to unravel how Orai dependent Ca^{2+} signaling rewires the SOX2-Nanog pluripotency axis and translates into differences in patient survival [2]. *In vitro*, Orai1 deficiency (knockout or knockdown) flipped the SOX2-Nanog switch, causing a profound loss of SOX2 and a marked upregulation of Nanog, together with a robust enrichment of the CD44+/CD24-CSC like subpopulation, indicating that Orai1 normally restrains the expansion of undifferentiated cells. In contrast, Orai3 knockdown enhanced SOX2 and reduced Nanog without significantly modifying the CSC compartment, whereas Orai2 silencing had no detectable effect on pluripotency markers or stemness. In silico, ORAI1 expression showed a positive correlation with SOX2, and low ORAI1 levels identified luminal A patients with poorer overall survival, particularly during early follow up. A combined low ORAI1/SOX2 signature and an ORAI1/SOX2 risk score further refined prognostic stratification, defining a high risk subgroup with nearly two fold increased mortality and time dependent ROC analyses indicating greater discriminative performance at early time points. Altogether, our data reveal an isoform specific control of the SOX2–Nanog axis by Orai1 mediated Ca^{2+} signaling in luminal A breast cancer and support a protective role of Orai1 in limiting CSC expansion, cautioning against indiscriminate Orai1 inhibition in this molecular subtype.

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ST5

Developmental and Epileptic Encephalopathy Associated with *KCNB1*: Toward Personalized Therapies

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Variants in the *KCNB1* gene, which encodes the voltage-gated potassium channel Kv2.1, are a known cause of developmental and epileptic encephalopathy (DEE) [1]. While Kv2.1 is pivotal in regulating neuronal excitability through membrane repolarization, the precise mechanisms by which specific *KCNB1* mutations disrupt channel function remain elusive [2] [3]. This work aims to characterize the functional consequences of disease-associated *KCNB1* variants and explore potential therapeutic strategies to restore channel activity.

To achieve this, wild-type and mutant Kv2.1 channels are expressed in heterologous cell systems to evaluate their expression, subcellular localization, and biophysical properties. Whole-cell patch-clamp recordings are employed to assess the impact of specific variants on current density, voltage-dependence, and gating kinetics. In parallel, biochemical and imaging approaches are used to investigate potential defects in protein expression, trafficking and stability. Furthermore, we explore the negative dominance of mutants, alongside their heteromerization with KvS subunits and the overall impact over Kv2.1 activity.

To identify potential treatments, computational structural modeling and virtual screening are utilized to identify candidate compounds capable of modulating Kv2.1 function and rescuing mutant channel phenotypes. Selected compounds will be subsequently validated through electrophysiological assays. Finally, to provide a disease-relevant cellular model, patient-derived fibroblasts are directly converted into neurons to study channel dysfunction and evaluate potential rescue strategies.

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ST6

MPI Inhibition as a Strategy to Counteract Pathogenic $Ca_v2.1$ Gain-of-Function in CACNA1A Disorders

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CACNA1A encodes the α1A pore-forming subunit of the $Ca_v2.1$ channel. Pathogenic gain-of-function variants in this gene are linked to neurological disorders including Familial Hemiplegic Migraine type 1 (FHM1) and Congenital Ataxia (CA), as they enhance Ca^{2+} entry and increase neuronal excitability. These alterations are consistent with mechanisms that favour cortical spreading depression in FHM1 and Purkinje cell hyperexcitability in ataxia (1). A similar pro-excitatory effect is also produced by $Ca_v2.1$ hypoglycosylation, as proposed for Phosphomannomutase 2 deficiency (PMM2-CDG) (2). Because Phosphomannose Isomerase (MPI) competes with PMM2 for mannose-6-phosphate (3), we proposed that MPI inhibition, combined with mannose supplementation, might improve N-glycosylation and thereby reduce abnormal $Ca_v2.1$ gain-of-function.

To test this idea, HEK293 cells expressing either wild-type or mutant (Y1245C and Δ1502) $Ca_v2.1$ were treated with the MPI inhibitor MLS0315771 in the presence of mannose. We examined N-glycosylation by Western blot and assessed channel function using whole-cell patch-clamp electrophysiology. The treatment increased N-glycosylation of the α2δ subunit ($p<0.01$, n=4). Δ1502 produced a marked gain-of-function phenotype, characterized by an approximately 26 mV negative shift in activation voltage ($p<0.0001$, n=4-16) and slower inactivation ($p<0.05$, n=5-13). MPI inhibition plus mannose partially corrected both abnormalities, shifting activation by 6.5 mV toward control values ($p<0.05$, n=7) and restoring inactivation kinetics ($p<0.01$, n=5-7). The Y1245C variant lowered activation voltage, by approximately 6 mV ($p<0.05$, n=7-13), but this effect was not modified by treatment.

PMM2 knockdown (modelling defective glycosylation) reduced α2δ N-glycosylation and membrane trafficking ($p<0.05$, n=3) and also slowed channel inactivation ($p<0.05$, n=16-18), reproducing a gain-of-function-like phenotype. These changes were reversed by the combination of MPI inhibition and mannose ($p<0.05$, n=9-16).

Our results indicate that promoting N-glycosylation through MPI blockade can alleviate pathogenic $Ca_v2.1$ gain-of-function in selected *CACNA1A* variants and in PMM2 deficiency, supporting this pathway as a potential targeted therapeutic strategy for $Ca_v2.1$-related channelopathies.

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ST7

Functional Effects of *KCNA5* p.G183R Mutation May Be Related to the Development of Ventricular Fibrillation in Acute Myocardial Infarction

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Aims: Acute myocardial infarction (AMI) is a leading cause of death in developed countries. One of the most dangerous complications that can occur during an AMI is ventricular fibrillation (VF), which drastically increases morbi-mortality. Evidence on rare genetic variants predisposing to VF during AMI remains limited and is confined to a small number of genes. This suggests that additional relevant variants may remain unidentified. Therefore, we analyzed a broad set of genes related to AMI and ventricular arrhythmia (VA) in a patient with VF during AMI.
Methods and results: Using Next Generation Sequencing (NGS) technologies, we sequenced and analyzed a total of 233 genes associated with MI and VA in a 68-year-old male Spanish patient. Genetic screening revealed the novel variant p.G183R in *KCNA5*, encoding the voltage-gated potassium channel Kv1.5. Bioinformatic prediction tools suggested a possibly damaging effect, hence, we assessed the Kv1.5 mutant function and trafficking employing electrophysiology (whole-cell patch clamp and two-electrode voltage-clamp) and immunofluorescence techniques, respectively. Functional assessment showed a complete loss of function for the homomeric mutated channel, and a 30% reduction of current in the heteromeric channel (wild-type (WT) + p.G183R) when compared to the WT channel. Immunofluorescence experiments showed no difference in protein expression or trafficking between p.G183R and WT.
Conclusions: The variant p.G183R in *KCNA5* identified in this study significantly reduced the ultrarapid potassium current and this effect could be related to the development of VF in AMI.

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ST8

AE3 p.G1070d Reveals a Chansporter Interaction in Short QT Syndrome

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Ion channels can assemble with solute transporters into macromolecular complexes, termed "chansporters", that coordinately regulate membrane excitability. We hypothesized that these complexes underlie Short QT Syndrome (SQTS) associated with variants in *SLC4A3*, which encodes the cardiac Cl^-/HCO_3^- exchanger AE3.
We studied a novel *SLC4A3* variant, c.3290G>A (p.G1070D), identified in a 35-year-old male with presyncope and a shortened QTc (340–360 ms). Using heterologous expression in HEK293 cells, co-immunoprecipitation demonstrated that AE3 associates with both hERG and Kv7.1-MinK. Patch-clamp recordings performed under bicarbonate-free conditions, in which AE3 transport is inactive, showed that AE3-p.G1070D shifted activation of both channels to more negative potentials, consistent with gain-of-function of repolarizing K^+ currents. In parallel, NH_4Cl-based intracellular pH assays showed that AE3-p.G1070D impairs AE3-mediated acid loading, indicating exchanger loss-of-function. Lysate-mixing controls supported that AE3–hERG complex formation requires co-expression. Furthermore, co-immunoprecipitation in HL-1 cells supported the presence of the AE3–hERG complex in a native cardiac model.
These findings identify AE3 as a chansporter partner of major cardiac potassium channels and suggest that p.G1070D combines loss of exchanger function with a gain-of-function effect on both hERG and Kv7.1 gating. This dual mechanism provides a plausible basis for accelerated repolarization in SLC4A3-associated SQTS and is consistent with recent proteomic (1) and functional studies (2) placing AE3 at the intersection of channel networks that govern cardiac repolarization.

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ST9

New Insights in the Modulation of the Electrophysiology of Kv1.5/Kvβ2.1 Channels by Lgi4

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The leucine-rich glioma-inactivated protein family is a four-member family (Lgi1-4) best known for their role as ligands for ADAM23/22/11 proteins in neurons, where they form part of larger multiprotein complexes that include Kv channels [1]. In this context, Lgis modulate Kv1-Kv4 channels, modifying their trafficking and/or biophysical properties [2]; however, little is known about their role in the heart. We demonstrated that Lgi3-4 are the only Lgis present in human myocardium, so we focused on studying their effects on cardiac potassium currents. In atrial cardiomyocytes, Kv1.5 channels, form complexes with regulatory subunits (such as Kvβs) to generate the repolarizing current I_{Kur}. Previous patch-clamp experiments conducted by our laboratory on transfected cells showed that Lgi3-4 modulate Kv1.5 channels only when coexpressed with Kvβs, impairing their interaction and causing, in the case of Kv1.5/Kvβ2.1: a decrease in the current amplitude, a positive shift in the activation curve, and a reduction in the degree of inactivation [3]. Given the well-documented role of Lgis as secreted molecules through their interaction with ADAM proteins, we cannot rule out the possibility that at least some of these effects on the currents are attributable to an extracellular function of Lgis. After verifying that Lgi4 is secreted in our heterologous system, we conducted conditioned-medium experiments by incubating cells expressing Kv1.5/Kvβ2.1 (with or without ADAM23) with medium derived from cells expressing Lgi4, and then analyzed their currents using the patch-clamp technique. In this communication, we show that the presence of Lgi4 in the conditioned-medium replicates the reduction in the degree of inactivation previously observed in cells expressing Kv1.5/Kvβ2.1, this effect enhanced
when ADAM23 is also expressed, while the current amplitude and the activation curve remain unchanged. These results suggest that Lgi proteins may exert their effects on the Kv1.5/Kvβ channelosome through a combination of extracellular and intracellular mechanisms.

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ST10

Evolutionary Tuning of TRPM8 Cold Sensitivity: Identification of Functionally Relevant Residues across Vertebrate Classes

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The Transient Receptor Potential Melastatin 8 (TRPM8) ion channel is the primary molecular sensor of cool and cold environmental temperatures. Despite sharing a conserved primary structure, TRPM8 orthologues display substantial variation in cold sensitivity across vertebrate species, suggesting that specific amino acid substitutions contribute to the ecological tuning of channel sensitivity to temperature. Identifying these residues could provide mechanistic insight into the molecular basis of cold thermal adaptation and would open new avenues for the modulation of cold-evoked nociception.
Here, we present a comparative evolutionary analysis of TRPM8 sequence across birds, mammals, and reptiles aimed at identifying candidate residues that modulate cold sensitivity. For each vertebrate class, we selected species with contrasting thermal ecologies and applied a comparative pipeline to evaluate amino acid variations. Candidate residues were systematically prioritized based on their conservation patterns across thermally distinct lineages, their structural location within the channel, and their predicted functional impact.
Following this analysis, the histidine residue at position 364 in cold-adapted bird species (such as Aptenodytes forsteri) emerged as a key candidate. As a proof of concept, we replaced the penguin residue with the chicken arginine variant. This substitution increased the proportion of transfected HEK293 cells responding to cold stimuli, suggesting a role for this aminoacid in the thermal sensitivity of the channel. This position belongs to a broader set of phylogenetically prioritized candidates currently under functional characterization across vertebrate classes. This integrative strategy — linking ecological adaptation, molecular evolution, and channel biophysics— contributes to decoding the structural basis of thermal sensitivity in TRPM8 and its evolutionary tuning across the vertebrate phylogeny.

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ST11

Sybody-Based Screening for State-and Subunit-Specific Recognition in AMPA Receptors

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AMPA receptors (AMPARs) play a central role in fast excitatory neurotransmission and operate as integral components of large, highly organized macromolecular signaling complexes in the mammalian brain. The functional and biophysical properties of AMPARs are tightly regulated across brain development by interactions with a broad array of auxiliary proteins, as well as by multiple post-transcriptional and post-translational modifications. Recent structural studies in cryo-EM have identified unique ligand-dependent conformational states that are selectively adopted by GluA3- (1) and GluA4-containing AMPA receptors (2), highlighting subunit-specific differences in receptor architecture. Capturing these transient conformations is essential for understanding receptor gating anddeveloping subunit-selective modulators.
Based on these findings, our approach focuses on the development of nanobodies capable of discriminating between specific AMPAR subunits and functional stales. In this study, we employed a robust synthetic nanobody (sybody) selection pipeline to target the ligand binding domain (LBD) of the GluA4 subunit. The GluA4 LBD was expressed as a soluble protein and site-specifically biotinylated *via* biotin ligase A (BirA) to ensure oriented immobilization during the selection process. Iterative enrichment cycles combining ribosome and phage display were performed against the Seeger synthetic sybody library (3), resulting in the isolation of high-affinity candidates. Ongoing efforts are directed toward ELISA-based validation of enriched sybodies and sequence analysis to identify binders that selectively recognize defined receptor conformational states, thereby providing a versatile platform for the structural and functional characterization of GluA4-containing signaling complexes.

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ST12

Integrated Computational and Experimental Strategies for TRPV2 Pharmacology and Vascular Therapeutics

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Transient receptor potential vanilloid 2 (TRPV2) channels are widely expressed cation channels implicated in cardiovascular, neuromuscular, and immune physiology, yet they remain orphan drug targets with limited and poorly specific pharmacology. Here, I present an integrated experimental and computational biophysics framework to elucidate TRPV2 modulation and to identify novel pharmacological probes with vascular activity. Using ligand-based chemoinformatics and structure-guided docking, a diverse set of candidate TRPV2 modulators was identified starting from known agonists, including probenecid. Molecular docking and Gaussian accelerated molecular dynamics simulations in an explicit lipid bilayer revealed a broad, continuous ligand-binding region in the TRPV2 transmembrane domain, proximal to the vanilloid and TM4–TM5 linker regions. These simulations highlighted a key role for membrane lipids in stabilizing ligand binding, indicating that lipids form an integral part of the functional drug-binding environment. To experimentally validate these findings, a scalable membrane-based calcium influx assay was developed using purified TRPV2 expressed in *Pichia pastoris*. This system enabled unbiased determination of agonist potency while avoiding confounding intracellular signaling. Screening of selected probenecid derivatives identified 4-(piperidine-1-sulfonyl)-benzoic acid (PSBA) as a novel TRPV2 activator with improved efficacy compared to probenecid. Computational analysis suggested that PSBA binds near the experimentally resolved 2-APB site and engages both protein residues and surrounding lipids.

Physiological relevance was assessed in an ex vivo mouse aorta model, revealing that TRPV2 activation induces vasodilation through a balance of endothelium-dependent relaxation and smooth muscle-mediated negative feedback involving potassium channels. Notably, PSBA exhibited sex-biased vasodilator responses, with stronger effects in female vessels.

Together, these results establish TRPV2 as a lipid-sensitive pharmacological target in the vasculature and demonstrate how combining computational modeling, membrane-based functional assays, and vascular physiology can accelerate ion channel drug discovery. This work highlights TRPV2 as a promising yet underexplored target for precision ion channel therapeutics.

Acknowledgements

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ST13

Loss of TRESK Potassium Channel Alters Hippocampal Neuronal Excitability, Synaptic Plasticity, and Shapes Behavior in a Sex-Specific Manner

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The brain's physiological dynamics depend on ion channel activity, which finely tunes neuronal intrinsic excitability, regulates the excitatory-inhibitory balance, and ultimately shapes behavior. Of particular interest is controlled potassium current leakage *via* K2P channels. TRESK (K2P18.1) acts as an excitability brake in peripheral nociceptive pathways, yet its function in the brain remains largely unexplored despite its widespread expression throughout the central nervous system. Here, we investigated the functional impact of TRESK on neuronal excitability, synaptic plasticity, and behavior. We combined RNAscope in situ hybridization to identify expression patterns, electrophysiological recordings in acute hippocampal slices to assess neuronal excitability, and a battery of behavioral tests to evaluate the consequences of TRESK deletion on mouse behavior. We observed *TRESK* mRNA in excitatory and inhibitory neurons throughout the hippocampus, with enriched expression in CA3 region and dentate gyrus. Field-potential and patch-clamp experiments in TRESK knockout mice revealed that the absence of TRESK increases neuronal excitability in CA3 pyramidal neurons and alters short-term and long-term hippocampal synaptic plasticity. Behavioral analyses uncovered sex-dependent alterations in exploration and emotional regulation, with female TRESK knockout mice exhibiting an increased anxiety-like behavior in the elevated zero maze and open field tests. In addition, mice lacking TRESK displayed impaired sociability in the three-chamber social test and enhanced spatial working memory performance in the Y-maze test. These findings support the role of TRESK as a regulator of hippocampal neuronal excitability and lay the groundwork for understanding its contribution to sex-dependent neural dynamics and behavioral changes.

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ST14

P2X Purinergic Receptors Are Required for Correct Cortical Development in Human Brain Organoids

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The human neocortex represents a crucial evolutionary advance, the formation of which requires the tight and precise orchestration of both intracellular and extracellular signals. Structures grown in three-dimensional cultures, specifically human-induced pluripotent stem cells (hIPSCs)-derived cerebral organoids (COs), have been fundamental to study the signals that regulate the formation of the cortex, overcoming the limitations of 2D cultures. Amongst these, purinergic signaling driven by extracellular ATP and other nucleotides may encode crucial intercellular communications that govern central nervous system (CNS) development. The ATP that accumulates in the extracellular milieu can interact with both ionotropic P2X and metabotropic P2Y receptors on cells to exert its modulating effects. Although widely studied in different animal models, little is known about the expression and function of this signaling system in the human cortex. Thus, here we analyzed the expression of P2X receptor subunits comprehensively throughout the entire process of CO development, confirming that P2X receptors are functional in ventricular structures of the human cortex. Specifically, we detected the expression of P2X1, P2X4, and P2X6 in CO, showing distinct distributions in Nestin$^+$ radial glial cells and/or DCX$^+$ newborn neurons. Significantly, we also show how prolonged pharmacological inhibition of P2X activity affects CO development, resulting in smaller organoids with fewer and less well-organized cortical ventricles. Altogether, our findings point to a relevant role of purinergic signaling during the formation of the human cerebral cortex.

Acknowledgements

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ST15

Mechanisms Involved in SARS-CoV 2 E Protein-Induced Calcium Release from Intracellular Stores in Rat Hippocampal Neurons Aged *In Vitro*

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Infection with SARS-CoV-2, the etiological agent of COVID-19, is associated not only with respiratory and vascular pathology but also with significant neurological alterations. The viral envelope (E) protein is a small structural component with ion channel–forming properties that plays an essential role during the viral life cycle. Nevertheless, its direct impact on neuronal cells has remained largely unexplored. We previously described that E protein rapidly penetrates hippocampal neurons and predominantly localizes to endoplasmic reticulum (ER) membranes in both short-term (6-8 DIV) and long-term (20-22 DIV) cultures of rat hippocampal neurons, considered as models of young and aged neurons, respectively. Notably, exposure to the E protein triggered apoptotic cell death selectively in aged neurons, whereas younger neurons remained largely resistant. Notably, Ca^{2+} release from intracellular stores triggered by the E protein was minimal in young neuronal cultures but substantially enhanced in aged neurons. In this study we have investigated mechanisms involved in Ca^{2+} release induced by E protein in young and aged hippocampal neurons, Ca^{2+} signaling alterations and mitochondrial dysfunction in aged rat hippocampal neurons, as well as potential neuroprotective mechanisms. Calcium imaging experiments performed in Ca^{2+}-free conditions to study the possible implication of inositol 1,4,5-trisphosphate receptors (IP_3Rs), using the pharmacological inhibitor 2-APB. Interestingly, this compound failed to reduce, and instead enhanced, E protein–evoked Ca^{2+} release. Given the close functional coupling between the ER and mitochondria, we next examined the role of mitochondria in buffering E protein–induced Ca^{2+} release. Mitochondrial depolarization using the uncoupler FCCP significantly increased cytosolic Ca^{2+} elevations elicited by the E protein, indicating that mitochondria actively take up part of the Ca^{2+} released from intracellular stores. Finally, we found that a novel antiviral inhibits both Ca^{2+} release and neuron cell death evoked by E protein suggesting a critical role of Ca^{2+} mobilization in Covid19 neurotoxicity.

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ST16

Persistent Nociceptor Hyperexcitability and Axonal Retraction Induced by Repeated Paclitaxel Exposure

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Paclitaxel-induced peripheral neuropathy is a common and important side effect that often forces doctors to reduce the dose or stop chemotherapy, which can impact patients' overall outcomes [1]. The symptoms may last for months, suggesting lasting changes in peripheral nociceptors. Yet, the specific cellular mechanisms behind these ongoing effects remain unclear [2]. In this study, we developed a primary culture of adult mouse nociceptors to explore how repeated exposure to paclitaxel affects them.

We treated the cells twice for 24 hours each, with a 96-hour recovery period in between, mimicking clinical chemotherapy cycles. Repeated administration of paclitaxel resulted in sustained elevations of both spontaneous and stimulus-evoked neuronal activity, accompanied by significant axonal retraction. After the initial treatment, neurons demonstrated a transient axonopathy with increased excitability that peaked at 48 hours and normalized within 96 hours. In contrast, a subsequent exposure led to marked and persistent axonal degeneration, as well as prolonged hyperexcitability, especially in $IB4^-$ sensory neurons. Neuronal excitability increased, accompanied by greater depolarizing membrane fluctuations and elevated somatic input resistance. These functional changes are probably connected to higher levels of $Na_v1.8$ and TRPV1 channels following paclitaxel treatment, which matches earlier findings on ion channel disruption in chemotherapy-related neuropathy [3]. Furthermore, repeated exposure led to additional rises in the expression of TRPM8, TRPA1, and $K_v3.4$ channels. Collectively, these results indicate that $Na_v1.8$ and TRPV1 may serve as promising targets for treating paclitaxel-induced neuropathic pain. They also endorse the use of long-term nociceptor cultures as an effective preclinical model for researching cumulative neurotoxicity.

Acknowledgements

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ST17

Rescuing Kv1.5 Channels In Pulmonary Arterial Hypertension By Modulating Sigma 1 Receptors With Repurposed Drugs

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Background and Purpose: Repurposing clinically used drugs may offer an attractive strategy to accelerate the development of novel therapies in pulmonary arterial hypertension (PAH). In this context, Kv1.5 channels, whose dysfunction is a hallmark in PAH, are modulated by the chaperone sigma-1 receptor (S1R). S1R activation increases Kv1.5 currents in pulmonary artery smooth muscle cells (PASMC) and limits pulmonary vasoconstriction. Notably, the marketed drugs dimemorfan and fluoxetine display S1R agonist actions; hence, the present study aimed to assess their potential efficacy to restore impaired Kv1.5 channel function in PAH.
Experimental Approach: The effects of dimemorfan and fluoxetine were assessed *in vitro*. Kv1.5 currents were recorded using the patch-clamp technique in freshly isolated PASMC from rats, humans, and from the S5416 plus hypoxia (SuHx) PAH rat model. Vascular responses and protein expression were evaluated in rat pulmonary arteries by wire myography and Western blot, respectively.
Key Results: Kv1.5 currents were increased in PASMC through S1R activation by dimemorfan and fluoxetine. The improvement of Kv1.5 channel activity by these drugs resulted in attenuated pulmonary vasoconstriction. Crucially, this Kv channel enhancing effect was also present in human samples and preserved in PA from experimental PAH.
Conclusion and Implications: Our findings highlight that activation of S1R through the use of repurposed drugs may be a promising strategy for restoring altered Kv1.5 channel activity in the context of PAH and other pulmonary vascular diseases, supporting its potential translational relevance.

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ST18

TRPA1 and TRPM8 Mediate Intrinsic Cold Sensitivity of Cutaneous Arteries

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The response of cutaneous vasculature to thermal stimuli is a homeostatic function critical for mammalian survival. Local cold exposure typically triggers a rapid, protective vasoconstriction to minimize heat loss, followed by restorative vasodilation to perfuse the tissue and prevent ischemia. This adaptative physiological mechanism was first described by Thomas Lewis (1). The cold-sensitive Transient receptorpotential (TRP) channels, TRPA1 and TRPM8, have been identified as key molecular sensors in this complex vascular response, acting through both sensory and sympathetic nerve fibers (2).
In this work, we sought to determine whether isolated peripheral cutaneous arteries possess intrinsic cold sensitivity and to characterize the functional contribution of TRPA1 and TRPM8 channels to this local response.
We performed pressure myography experiments in isolated plantar arteries from C57BL/6J, Trpa1 and Trpm8 KO mice to determine changes in diameter upon exposure to low temperature and explore the expression of these channels by qPCR and immunofluorescence in vascular and nervous tissues. Our findings reveal that cold (15°C) induces a significant intrinsic contraction in isolated plantar arteries (28 ± 3.5%), whereas mesenteric arteries showed no reaction. This vasoconstriction was significantly attenuated in plantar arteries from Trpa1 KO (11.04 ± 2.5%) and Trpm8 KO mice (10.9 ± 1.9%) and did not change in plantar arteries form Tpv4 KO and Trpm3 KO. The simultaneous blockade of both channels virtually abolished the response. In the presence of CGRP receptor inhibitor BIBN (blockade of sensory fibers), the cold-induced vasoconstriction response was potentiated while blockade of sympathetic activation led to a significant vasodilation on the cold-induced response. TRPA1 and TRPM8 channels mRNA were detected in sympathetic ganglia, and confocal images of plantar arteries confirmed Trpm8 presence in perivascular sympathetic fibers. We conclude that TRPM8 and TRPA1 channels could represent new targets for temperature regulation through local control of cold-induced vascular responses.

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ST19

Tetrodotoxin (TTX) Attenuates Post-Operative Pain in Mice: Involvement of the Endogenous Opioid System

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Introduction: Postoperative pain remains inadequately managed. Opioids are still the mainstay for treating acute postoperative pain, but they produce several significant side effects, such as constipation [1]. Tetrodotoxin (TTX) is a potent neurotoxin that selectively blocks a subset of voltage-gated sodium channels (VGSCs) and is currently under clinical development for cancer-related pain [2]. One of the TTX-sensitive VGSCs is Nav1.7, and its blockade leads to an upregulation of endogenous opioids in sensory neurons [3]. However, to our knowledge, the involvement of the endogenous opioid system in the analgesic effects of TTX has not yet been investigated.
Aim: To evaluate the potential therapeutic effects of TTX in two models of postoperative pain and to assess the contribution of opioidergic mechanisms to these effects.
Methods: Two postoperative pain models were performed in female CD-1 mice. In the plantar incision model, a superficial incision was made in the plantar surface of the paw, and changes in hind paw weight-bearing distribution were assessed using dynamic weight bearing. In the laparotomy model, a transverse laparotomy was performed, and mechanical sensitivity in the abdominal area (von Frey test), movement-induced pain (actimetry), and pain-related facial expressions (Face Analyzer) were assessed.
Results: Systemic administration of TTX (3–9 μg/kg) and morphine (0.25–1 mg/kg) dose-dependently reduced alterations in hind paw weight bearing following plantar incision. In the laparotomy model, TTX (1–3 μg/kg) and morphine (0.25–1 mg/kg) reduced tactile allodynia, movement-induced pain, and the percentage of pain-related facial expressions, with TTX showing greater efficacy than morphine. The combination of low doses of TTX and morphine produced only additive effects in reducing allodynia and movement-induced pain. However, naloxone administration prevented the effects of TTX on tactile allodynia and movement-induced pain induced by laparotomy.
Conclusions: TTX attenuates postoperative pain in mice, likely through the involvement of the opioidergic system.

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ST20

DECA-11 Selectively Restores Nav1.5 and Kir2.1 Channel Expression in the Membrane

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Heart failure (HF) electrical remodeling downregulates Nav1.5 and Kir2.1 channels (encoded by *SCN5A* and *KCNJ2* genes, respectively) at the cardiomyocyte membrane, reducing the Na^+ (I_{Na}) and inward rectifier K^+ (I_{K1}) current densities, thereby impairing ventricular excitability and delaying repolarization. These derangements greatly increased the risk of ventricular arrhythmias (VA) and mortality in HF patients.
We designed a gene therapy encoding an 11-mer peptide named DECA-11 (WO2025068623), capable of increasing I_{Na} and I_{K1} in heterologous expression systems, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), and mouse ventricular cardiomyocytes that reduces VA risk in a mouse model of HF.
Here we demonstrate that DECA-11 did not affect the density of the current generated by other cardiac channels involved in the genesis of the cardiac action potentials (Cav1.2, Kv4.3, hERG, and Kv7.1+minK) or neuronal Na^+ channels (Nav1.1, Nav1.2, Nav1.7 and Nav1.8). Furthermore, it did not augment the late I_{Na} nor modify the amplitude and duration of intracellular Ca^{2+} transients or the contraction and relaxation speed in spontaneously beating hiPSC-CMs at three frequencies (0.5, 1, and 1.8 Hz). Furthermore, DECA-11 did not modify the biophysical properties of Nav1.5 and Kir2.1 channels, as it had no effect on their conductance (γ), open probability (Po) and kinetics characterized in single-channel experiments (cell-attached configuration). However, DECA-11 significantly upregulated the promoter activity of the human *KCNJ2* gene ($P=0.006$) and increased its mRNA expression levels ($P=0.0003$), while it did not modify the *SCN5A* gene transcription or mRNA expression levels. Importantly, DECA-11 increased sarcolemmal and total expression of Nav1.5 and Kir2.1 channels ($P\leq0.0001$) in ventricular samples of mice subjected to transverse aortic constriction. The results provide compelling evidence that DECA-11 selectively and effectively increases I_{Na} and I_{K1} by augmenting the sarcolemmal Nav1.5 and Kir2.1 channel expression supporting its potential and leading the bases for a promising targeted antiarrhythmic therapy.

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POSTERS

P1

Tresk Potassium Channels Differentially Regulates Cerebellar Motor Learning and Coordination in Male and Female Mice

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Ion channel function is critical for establishing neuronal excitability and regulating synaptic transmission throughout the nervous system, thereby influencing motor control and behavioral output. K2P potassium channels, modulate the intrinsic excitability of neurons by controlling background potassium conductance. TRESK (K2P18.1), a two-pore domain potassium channel, is expressed across multiple brain regions including the cerebellum, yet its specific role in cerebellar function and motor behavior remains unclear. This study investigates how TRESK influences cerebellar neuronal properties and motor performance using a TRESK knockout (KO) mouse model. RNAscope in situ hybridization shows significant TRESK expression in both excitatory and inhibitory cerebellar neurons. Analysis of available databases of single-nucleus transcriptomic data revealed that TRESK is preferentially enriched in cerebellar inhibitory interneurons, particularly Golgi cells and Purkinje layer interneurons, suggesting a specialized role in modulating cerebellar inhibitory tone. Electrophysiological analysis is currently underway to characterize how TRESK deletion affects cerebellar neuron firing properties and synaptic function. Behavioral phenotyping of TRESK knockout mice revealed sex-dependent motor deficits: in the accelerated rotarod test, male KO mice demonstrated altered motor learning trajectories with greater trial-to-trial variability compared to wild-type or female KO mice. Female KO mice displayed significantly poorer balance and coordination in the ledge test and a higher kyphosis score.
These findings demonstrate that TRESK modulates cerebellar motor circuits in a sex-dependent manner and suggest that altered TRESK function may contribute to motor coordination deficits. This work provides insights into the role of leak potassium channels in cerebellar physiology and motor behavior.

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P2

Ubiquitin-Dependent Endocytosis of the Kv1.3 Potassium Channel

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The voltage-gated potassium channel Kv1.3 is a pivotal regulator of both action potential propagation in sensory neurons and calcium-dependent signalling cascades essential for immune cell activation. This study elucidates a convergent regulatory mechanism wherein two distinct endocytic stimuli, (i) epidermal growth factor receptor (EGFR) activation, which recruits tyrosine kinase activity critical for neuronal differentiation, and (ii) protein kinase C (PKC), a serine/threonine kinase central to the attenuation of inflammatory signalling, exploit identical molecular determinants on Kv1.3 to modulate channel surface density and function. We demonstrate that both pathways trigger ubiquitin-dependent endocytosis of Kv1.3, directing the channel to lysosomal degradation compartments. Functional mapping identified two specific lysine clusters situated within the N- and C-terminal domains of the channel. Both motifs undergo ubiquitination following stimulation of either tyrosine- or serine/threonine-directed kinase cascades. Mutational ablation of these lysine residues exhibited an additive effect, significantly attenuating both ubiquitin conjugation and subsequent internalization of the channel. Furthermore, this resulted in pronounced accumulation of Kv1.3 at the immunological synapse and a concomitant increase in channel activity in primary human T lymphocytes. Sustained localization of Kv1.3 at this interface is predicted to potentiate leukocyte effector responses and may drive the transition to chronic inflammatory pathologies. Collectively, these findings position ubiquitination as a precise modulator governing Kv1.3 turnover, thereby fine-tuning cellular excitability and immune homeostasis.

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P3

Functional expression of Kir2 channels in pulmonary vascular cells and their role in pulmonary arterial hypertension

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Strongly inward-rectifier K^+ (Kir2) channels are key determinants of vascular membrane potential (V_M), contributing to endothelium-dependent hyperpolarization and active hyperemia in multiple vascular beds, including the cerebral circulation. However, their expression and functional relevance in the pulmonary vasculature remain poorly defined, with available evidence largely limited to cultured bovine cells. Although mutations in Kir2-encoding genes have not been directly linked to pulmonary arterial hypertension (PAH), previous studies report a modest downregulation of Kir expression in lungs from PAH patients.

Here, we investigated the functional expression of Kir2 channels in pulmonary arteries (PAs) and their potential involvement in PAH pathophysiology. Using patch-clamp electrophysiology, immunofluorescence, and wire myography, we assessed Kir2 channel expression and function in freshly isolated pulmonary artery smooth muscle cells (PASMC) and endothelial cells (PAEC) from rats. Kir2 currents were identified as Ba^{2+}-sensitive inward currents displaying strong inward rectification and were supported by membrane localization of Kir2.1 and Kir2.2 subunits. Under physiological extracellular K^+ conditions, $BaCl_2$ (100 μM) induced marked membrane depolarization in current-clamp recordings. Functionally, $BaCl_2$ elicited concentration-dependent contractions in PA rings and significantly attenuated acetylcholine (ACh)-evoked, endothelium-dependent relaxation, indicating a tonic vasodilatory role of Kir2 channels. In a rat model of PAH (SU5416 plus hypoxia), both Kir2 current density and Kir2.1/Kir2.2 protein expression were significantly reduced in PASMCs and PAECs. Consistently, Ba^{2+}-induced depolarization and vasocontraction were attenuated, and Kir2 blockade impaired ACh (1 nM–100 μM)-induced endothelium-dependent relaxation.

Collectively, these findings provide the first direct evidence of functional Kir2 channel expression in native pulmonary vascular cells, where they play a critical role in regulating V_M and vascular tone. Their downregulation in PAH highlights Kir2 channels as potential therapeutic targets in this severe and currently incurable disease.

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P4

Adaptor-Dependent Recruitment of the Nedd4-2 E3 Ubiquitin Ligase to the Kv1.3

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Kv1.3, a voltage-gated potassium channel constitutes a critical determinant in the immune cell reactivity. Proinflammatory stimuli drive increased Kv1.3 plasma membrane density, thereby augmenting calcium-mediated signaling pathways essential for leukocyte effector function. Homeostatic constraints demand the removal of excess channel protein from the cell surface to attenuate sustained inflammatory states. The E3 ubiquitin ligase Nedd4-2 executes this negative regulation by marking Kv1.3 for degradation *via* ubiquitin conjugation and lysosomal routing. Given the absence of canonical PY interaction motifs within Kv1.3, direct ligase binding is excluded, necessitating the intervention of adaptor proteins. Our investigation reveals that Ndfip1, alongside particular 14-3-3 protein variants, enables the functional association between Nedd4-2 and Kv1.3. In response to PKC stimulation, a rapid membrane-proximal interaction ensues, triggering ubiquitin tagging, internalization into transport vesicles, and terminal lysosomal proteolysis. These findings outline the adaptor landscape required for Nedd4-2-mediated Kv1.3 turnover, establishing Ndfip1 as a principal regulator while the functions of specific 14-3-3 isoforms await further resolution.

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P5

Targeting TRPM8 with Novel 4-Trans-Hypdroxyproline Derivatives

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Transient Receptor Potential Melastatin 8 (TRPM8) is a non-selective cation channel widely expressed in the body, particularly in sensory neurons.[1] It plays a central role in cold sensing and in initiating cellular responses to low temperatures, contributing to thermoregulation and pain perception. TRPM8 has also been implicated in various pathological conditions, including inflammatory and neuropathic pain, as well as several cancers.[2]

During an *in vitro* screening campaign aimed at identifying TRPM8 modulators, a family of 4-*trans*-hydroxyproline (Hyp) derivatives emerged with moderate antagonist activity. The therapeutic relevance of Hyp containing motifs is supported by their presence in several FDA approved drugs.[3] Building on the initial observations, a series of structural modifications was introduced at the N , and C positions, including the incorporation of amide functionalities and amino acid derivatives, for enhancing biological activity and establishing structure–activity relationships (SAR). This study includes the synthesis of Hyp derivatives, their biological characterization, including TRPM8 antagonist activity bythrough Ca^{2+} microfluorometry and patch clamp *in vitro* assays, selectivity profiling across additional TRP channels, cytotoxicity evaluation (MTT), ADME analysis, and *in vivo* efficacy in a mouse model of oxaliplatin induced peripheral neuropathy.

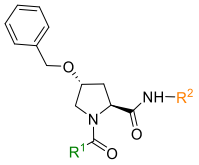

Figure 1. General structures of 4-*trans*-hydroxyprolines

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P6

On The Importance of Metabolic Stability in Bioactive Peptides: DECA-11 Analogues as a Case Study

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Within peptide-based medicinal chemistry programs, robust synthetic access to analogues, suitable functionalization to fine-tune biological activity and physicochemical properties, and the control of conformational preferences are essential. However, metabolic instability remains one of the major limitations of peptide therapeutics, as rapid proteolytic degradation restricts systemic half-life and therapeutic impact. Improving resistance to endogenous peptidases is therefore critical to ensure that bioactive peptides reach their molecular targets at effective concentrations.[1]

DECA-11 is a peptide able to increase I_{Na} and I_{K1} currents downregulated as a consequence of the electrical remodeling process induced by heart failure with reduced ejection fraction (HFrEF), avoiding the development of fatal ventricular arrhythmias. Therefore, this peptide could be of potential interest for reducing morbidity and mortality in HFrEF. Using Ac-DECA-11 analogues as a case study, we investigated how rational structural modifications can overcome metabolic liabilities and substantially improve pharmacokinetic properties. Metabolic stability was assessed through incubation in human serum and liver microsomes.[2] Our findings highlight specific sequence modifications that markedly extend peptide half-life and convert metabolically labile prototypes into viable candidates with prolonged stability.

This communication will highlight the central role of metabolic stability engineering within the DECA-11 peptide program, providing a comparative analysis of metabolic half-life values across the analogue series and mapping the fragmentation spectra associated with the dominant degradation pathways. We will outline the progression from the native DECA-11 sequence to a lead analogue that demonstrates substantially improved metabolic stability, prolonged serum persistence, and a suitable microsomal half-life. Together, these advances represent a significant step toward establishing a peptide-based modulator with properties aligned to the requirements for preclinical development.

Acknowledgements

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P7

State-Dependent CaV2.1 Blockade Reduces Ca^{2+} Overload and Hyperneurotransmission in Both *In Vitro* and *In Vivo* Models of Migraine-Linked *CACNA1A* Gain-of-Function

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Hemiplegic migraine (HM) is a severe form of migraine characterized by transient hemiparesis, mainly associated with gain-of-function mutations in *CACNA1A* (encoding the $Ca_v2.1$ channel)(1). Because currently available $Ca_v2.1$ inhibitors have limited therapeutic utility owing to poor bioavailability and insufficient brain penetration (2), we explored whether state-dependent $Ca_v2.1$ inhibition could provide a more suitable pharmacological strategy for HM.

We screened eight small molecules derived from a previously known Ca_v inhibitor using automated patch-clamp recordings in CHO cells expressing human $Ca_v2.1$, $Ca_v1.2$ or $Ca_v2.2$. Specific CaV2.1 inhibitors were evaluated in HEK293 cells expressing either wild-type $Ca_v2.1$ or the HM-associated Y1245C variant. We also examined their effects in a broader disease-relevant context by measuring Ca^{2+} responses in cultured cortical neurons obtained from wild-type and $Ca_v2.1$-R192Q knock-in mouse embryos after high-potassium stimulation, and by testing aldicarb sensitivity in live *C. elegans* carrying the human-equivalent $Ca_v2.1$-D1640N gain-of-function mutation or the wild-type channel as an *in vivo* readout of synaptic neurotransmission. Animal procedures complied with EU Directive 2010/63/EU. UOS-00021171 and UOS-00021174 showed preferential inhibition of $Ca_v2.1$ over other Ca_v channels and suppressed mutant currents more strongly than wild-type currents. At +5 mV, UOS-00021171 decreased maximal Ca^{2+} current by 31±8% in Y1245C channels compared with 5±7% in wild-type channels (n=6-8). UOS-00021174 reduced current by 49±5% in the mutant and by 24±11% in the wild-type background (n=6-7, $p<0.05$). In cortical neurons from R192Q embryos, UOS-00021174 normalized the abnormally increased Ca^{2+} responses, and in live D1640N C. elegans it reduced aldicarb hypersensitivity ($p<0.05$), consistent with a rescue of excessive cholinergic neurotransmission at the nematode neuromuscular junction. These effects were achieved with minimal impact on wild-type neurons.

These data identify UOS-00021174 as a promising therapeutic candidate for HM and support state-dependent $Ca_v2.1$ inhibition as an approach capable of dampening pathological neuronal hyperactivity while preserving physiological channel function, potentially reducing adverse effects.

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P8

Expression of Kv1.3 in Lung Immune Cells in Models of Pulmonary Arterial Hypertension

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Pulmonary arterial hypertension (PAH) is a severe disease characterized by sustained pulmonary vasoconstriction, progressive vascular remodeling, and eventual vessel occlusion. The pathogenesis of PAH is complex and multifactorial, involving processes such as endothelial dysfunction, hypoxia, inflammation, and alterations in ion channel activity, particularly potassium channels (Kv). Although Kv channels have been widely studied in pulmonary vascular cells, their role in immune cells, especially T lymphocytes, remains poorly understood. Among them, Kv1.3 channels regulate T cell activation, proliferation, and cytokine secretion, suggesting their involvement in the immune and inflammatory components of PAH.

The aim of this study was to characterize Kv1.3 expression in pulmonary immune cells using the Sugen/hypoxia rat model of PAH. Rats received a subcutaneous injection of Sugen and were exposed to hypoxia (10% O_2) for three weeks to induce disease. Subsequently, animals were returned to normoxia for three additional weeks to evaluate progression and recovery. Analyses were conducted at the end of hypoxia (3 weeks) and after recovery (3+3 weeks). Lung tissues were collected and analyzed by flow cytometry. Results showed that after 3 weeks of hypoxia, no significant changes were detected in Kv1.3 expression in αβ T cells. However, in the 3+3 model, a reduction in Kv1.3+ cell frequency was observed in total αβ T cells, including $CD4^+$ and $CD8^+$ subsets, without differences in mean fluorescence intensity (MFI). Similarly, γδ T cells showed no changes at 3 weeks but a decreased percentage of Kv1.3+ cells later. Nevertheless, MFI was increased in γδ T cells in the 3+3 group, suggesting greater Kv1.3 expression.

In conclusion, Kv1.3 expression in lung T lymphocytes is dynamically regulated during PAH progression, with higher expression in γδ T cells, suggesting a role in disease immunopathology and potential functional relevance

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P9

Role of Voltage-Gated Ca^{2+} Channels in PACAP-38-Driven Effects in Adrenomedullary and Primary Sensory Cells under Neuropathic Conditions in Rats

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In the rat chronic constriction injury (CCI) model of sciatic nerve neuropathic pain, we previously demonstrated parallel neuroplastic adaptations in adrenomedullary chromaffin cells (CC) and primary sensory neurons. These alterations involve the functional expression of nAChRs, T-type Cav channels, and P2X receptors (1) underlying enhanced excitability in both cell types and increased catecholamine release from chromaffin cells, which exacerbate the CCI-induced mechanical and thermal allodynia (2). Pituitary adenylate cyclase-activating polypeptide (PACAP) acts as a crucial co-transmitter at the splanchnic nerve–CC synapse during high secretory demand and is significantly overexpressed in sensory neurons following nerve injury, where it modulates sodium channel activity (4). Utilizing patch-clamp electrophysiology and amperometry, we examined the involvement of Cav channels in the PACAP-38-mediated effects on excitability and catecholamine release from cultured CC and isolated adrenal glands, as well as dorsal root ganglion (DRG) neurons, from control and CCI rats.

CC and DRG neurons from CCI rats exhibited increased electrical excitability. In CC, this state was associated with a depolarized resting membrane potential and augmented sodium currents. PACAP-38 further potentiated electrical activity, a process in which we identified an enhanced Ca^{2+} influx through L-type (Cav1) as well as T-type (Cav3) channels in both cell types in the CCI group. Notably, PACAP-38-induced excitability was significantly attenuated by T-type (Ni^{2+}, mibefradil) and L-type (nifedipine) Ca_v blockers. Furthermore, PACAP-38 evoked catecholamine secretion from CC, which was significantly heightened in adrenal glands from CCI animals. This effect was Ni^{2+}, sensitive, underscoring the pivotal role of T-type channels in the response.

Our data suggest that Cav3 channels are key elements mediating PACAP-38-driven cell excitability and catecholamine secretion. These findings identify them as potential pharmacological targets within the neuroendocrine pain circuit, particularly in the context of peripheral sensitization associated to neuropathic pain, where PACAP-38 would act as a key mediator.

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P10

An In Silico Approach to the Different Penetrance of Congenital Long QT Syndromes (LQTS) Types 1 and 2

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Background: Congenital Long QT Syndrome types 1 and 2 (LQT1 and LQT2) are caused by mutations in the *KCNQ1* and *KCNH2* genes, responsible for the I_{Ks} and I_{Kr} currents, respectively. However, the penetrance of these mutations is highly variable, since some carriers remain asymptomatic, while others exhibit severe clinical manifestations. Objective: Elucidate how physiological variability in other cardiac ionic currents may influence the arrhythmic phenotype.
Methods: Human ventricular action potentials were simulated in silico using the O'Hara-Rudy dynamic model implemented in the Virtual Assay software. Conductances of nine key ionic currents were randomly modified within physiologically plausible ranges and the resulting population of models was validated under stress conditions. LQT1 and LQT2 cohorts were generated by reducing I_{Ks} by 80% and I_{Kr} by 60%, respectively. These cohorts were stratified into four risk categories based on action potential duration (APD) under different conditions and the occurrence of arrhythmic events.
Results: LQT1 models demonstrated impaired adaptation to adrenergic stimulation, whereas LQT2 models showed marked APD prolongation at rest. Risk stratification revealed a higher incidence of arrhythmic events in LQT2 (7.6%) compared to LQT1 (0.55%). Regression analyses identified I_{Kr} and I_{K1} as protective currents, while I_{NaL} and I_{CaL} were major contributors to APD prolongation in both types.
Conclusion: Variations in the relative expression of other ionic currents involved in shaping the AP -within the physiological ranges- can modulate the phenotype of congenital arrhythmic syndromes and the associated risk of sudden death. Consequently, these currents represent promising targets for therapeutic intervention.

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P11

Differential Effects of a KCNE4 C-Terminal Domain Polymorphism on Kv1.3 Activity

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Kv1.3 channels play a crucial role in leukocyte physiology, including activation and proliferation processes. Kv1.3 activity and localization are modulated by the regulatory subunit KCNE4, which acts as a negative regulator. Both proteins interact *via* their C-terminal domains. While KCNE4 C-terminal is mainly responsible for impairing plasma membrane trafficking of the channel, the transmembrane domain participates in accelerating Kv1.3 C-type inactivation. Overall, KCNE4 reduces the macroscopic currents of Kv1.3. Here, we present a KCNE4 C-terminal polymorphism 145D/E involving two negatively charged residues. Each variant is associated with a different immune system related disease: childhood acute lymphoblastic leukemia (145D) and allergic rhinits (145E). We investigated the functional effects of each KCNE4 variant on Kv1.3 activity, and we observed how KCNE4 145E had a two-fold higher effect in decreasing Kv1.3 currents compared to KCNE4 145D. While both variants had a similar effect in stopping Kv1.3 plasma membrane trafficking, we observed a difference in active channel abundance in the form of gating currents. A deeper study of the position and side chain composition of this residue revealed that polarity is crucial for the difference in effects. Overall, we present KCNE4 145D/E polymorphism as a key controller of Kv1.3 current density magnitude.

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P12

Kv1.3 Regulates Early Vascular Remodeling and Arterial Stiffness in Uremia-Induced Calcification

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Vascular calcification (VC) is the pathological deposition of hydroxyapatite within the arterial wall and a major contributor to cardiovascular morbidity, including increased risk of heart disease, stroke, and atherosclerotic plaque rupture[1,2]. Although traditionally considered a passive process, VC is now recognized as an active, cell-mediated phenomenon driven by phenotypic modulation (PM) of vascular smooth muscle cells (VSMCs) toward an osteochondrogenic phenotype. In chronic kidney disease (CKD), vascular medial calcification arises early, promoted by uremia and alterations in mineral metabolism.

Early stages are characterized by loss of contractile markers, activation of osteogenic programs, and extracellular matrix (ECM) remodeling, particularly elastin, preceding overt mineral deposition. Ion channels have emerged as key regulators of vascular cell behavior beyond their electrophysiological roles. Among them, the voltage-gated potassium channel Kv1.3 has been implicated in proliferation, migration, and phenotypic modulation, although its role in early uremia-induced remodeling remains unclear.

We have previously shown that uremic serum upregulates Kv1.3 in human VSMCs and that its inhibition partially prevents calcification *in vitro*[3]. Here, we investigated the contribution of Kv1.3 to early phenotypic modulation in an ex vivo model using mouse aortic rings exposed to human uremic serum (US).

US induced a marked upregulation of the osteogenic marker alkaline phosphatase (ALPL) in wild-type (WT) vessels, which was prevented by Kv1.3 blockade (PAP-1) and reduced in Kv1.3$^{-/-}$ aortas. These changes occurred in the absence of detectable mineral deposition, supporting a role for Kv1.3 in early remodeling. Functionally, US increased arterial stiffness in WT vessels, an effect attenuated by Kv1.3 inhibition. Furthermore, elastin analysis revealed increased structural heterogeneity in US-treated vessels. Ongoing atomic force microscopy studies aim to further characterize local mechanical properties associated with ECM remodeling.

Our findings identify Kv1.3 as an upstream regulator of early PM, ECM disorganization, and arterial stiffening in uremia; its inhibition may prevent early VC.

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P13

Effects of a Novel FKBP12 Ligand (AHK) on Action Potentials and Cardiac Channels

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A novel Calstabin2 (FKBP12.6) ligand, also known as AHK (Patent Ref#3466933), was designed to finely tune Ryanodine receptor 2 (RyR2) activity in neurons and cardiomyocytes. AHK (4-arylthioalkyl-1-carboxyalkyl-1,2,3-triazol) targets the FKBP12/RyR2 complex hot-spot interface contact residues and shows efficacy as Ca^{2+} normalizer in myotubes under nitro oxidative stress conditions [1]. Dysregulation of RyR2 is highly significant across various pathological states, such as atrial fibrillation, heart failure, and Duchenne muscular dystrophy (DMD), and, thus, RyR2 is thus a promising therapeutic target for these disorders [2].

Here we analyzed possible AHK effects on the characteristics of action potentials (APs) and the main cardiac ionic currents recorded in human induced pluripotent stem cell cardiomyocytes (hIPS-CMs). In human cardiomyocytes paced at 1 Hz, AHK (0.5 μM) significantly shortened the AP duration measured at 20, 50, and 90% of repolarization ($n \geq 7$, $P < 0.05$) without modifying the resting membrane potential and the action potential amplitude ($n \geq 7$, $P > 0.05$). The treatment with AHK did not affect the density and voltage- and time-dependent parameters of Na^+ current (I_{Na}) or its late component ($I_{Na,L}$) ($n \geq 10$, $P > 0.05$). Conversely, AHK significantly reduced the density of the L-type Ca^{2+} current ($I_{Ca,L}$) ($n \geq 7$, $P < 0.05$) without affecting its voltage- and time-dependent characteristics. Furthermore, AHK inhibited the inward and outward components of the current generated by the Na^+/Ca^{2+} exchanger (I_{NCX}). These results suggest that AHK directly interacts with human cardiac L-type Ca^{2+} channels an effect that could contribute either to an antiarrhythmic action or being deleterious by decreasing cardiac contractility. Furthermore, and according with its effects as a rycal, AHK decreases I_{NCX} which could be antiarrhythmic in the context of heart failure or atrial fibrillation.

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P14

Kv1.3 Controls VSMC Phenotypic Modulation through ECM-Dependent Adhesion and Migration.

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Kv1.3 channels are central to vascular smooth muscle cell (VSMC) phenotypic modulation, the switch from contractile to proliferative state underlying many vascular lesions. A switch from Kv1.5 to Kv1.3 expression is an early, necessary step that drives this transition, facilitating vascular lesion (1), but mechanistically unclear. Kv1.3 has been shown to bind β1-integrin, an extracellular matrix (ECM) receptor, and channel–integrin interactions regulate adhesion, migration, and signaling in other cell types (2). Since VSMC–ECM interactions influence lesion development (3), we tested whether Kv1.3–β_1-integrin coupling controls VSMC adhesion and mobility during vascular remodeling. We used Kv1.3KO mice to explore *in vivo* vascular remodeling (femoral artery wire- injury) and to obtain primary VSMC cultures from femoral arteries. Cell adhesion and mobility was explored with time-lapse microscopy (TLM) in WT and Kv1.3KO VSMCs and in HEK cells overexpressing Kv1.3, Kv1.5, or a poreless Kv1.3 (WF-Kv1.3).

Kv1.3KO femoral arteries exhibit thicker walls with fewer, but larger VSMCs. After wire injury, lumen stenosis was significantly reduced in Kv1.3KO vs WT mice, confirming that Kv1.3 is required for acute injury-driven remodeling. This dual role highlights Kv1.3's distinct contributions to baseline structure versus pathological remodeling.

Adhesion assays on various ECM substrates showed fibronectin elicited the strongest responses. Kv1.3-transfected HEK cells exhibited increased adhesion rates compared to Cherry-transfected cells. Overexpressing Kv1.3 or WF-Kv1.3 HEK cells increased maximum speed and travel distance compared to those expressing Kv1.5 or Cherry alone. WT VSMCs adhered better than Kv1.3KO cells, and β_1-integrin blockade reduced adhesion only in WT, not KO. 48h-TLM experiments showed decreased speed and travelled distance, in Kv1.3KO VSMCs, together with reduced cell size change compared to WT. All these were reversed upon Kv1.3 overexpression in the KO cells. Altogether, our data demonstrate that Kv1.3 channels are key regulators of VSMC–ECM interactions and motility, thereby influencing vascular structure and remodeling

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P15

Functional Mapping of DECA-11 Identifies a Minimal Peptide Motif Mediating I_{Na} and I_{K1} Potentiation

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Heart failure is a severe condition associated with high rates of hospitalization and increased mortality, largely due to ventricular arrhythmias (VA) and fibrillation. Most antiarrhythmic drugs are contraindicated due to their potential proarrhythmic effects and increased mortality risk, which underscores the urgent need for new, precise and safe advanced therapies. Electrical remodeling in cardiac diseases is often associated with reduced Nav1.5 and Kir2.1 expression, increasing the risk of VA. In prior studies, we characterized the peptide DECA-11 (RFTRESLAAIE, Patent WO2025068623), an 11-mer peptide capable of selectively increasing the densities of the Na^+ (I_{Na}) and inward rectifier K^+ (I_{K1}) currents in heterologous expression systems, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), and mouse cardiomyocytes. Here, we aimed to identify the minimal motif within the peptide required to preserve its enhancing activity. To this end, fluorescein thiourea-tagged DECA-11 was functionalized with common conjugations such as palmitoylation, acetylation, and the cell-penetrating TAT peptide in order to assess their cellular internalization by immunofluorescence in HEK293 cells. It was found to be the palmitoylation of DECA-11 at its N-terminus was essential for its internalization; therefore, this derivative was used as a lead compound for subsequent experiments. Serial microdeletions of palmitoylated DECA-11 generated peptides whose effects were tested in Chinese hamster ovary cells previously transfected with Nav1.5 or Kir2.1 channels. Patch-clamp experiments demonstrated that the shortest peptide retaining the ability to increase I_{Na} and I_{K1} was TRESLAA. This domain was then subjected to alanine scanning to determine the key residues mediating its activity. This approach established RES as the minimal essential motif, identifying S6 (DECA11 numbering) as a critical residue. Furthermore, mutagenesis studies demonstrated that p.S6D DECA-11 also increased I_{Na} and I_{K1}. These findings define the molecular determinants of DECA-11 activity and suggest that phosphorylation of the serine at position 6 may underlie the peptide's activity.

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P16

A SAR Driven Strategy for Developing K_V/KChIP2 Channelosome Modulators Ion Channel Pharmacology and Therapeutics

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The heart's normal electrophysiological function relies on the orderly propagation of action potentials (APs). APs are electrical signals generated by cardiomyocytes, that enable and coordinate the contraction of the heart muscle. The disruptions of these signals form the underlying causes of cardiac arrhythmias.[1]

AP's shape and duration are determined by a subtle interplay between a few different ion channels. Channels assembly with several regulatory subunits to form the so called channelosomes. Among them, we focus on the voltage-gated K^+ (K_v) K_v1.5, predominantly localized in atrial tissue, and KV4. It has been described that subunit KChIP2 (Potassium Channel Interacting Protein 2) interacts with K_v1.5. and K_v4 modifying their currents. KChIP2 is mainly expressed in heart and it's a key regulator of cardiac AP duration.[2]

Our research strategy focuses on targeting the regulatory subunits through the identification of novel KChIP2 ligands as useful tools to modulate K_v/KChIP2 channelosome.[3] Additionally, we seek to develop a solid Structure Activity Relationship (SAR) model to guide the identification of new KChIP2 binding compounds.

In this communication, we described a multidisciplinary approach for the identification of KChIP2 ligands *via* a divergent synthetic strategy followed by a FRET based biological evaluation. This allowed us to design SAR studies that elucidated the key structural determinants responsible for KChIP2 binding.

Altogether, these results highlight the usefulness of our SAR driven approach for the development of KChIP2-targeting chemical probes and support its potential application in the discovery of modulators of K_v/KChIP2 channelosomes. Electrophysiological studies are currently ongoing.

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P17

Calcium Remodeling in Human Glioblastoma and Surrounding Tissues with High and Low Tumor Probablity of Recurrence as Shown by AI

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Glioblastoma (GBM) is the most aggressive primary brain tumor, characterized by marked molecular heterogeneity and high recurrence rates. Alterations in intracellular calcium (Ca^{2+}) homeostasis may contribute to tumor progression phenotype. In this study, we performed a transcriptomic analysis of genes involved in intracellular Ca^{2+} transport and regulation in GBM and peritumoral tissues to identify tumor molecular signatures. RNA-Seq was conducted on 33 human samples, including 9 unpaired GBM tumors and 24 paired blind samples (α,β,γ) from tumor and peritumoral regions with different recurrence probabilities based on Artificial Intelligence analysis of 8 additional patients. RNA was extracted, quality-controlled, processed and analyzed in R enviroment. Regarding differential expression analysis, significant transcriptional differences were found only between α vs. γ and GBM vs. γ, with no differences between GBM and α or β, nor between α and β. These results suggest two similar phenotypes (GBM/α and β) representing tumor cells, and a distinct γ phenotype corresponding to normal tissue. A total of 1,515 and 1,495 differentially expressed genes (DEGs) were identified in α vs. γ and GBM vs. γ, respectively, with 673 shared genes. Focusing on Ca^{2+}-related genes, 20 DEGs were identified in each comparison (>1% of total), with 14 overlapping. Voltage-operated calcium channel analysis showed downregulation of $Ca_v1.4$ and upregulation of $Ca_v3.2$ in α and GBM samples. SERCA1 was selectively upregulated in α samples, while purinergic receptor genes wereconsistently downregulated. TRP channel analysis revealed downregulation of TRPV3,5,6, and TRPM6, and upregulation of TRPC6 and TRPV4. Additionally, components of store-operated calcium entry (SOCE) signaling and genes involved in endoplasmic reticulum and mitochondrial Ca^{2+} handling were mostly downregulated. In conclusion, this study identifies a distinct Ca^{2+}-related transcriptional signature distinguishing GBM-like samples from a divergent peritumoral phenotype, highlighting Ca^{2+} homeostasis pathways as potential biomarkers and therapeutic targets associated with tumor aggressiveness and recurrence.

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P18

Changes in Intracellular Ca^{2+} Homeostasis and Molecular Players Involved in Rat Hippocampal Neurons During *In Vitro* Aging

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Neuronal aging is associated to changes in Ca^{2+} signaling, a key integrator of excitability, plasticity, and cellular resilience. Here, we combined single-cell Ca^{2+} imaging with bulk transcriptomic profiling and linear modelling to elucidate the basis of Ca^{2+} remodeling in rat hippocampal neurons during *in vitro* aging. Accordingly, primary hippocampal cultures (neurons and glia) from neonatal Wistar rats were cultured for 6–8 days *in vitro* (DIV) or 18–21 DIV, representing young and aged neurons. Intracellular Ca^{2+} responses to different stimuli and store-operated Ca^{2+} entry (SOCE) were monitored. IP3R-mediated Ca^{2+} release was assessed using caged-IP3 photolysis and confocal microscopy. In parallel, Clariom D (rat) microarrays of the same samples were analyzed using limma (FDR<0.05), controlling for the random effect of culture origin (paired young/aged samples from the same primary preparation) and applying a Bayesian framework to infer neuron-specific expression changes from paired mixed and glia-enriched cultures within a curated Ca2+ gene panel. We found that Ca^{2+} responses mediated by voltage-gated Ca^{2+} channels, glutamate and acetylcholine receptors were enhanced in aged neurons, while SOCE nearly disappeared with age. IP3R-dependent Ca^{2+} release also declined in aging neurons. Transcriptomic analysis revealed overexpression of a number of plasma membrane receptors including GluN2C, GluA1/2, GluK4/5, CHRM1/2, P2X5/6, P2Y4, and Cav3.1, the infraexpression of P2Y1 and CHRM3, along with altered expression of pumps/exchangers, SOCE components, ER channels, and the loss of mitochondrial Ca^{2+} transporters. In summary, aging shifts Ca^{2+} signaling in hippocampal neurons towards enhanced Ca^{2+} influx but impaired SOCE, ER Ca^{2+} release and mitochondrial Ca^{2+} handling leading to enhanced susceptibility to neuron cell death and loss of dendritic spine stability required for memory formation. Thus, posing the question on whether Ca^{2+} remodeling may be a novel aging hallmark.

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P19

Functional Analysis of the *SCN5A-SCN10A* Haplotype in Brugada Syndrome

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Brugada syndrome (BrS) is a rare inherited cardiac disorder, more common in males, characterized by arrhythmias and a high risk of sudden cardiac death. Loss-of-function variants in *SCN5A*, encoding the pore-forming α-subunit of the main cardiac sodium channel $Na_v1.5$, account for ~25% of BrS cases. However, recent GWAS have shown that common non-coding variants also contribute to disease susceptibility through a polygenic pattern. In this context, we previously reported that a 7-SNV haplotype within the *SCN5A-SCN10A* locus is associated with differential susceptibility to BrS, supporting an impact of non-coding cis-regulatory variants on the phenotype (1).

Here, we performed a haplotype analysis of the *SCN5A-SCN10A* locus in BrS cases and controls using nanopore sequencing. Preliminary data show that the haplotype composition of subjects carrying an exonic pathogenic SCN5A variant (SCN5A+) is more similar to that of healthy controls than to that of subjects without a pathogenic variant (SCN5A−). Since we had previously shown that SCN5A− individuals homozygous for the risk haplotype Hap1 have increased susceptibility to BrS, these results suggest that an exonic pathogenic variant accounts for the phenotype only when Hap1 is in heterozygosis.

We also examined functional effects of SCN5A-SCN10A haplotypes in cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs). In Hap1/1 hiPSC-CMs, expression of the cardiac short SCN10A transcript (SCN10A-short) (2) was decreased compared with a healthy Hap1/3 control.

Although SCN5A mRNA and $Na_v1.5$ levels were unchanged, sodium current was reduced by ~50%, and the steady-state inactivation curve shifted to more hyperpolarized potentials, consistent with loss of function and a BrS phenotype (1). Finally, partial CRISPR-Cas9 editing of BrS Hap1/1 hiPSCs toward a protective genotype moderately restored *SCN10A*-short expression and, importantly, sodium current defects by reverting a common *SCN10A* variant known to modulate cardiac *SCN5A* expression (3).

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P20

Mapping Binding Sites of Known Ligands in TRPM8

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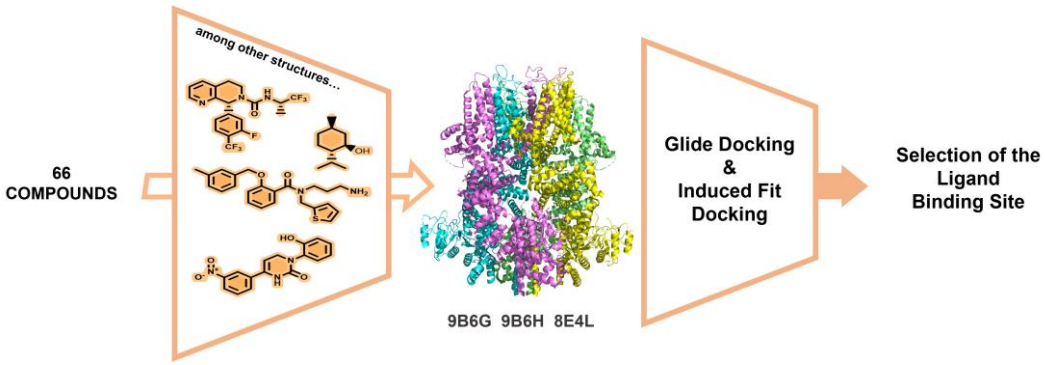
The transient receptor potential melastatin 8 (TRPM8) channel is a calcium-permeable, non-selective cation channel involved in the detection of innocuous cold. In addition to its established role in thermosensation, TRPM8 has emerged as a therapeutic target in conditions such as neuropathic pain, cold hypersensitivity, and various cancer types, including prostate, breast, and bladder cancer (1,2). As a polymodal sensor, TRPM8 can be activated by cool temperatures, membrane depolarization, mechanical forces, osmotic changes, and cooling agents such as menthol and icilin.

Cryo-electron microscopy (cryo-EM) studies have shown that TRPM8 forms a domain-swapped tetramer. Each subunit consists of a cytoplasmic region and a transmembrane domain, which encompasses the voltage sensor-like domain (VSLD) and the pore-forming region (3). Structural data of TRPM8 in complex with ligands have revealed different binding sites within the VSLD or at the interface between adjacent subunits. However, the specific binding site preferences of many known ligands remain unclear.

Docking studies were carried out to identify the binding site for known ligands in TRPM8 (Fig. 1). These simulations provide insights into binding preferences and the structural requirements for ligand recognition. This contributes to a better understanding of TRPM8 modulation and provides a foundation for the rational design of novel ligands for the treatment of TRPM8-associated pathologies.



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P21

Role of Vascular Cold-Sensitive Channels in a Complex Regional Pain Syndrome Model

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Chronic pain conditions like Complex Regional Pain Syndrome type I (CRPS-I) are rare disorders characterized by disproportionate pain sensitivity, vasomotor and sudomotor dysfunction, and motor and trophic changes in the absence of major nerve damage. Increasing evidence suggests that vascular mechanisms contribute to CRPS-I pathophysiology, yet the precise mechanisms remain elusive (1). The Chronic Post-Ischemia Pain (CPIP) model effectively recapitulates CRPS-I features (2), including thermal hyperalgesia, mechanical allodynia, and autonomic dysfunction, making it an ideal model to investigate the vascular contribution to altered nociception (3). Given that TRP channels (TRPV1, TRPA1, TRPM8) are primary nociceptors driving pain hypersensitivity in chronic pain, understanding how vascular dysfunction modulates TRP activity could reveal novel mechanisms linking circulation to pain in CRPS-I.

Here, we investigate the role of cold-sensitive TRP channels (TRPA1 and TRPM8) in vascular responses in CRPS-I using the CPIP model. CPIP was induced in male C57BL/6 mice (9–16 weeks) by hind paw ischemia-reperfusion. Mechanical hypersensitivity (von Frey) was significantly increased in CPIP mice from 3 hours up to 60 days post-reperfusion, while heat sensitivity (hot plate test) showed no differences. Cold allodynia (acetone test) was observed only at 3 hours and 1 day post reperfusion, with no significant changes later.

Focusing on this time window showing altered cold sensitivity, we performed pressure myography on isolated plantar arteries from CPIP and sham mice 1 day after lesion. CPIP arteries showed increased phenylephrine sensitivity, indicating enhanced vasoconstrictor responsiveness. We are now evaluating vascular responses to cold stimuli to determine if they persist beyond the behavioral cold hypersensitivity phase and exploring the contribution of TRPM8 and TRPA1 to vascular function in CPIP. This work could uncover new vascular targets for treating CRPS-I and other chronic pain conditions where circulatory and nociceptive mechanisms converge.

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P22

The Bioactive Paralipidome of TRPV Channels Reveals Conserved Lipid and Drug-Binding Hotspots

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Transient Receptor Potential Vanilloid (TRPV) channels are polymodally regulated ion channels whose activity depends critically on the membrane environment. Although individual lipids have been implicated in TRPV regulation, a systematic characterization of lipid–protein interactions across the entire TRPV subfamily has been lacking. Here, we define the bioactive paralipidome of all six TRPV channels (TRPV1–6) using long timescale coarse grained molecular dynamics simulations in a complex, asymmetric plasma membrane.

Each TRPV channel was simulated for a total of 15 μs, enabling extensive sampling of lipid diffusion and binding. Quantitative analysis of lipid enrichment, residence time, and binding site formation reveals that TRPV channels actively recruit a specific subset of membrane lipids, dominated by cholesterol and phosphatidylinositols. These lipids engage discrete, recurrent binding regions rather than forming a nonspecific lipid annulus. We identify six lipid binding sites across the TRPV subfamily, including the canonical vanilloid pocket and two previously undescribed sites, which we term the upper vanilloid and shallow S2–S3 sites.

The physicochemical nature of the binding sites is lipid dependent: cholesterol preferentially occupies hydrophobic cavities within the transmembrane domain, while phosphatidylinositols localize to conserved polybasic regions near the cytosolic membrane interface. Evolutionary conservation analysis demonstrates that these sites are preserved across TRPV channels, supporting their functional relevance. Strikingly, several lipid binding sites spatially overlap with known agonist and antagonist binding pockets resolved in cryo EM structures, suggesting direct competition or cooperative interactions between endogenous lipids and pharmacological ligands.

Together, our results establish the TRPV paralipidome as an integral component of channel structure and regulation. This lipid centric view provides a mechanistic framework to understand TRPV modulation in physiological membranes and highlights conserved lipid binding sites as underexplored targets for structure based drug discovery.

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P23

The β1 Subunit Is a Common Determinant of Distinct NaV1.5 Modulatory Mechanisms in Brugada Syndrome

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Background: Brugada syndrome (BrS) is an inherited arrhythmogenic disease associated with an increased risk of ventricular fibrillation and sudden cardiac death. SCN5A, which encodes the α-subunit of the cardiac sodium channel $Na_v1.5$, is the major gene associated with BrS. $Na_v1.5$ is regulated by several auxiliary subunits, including the β1 subunit encoded by *SCN1B*, although its precise role remains controversial. Here, we investigated whether the β1 subunit participates in two sodium current (I_{Na}) regulatory mechanisms proposed to involve α–α interactions: (i) dominant-negative effects exerted by mutant $Na_v1.5$ channels and (ii) regulation mediated by the short *SCN10A* transcript $Na_v1.8$-short. Previous work from our group showed that the BrS-associated variant *SCN5A* c.4573G>A ($Na_v1.5$ p.V1525M) exerts a dominant-negative effect on I_{Na} in hiPSC-derived cardiomyocytes, but not in HEK293T cells lacking the β1 subunit (1).
Aim: To investigate the role of the β1 subunit in (i) the dominant-negative behavior of the $Na_v1.5$p.V1525M variant and (ii) the regulation of I_{Na} mediated by $Na_v1.8$-short.
Methods: HEK293T cells were transfected with different combinations of *SCN5A*, *SCN5A*_c.4573G>A, *SCN10A*-short and *SCN1B* to evaluate both the dominant-negative effect and $Na_v1.8$-short-mediated modulation. Whole-cell patch-clamp recordings were performed to assess I_{Na} amplitude, gating kinetics, and voltage dependence.
Results: The dominant-negative effect of $Na_v1.5$ p.V1525M was observed in the heterologous expression system only when SCN1B was co-expressed. Likewise, co-expression of *SCN1B* in cells transfected with $Na_v1.5$ and $Na_v1.8$-short produced a significant increase in I_{Na}, whereas this effect was absent in the absence of *SCN1B*.
Conclusions: Our data identify the β1 subunit as a common determinant of two distinct $Na_v1.5$ regulatory mechanisms: the dominant-negative effect caused by $Na_v1.5$_p.V1525M and the $Na_v1.8$-short-dependent gain of function. These findings support the idea that the β1 subunit acts as a molecular mediator of distinct α–α interactions with potential pathological relevance.

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P24

Endocrine-Disrupting Chemical Mixture Exposure During Pregnancy: Effects on Maternal Glucose Homeostasis and Ion Channel Activity

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Endocrine-disrupting chemicals (EDCs) are exogenous substances that can interfere with the endocrine system by mimicking, blocking, or altering the action of natural hormones (1). Pregnancy represents a particularly sensitive period, during which maternal exposure may have important implications for both maternal physiology and fetal health (2) Despite this, most studies have examined EDCs individually, whereas real-world exposure occurs as complex mixtures, underscoring the need to assess their combined effects to more accurately characterize associated risks.

In this study, we assess the impact of a relevant EDC mixture on maternal metabolism in a pregnant animal model, with a particular emphasis on glucose homeostasis.

We found that gestational exposure to the mixture induces glucose intolerance and alters circulating glucagon levels in the mother. It impairs endocrine pancreatic function, reducing β-cell insulin secretion while disrupting α-cell regulation, leading to inappropriate glucagon secretion even under inhibitory conditions. These alterations are associated with changes in calcium currents and in the expression of key genes involved in β- and α-cell identity and function. *In vitro*, the same mixture also reduces calcium currents in both murine islets and human β-cells, suggesting a direct effect on calcium signaling that may underlie the observed defects in insulin and glucagon secretion.

Collectively, these findings indicate that gestational exposure to the EDC mixture disrupts pancreatic endocrine function and maternal glucose regulation, highlighting its potential impact on metabolic health during pregnancy.

Acknowledgements

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P25

A GPRC5B-Fyn Signaling Axis Regulates VRAC Activity by Targeting LRRC8A Channelopathies and Rare Diseases

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The mechanisms by which physiological signals gate the volume regulated anion channel (VRAC) remain poorly understood. Here, we identify a GPRC5B-Fyn signaling axis that directly controls VRAC activation by targeting the essential subunit LRRC8A. We found that the orphan G-protein coupled receptor GPRC5B binds LRRC8A and recruits the Src family kinase Fyn, which in turn suppresses hypotonicity-evoked VRAC activation. Disrupting Fyn binding to GPRC5B or substituting a predicted phosphorylation site on LRRC8A (Y362) abolishes this inhibition. Across primary astrocytes and reconstituted systems, GPRC5B prevents the inter-subunit conformational rearrangements associated with VRAC opening without altering basal channel expression. In astrocytes, the MLC protein MLC1 partially sequesters GPRC5B, thereby reducing the GPRC5B-LRRC8A interaction, providing a mechanistic link to VRAC dysfunction in megalencephalic leukoencephalopathy. These results reveal a receptor-kinase-channel pathway that gates VRAC through Y362 dependent modulation of LRRC8A, establishing a molecular framework for VRAC regulation and its disruption in disease.

P26

Mechanistic Insights into DCPIB Inhibition of VRAC: Electrostatic Control and Binding Plasticity

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The volume-regulated anion channel (VRAC), formed by LRRC8 heteromers, regulates cell volume and is a promising therapeutic target. DCPIB is the most widely used VRAC inhibitor, yet its binding mechanism remains poorly defined. Although structural studies suggested a role of residue R103 in LRRC8A in DCPIB binding, its functional relevance has been unclear.

Here, we combined electrophysiology in *Xenopus oocytes* and LRRC8$^{-/-}$ HEK293T cells with molecular dynamics simulations and rational design of DCPIB analogs. Mutations of R103 abolished ATP-dependent block of VRAC but did not affect inhibition by DCPIB or the related inhibitor carbenoxolone (CBX), indicating distinct binding mechanisms. Simulations revealed two DCPIB binding modes: one involving R103 and another engaging K51 and hydrophobic residues. In R103F mutants, DCPIB retained binding *via* π-stacking and alternative electrostatic contacts, explaining its persistent inhibition. Attempts to disrupt these interactions by mutating K51 failed due to channel loss of function.

To probe electrostatic contributions, we synthesized DCPIB derivatives varying charge and hydrophobicity. Functional assays and steered molecular dynamics (MD) showed that analogs retaining the carboxylate group maintain high-affinity binding, whereas esterified forms exhibit rapid unbinding and reduced potency. Biphenyl substitutions increased binding energy but imposed steric penalties, suggesting a trade-off between affinity and accessibility.

Our findings reveal an adaptable binding mechanism for DCPIB, contrasting with described lipid-displacement mechanisms for other VRAC inhibitors, and establish electrostatic complementarity as the primary determinant of high-affinity block. These insights provide a framework for rational design of next-generation VRAC inhibitors with improved specificity.

P27

Structure-Guided Optimization of a TRPM8 Hit

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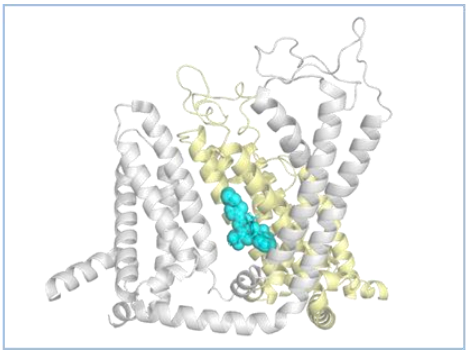
The Transient Receptor Potential Melastatin 8 (TRPM8), is a non-selective calcium channel recognized as the receptor for cold and menthol. It has drawn attention as a potential therapeutic target for several diseases, including neuropathic pain, chronic migraine, cold hypersensitivity and cancer.[1] TRPM8 is made up of a domain-swapped tetramer, in which each monomer has a transmembrane (TM) and a cytosolic domain.[2] Cryo-EM TRPM8 structures have revealed different ligand binding pockets within the TM region of TRPM8.[3]



A screening of our in-house compound collections identified a derivative containing a nitrogen six-membered heterocyclic core as a TRPM8 ligand. Induced fit docking (IFD) studies revealed that this hit binds at the interface between two monomers in a similar pocket to the ligand TCI-2014 in the structure of PDB code 9B6H (Fig. 1).3 A 200 ns molecular dynamic simulation (MD) confirmed the stability of this complex and the establishment of a hydrogen bond with Ser966. Based on this findings, a focused virtual library was designed, in which compounds retained the nitrogen six- membered heterocyclic core. The filtering of this library through IFD studies allowed the selection of the most promising candidates for synthesis. Biological evaluation revealed that several compounds exhibited improved affinity compared with the hit, although still in the low micromolar range. Overall, these results provide insight into the binding site of these derivatives and support the rational design of more potent TRPM8 modulators.

Acknowledgements
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P28

Conformational Dynamics and Residue Ionization in Protein Channels: Insights from Constant-PH MD Simulations

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Electrostatic interactions are crucial for protein structure and function, especially in mesoscopic protein channels where ion selectivity is largely ruled by the protein's electrostatic properties. Understanding the protonation state of ionizable residues across pH values —often described by their pKa— is key to linking structure and function. Here we study the charge state of a general diffusion porin, OmpF, as a test model with a large number of ionizable residues. We use Constant pH Molecular Dynamics (CpHMD) simulation (1,2), arguably one of the most accurate pKa prediction methods in proteins that contain many charged residues since it captures the coupling between conformational dynamics and residue protonation. We show how residue conformation significantly impacts protonation curves, deviating from the Henderson-Hasselbalch model. CpHMD simulations in OmpF reveal that side-chain fluctuations create heterogeneous environments, resulting in non-ideal titration behavior. Titration curves of key residues such as D97 exhibit Hill coefficients below 1, suggesting an apparent negative cooperativity possibly resulting from microstate heterogeneity rather than from simple electrostatic interactions. We also report CpHMD simulations proving that the applied voltage modulates local pH gradients across membrane-spanning regions, directly affecting apparent pKa values (3). These dynamic interactions could explain discrepancies between static pKa predictions and functional data under physiological conditions. The asymmetric pKa shift might influence channel rectification. CpHMD simulations emerge as a valuable tool to study pH-dependent changes in channel conformation which may trigger conductive or nonconductive states as well as changes in selectivity.

Acknowledgements

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P29

Optochemical Modulation of Cold-Activated TRPM8 Channels

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TRPM8 is a TRP ion channel activated by cold and cooling compounds, expressed in a subpopulation of sensory neurons. TRPM8 is the principal sensor of environmental cold. There is substantial evidence the downmodulation of TRPM8 by Gq-coupled GPCRs in sensory neurons and heterologous expression systems. However, the mechanisms underlying this modulation are disputed. Some studies propose a direct inhibition of TRPM8 by Gαq (5) while others propose the activation of PLC and reduced membrane PI(4,5)P2 availability as the primary mechanism (1, 3).

HEK293 cells overexpressing TRPM8 and the muscarinic M1 receptor (M1R), we monitored whole-cell currents activated by cold or menthol. In both cases, application of 10 μM carbachol (Cch) produced a drastic reduction of inward and outward currents. These effects were replicated by brief application of blue light (460 nm) in cells overexpressing melanopsin, a light-sensitive GPCR. In cells expressing *Danio rerio* Dr-VSP, a voltage-sensitive lipid 5-phosphatase (2) which dephosphorylates PI(4,5)P2, brief depolarizing voltage pulses produced a strong and immediate suppression of menthol-activated currents. In cells co-expressing Dr-VSP, melanopsin and TRPM8, the inhibition produced by a +100 mV voltage pulse was not potentiated by melanopsin activation. This result suggests that activation of a GqPCR does not increase inhibition evoked by exclusive PI(4,5)P2 depletion.

In cells co-expressing M1R and the PI(4,5)P2 optical sensor PH-GFP, stimulation of PLC by M1R activation caused the rapid translocation of fluorescence from the membrane to the cytosol, reflecting the cleavage of PI(4,5)P2. Decay of TRPM8 current kinetics mirrored the time course of PI(4,5)P2 depletion, suggesting a strong coupling between both processes (4). In these cells, a short incubation with edelfosine, a PLC inhibitor, prevented the translocation of the PH-GFP probe by Cch, and the inhibition of TRPM8 currents was drastically reduced.

Our results indicate that PI(4,5)P2 depletion is crucial for TRPM8 inhibition after Gq-coupled GPCR activation.

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P30

Role of Orai Isoforms in Angiogenesis Induced by Blood Serum of Breast Cancer Patients

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Background: Breast tumour progression requires an angiogenic switch in which tumour-associated inflammation stimulates new vessel growth. Calcium influx is a key regulator of endothelial signaling during tumour vascularization, however, its contribution remains insufficiently explored. To better mimic the tumour microenvironment, we investigated the pro-angiogenic effects of blood serum (BS) from breast cancer patients on human umbilical vein endothelial cells (HUVECs), focusing on the activation of Orai-dependent store-operated calcium entry (SOCE) and its impact on angiogenic gene regulation.
Methods: HUVECs were stimulated with 5% BS obtained from healthy controls or breast cancer patients (triple-negative, HER2+, and luminal subtypes). Angiogenesis was assessed by tube formation and wound-healing assays. Protein expression was analyzed by western blot.
Results: Stimulation with BS of breast cancer patients induced a pro-angiogenic activation of HUVECs, characterized by increased tube formation and enhanced migration relative to control serum. These functional changes were accompanied by elevated expression of VEGFR2, the Notch1 intracellular domain (NTM), the transcription factor MEF2A, and both Orai1 and Orai3 isoforms. To determine the contribution of Orai channels, we silenced Orai1 and Orai3. Orai1 k nockdownsignificantly inhibited BS-induced tube formation, whereas Orai3 silencing led to disorganized, aberrant vessel-like structures. Consistently, Orai1, but not Orai3, was required for BS-induced activation of the survival pathway PI3K/AKT. Of note, Orai1 but not Orai3 silencing seems to affect NTM expression. Furthermore, we examined the role of MEF2A. MEF2A knockdown prevented the BS-induced upregulation of Orai1, Orai3, and VEGFR2, while not affecting NTM levels. These findings indicate that MEF2A drives a pro-angiogenic transcriptional action upstream of calcium entry, whereas Notch1 signaling doesn't appear relevant in this context.
Conclusions: Altogether, our data demonstrate that BS from breast cancer patient induces a pro-angiogenic endothelial phenotype through a MEF2A/Orai1 axis, which promote cell survival, a key step in new vessel formation.

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P31

Mechanisms of AMPA Receptor Assembly by ER-Associated Proteins

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AMPA receptors (AMPARs) are glutamate-gated ion channels responsible for fast excitatory synaptic transmission. Their assembly into functional tetramers occurs in the endoplasmic reticulum (ER), where resident proteins such as ABHD6, FRRS1L, and CPT1C contribute to receptor maturation, although their specific roles in assembly steps remain incompletely defined. In this work, we employ a combination of biochemical techniques and cryo-electron microscopy (cryo-EM) to characterize the molecular basis of AMPAR assembly.

Native gel analyses indicate that ABHD6 associates with monomeric GluA subunits (GluA1–4), including both full-length receptors and variants lacking the N-terminal domain (ΔNTD). Experiments performed with these constructs suggest that the ligand-binding (LBD) and transmembrane (TMD) domains are sufficient to support the interaction with ABHD6 and are likely involved in early oligomerization steps.

In contrast, co-purification of FRRS1L with GluA1 results in tetrameric assemblies. Image classification reveals two main particle populations: free GluA1 tetramers and FRRS1L-bound complexes. Biochemical analyses using FRRS1L deletion constructs demonstrate that truncation of residues 30–93, together with the transmembrane domain, is sufficient to support complex formation, pointing to a minimal binding region. Additional interaction studies using GluA ΔNTD and TMD–LBD constructs indicate that FRRS1L forms stable complexes with all GluA subunits and seems to engage receptors through multiple interaction interfaces. Binding is not strictly dependent on the N-terminal domain, as both N-terminal and non NTD regions can support complex formation, with subunit-dependent differences in interaction stability. Surface expression assays indicate that GluA1–FRRS1L complexes are largely retained in the endoplasmic reticulum.

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P32

Orai1 Dependent Ca^{2+} Entry Enables cAMP–CREB Signaling to Regulate Endothelial Function

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Angiogenesis plays essential roles in physiological and pathological processes, including fetal development, wound healing, and cardiac repair. Store-operated calcium entry (SOCE) has been shown to be a critical mechanism in endothelial cell activation and neovascularization. Among intracellular signaling pathways, Ca^{2+} and cyclic AMP (cAMP) are two major second messengers governing fundamental cellular functions.
Recently, we demonstrated that Orai1-mediated Ca^{2+} entry stimulates cAMP-dependent CREB activation in cardiac and smooth muscle cells. However, the role of CREB and its regulation by Orai1-dependent Ca^{2+} entry in angiogenesis remain poorly understood. Therefore, we investigated CREB as a key component of the SOCE–angiogenesis axis, acting as a downstream decoder of Ca^{2+} signals that link Orai1-mediated Ca^{2+} entry to transcriptional programs driving angiogenesis. To examine the role of the cAMP/CREB pathway in endothelial cells, we increased intracellular cAMP levels using dibutyryl-cAMP (db-cAMP) and ibudilast, a phosphodiesterase inhibitor with anti-inflammatory properties. Using classical angiogenesis assays, we found that both db-cAMP and ibudilast significantly enhanced migration and tube formation in human umbilical vein endothelial cells (HUVECs). Moreover, both drugs induced significant and time-dependent CREB phosphorylation. Next, we investigated whether SOCE activates CREB in HUVECs. Thapsigargin-induced Ca^{2+} entryresulted in CREB phosphorylation, an effect that was inhibited by CM4620, a specific Orai1 inhibitor. Consistently, genetic silencing of Orai1 also prevented thapsigargin-induced CREB activation. Finally, genetic silencing of CREB in HUVECs led to a significant downregulation of Orai1 expression, while Orai3 levels remained unchanged, confirming that CREB activation promotes Orai1 expression in HUVECs.
Altogether, these data identify CREB as an important transcriptional regulator of angiogenesis andreveal its regulation by intracellular Ca^{2+} entry through Orai1. Our findings provide new insights into the molecular mechanisms underlying vascular growth and highlight a dynamic feedback loop between Ca^{2+} signaling and transcriptional control in endothelial cells.

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P33

Orai1 Drives Angiogenesis after Myocardial Infarction through Notch1 Signaling Pathway

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Neovascularization is crucial for cardiac repair after myocardial infarction (MI), and Ca^{2+} signaling plays a critical role in endothelial activation. While Orai1-dependent store-operated calcium entry (SOCE) is known to support angiogenesis, its specific role after MI remains unclear.

To analyze pro-angiogenic mechanisms and to mimic the post-infarct systemic environment we used serum of patients with ST-elevation MI (STEMI) to stimulate human umbilical vein endothelial cells (ECs). We performed integrative analysis, including transcriptomics, proteomics, single-cell RNA sequencing.

Our results demonstrate that STEMI serum boosted angiogenesis by upregulating VEGF, Notch, and Ca^{2+} signaling pathways in ECs. Notably, it increased Orai1 expression and SOCE activity, required for EC migration and proliferation. Orai1 inhibition with CM4620 significantly impaired subintestinal venous plexus (SIVP) development in zebrafish embryos. Single-cell RNA sequencing confirmed Orai1 upregulation, particularly in tip and proliferating EC clusters, which was confirmed in peri-infarct regions of mouse hearts. Proteomics analysis revealed that Orai1 silencing dysregulated VEGF and Notch1-related pro-angiogenic proteins. Furthermore, interleukin-17A (IL-17A) mimicked patient serum effects, inducing Orai1-mediated SOCE and EC migration. Conclusions: Together, these findings reveal a novel role for Orai1-dependent mechanism in post-MI angiogenesis, highlighting Orai1 as a potential therapeutic target for cardiac repair.

Acknowledgements

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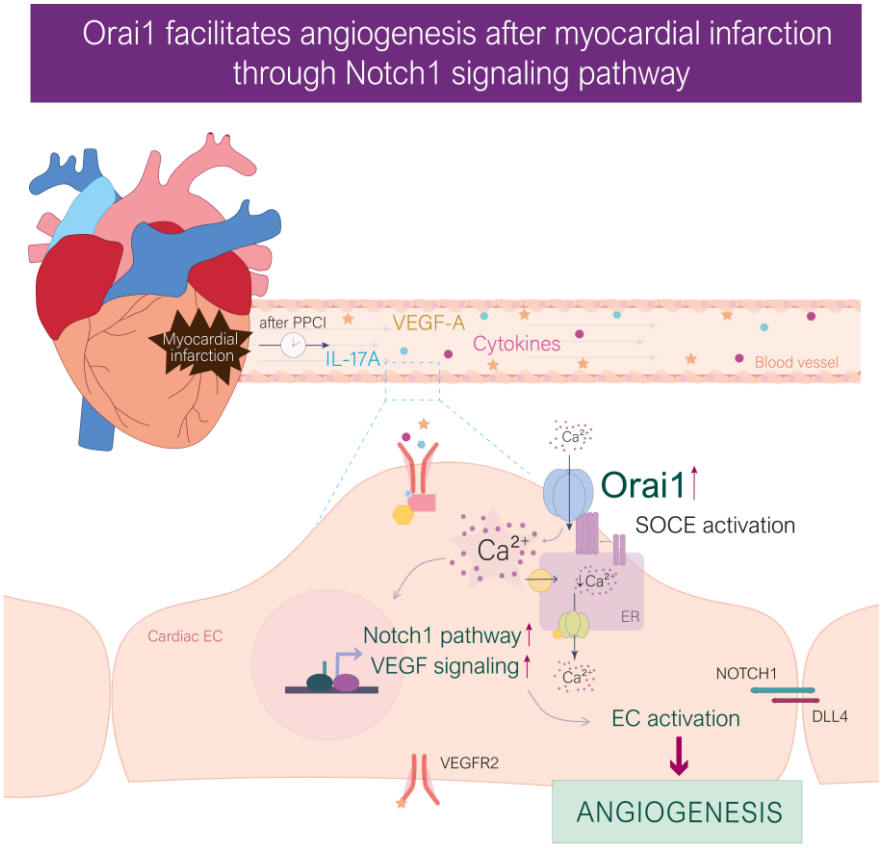
Orai1 facilitates angiogenesis after myocardial infarction through Notch1 signaling pathway
Myocardial infarction
after PPCI
IL-17A
VEGF-A
Cytokines
Blood vessel
Orai1
SOCE activation
Ca2+
ER
Cardiac EC
Notch1 pathway
VEGF signaling
NOTCH1
DLL4
EC activation
VEGFR2
ANGIOGENESIS

P34

Ion Channel Dysfunction in Pancreatic α- and β-Cells in Gestational Diabetes Mellitus: Role of K_{ATP} Channels, Na^+ and Ca^{2+} Currents.

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Gestational diabetes mellitus (GDM) is defined as any degree of glucose intolerance with onset or first recognition during pregnancy and represents the most common metabolic complication of gestation[1]. Its prevalence has increased over the past two decades, largely driven by rising rates of obesity among women of reproductive age. GDM develops when pancreatic β-cells are unable to sufficiently compensate for the progressive insulin resistance of pregnancy, resulting in impaired maternal glucose homeostasis. Given the established role of β-cell dysfunction and growing evidence of altered α-cell function in GDM, this study aims to characterize their electrical activity and define their contribution to impaired glycaemic regulation in GDM. To this end, we previously established a preclinical animal model of GDM that recapitulates the key features of the human condition. Single channel recordings revealed a significant increase in K_{ATP} channel activity in β-cells from GDM mice, along with an impaired ability of glucose to induce channel closure. This was associated with a reduced occurrence of action currents, indicating decreased cellular excitability. Given that membrane depolarization driven by K_{ATP} channel closure is essential for the activation of voltage-gated Ca^{2+} channels, these findings suggest a disruption of the stimulus–secretion coupling mechanism. Consistently, voltage-gated Ca^{2+} currents were markedly reduced in β-cells from GDM mice. In contrast, voltage-gated K^+ currents remained unchanged across experimental groups, indicating a selective impairment of ion channels directly involved in insulin secretion. At the molecular level, increased expression of Kir6.2 and Sur1 was observed in islets from GDM mice, potentially contributing to enhanced K_{ATP} channel activity. These functional alterations were accompanied by a significant reduction in glucose-stimulated insulin secretion. Furthermore, Na^+ currents were modified in pancreatic α-cells from GDM mice, together with altered expression of key genes involved in α-cell function and identity.

Our results show that GDM is associated with specific alterations in ion channels in pancreatic α- and β-cells, leading to impaired excitability and dysregulated islet hormone

secretion. These findings highlight ion channels as key contributors to α- and β-cell dysfunction in GDM and as potential therapeutic targets.

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P35

Electrophysiological Evaluation of Novel KChIP2 Ligands on Kv4.3 and Kv1.5 Channelosomes: Insights into Promising Therapeutic Avenues for Atrial Fibrillation

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Atrial-selective modulation of potassium currents represents a promising strategy to improve the safety of antiarrhythmic therapies for atrial fibrillation (AF). The I_{Kur} current, mediated by Kv1.5 channels, is atrium-specific, and its inhibition prolongs the effective refractory period (ERP) and action potential duration (APD) without significantly affecting ventricular repolarization [1]. Conversely, the I_{to} current, generated by Kv4.3 channels and typically reduced in AF, plays a key role in early repolarization. The enhanced activation of Kv4.3 may help restore a more physiological action potential, closer to sinus rhythm. Current therapeutic options for AF treatment have shown limited efficacy, underlining the importance of identifying novel avenues for its intervention [2]. Recently, we have described KChIP2 as an interactor of the Kv1.5 channelosome [3]. Several studies have revealed marked reductions of I_{to} density in chronic AF due to downregulation of Kv4.3 and KChIP2 expression. In our current coordinated project, following a multidisciplinary approach that integrates medicinal chemistry and electrophysiology, we have identified a new family of potent KChIP2 ligands (in the nanomolar range). We then studied their pharmacological effects on Kv1.5 and Kv4.3 channels transiently expressed in HEK293 or CHO cells, respectively, in the absence or presence of KChIP2. In this communication, we will present the electrophysiological evaluation of the new KChIP2 ligands using whole-cell patch-clamp recordings. Our results indicate that this novel family inhibits the Kv1.5 current and increases the Kv4.3 current in a KChIP2-dependent manner. This dual effect is particularly relevant, given the shortened ERP and APD and the reduction of the Kv4.3 current that happen in AF. Consequently, the simultaneous modulation of Kv1.5 and Kv4.3 could restore some of the atrial electrical remodeling that occurs during AF. Thus, this newly uncovered KChIP2-ligand family comprises promising modulators for atrial-selective antiarrhythmic strategies, combining efficacy with a low risk of proarrhythmic effects.

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