CANAUX IONIQUES

Plenary lecture : **Annette Dolphin (London, UK)**
Drugs and trafficking: lessons from calcium channels

Techniques for studying ion channels and their environment
Scott Hansen (Jupiter, USA)
Lucie Bergdoll (Marseille, France)
Laetitia Mony (Paris, France)

Channelopathies : modeling disease
Dominique Glauser (Fribourg, Switzerland)
Nadine Ortner (Innsbruck, Austria)
Carol Ann Remme (Amsterdam, The Netherlands)

Glutamate receptors: trafficking, turnover, plasticity, agonists
Mark Farrant (London, UK)
Aude Panatier (Bordeaux, France)
Nelson Rebola (Paris, France)

Channelopathies : from structure to pharmacology
Jean-François Desaphy (Bari, Italy)
Henry Colecraft (New York, USA)
Barbara Ribiero (Nantes, France)

Ion channels and cancer
Barbara Ehrlich (Yale, USA)
Rainer Schindl (Graz, Austria)
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We thank them for their financial and material support.

FOREWORDS/AVANT-PROPOS

Dear Colleagues,

We are delighted to meet again in Sète for the 33rd edition of the Ion Channels Meeting. For this new edition, we will have the chance to listen to talented researchers, senior and junior, working on different fields of biology (neurosciences, stem cells, hypoxia, cardiac physiology, etc.) and pathophysiology (cancer and metastases, autism, neuromotor and cardiac disorders...) having as common target the ion channels.

As a new feature, the 2024 edition will include two symposia dedicated to genetic channelopathies. This will be an opportunity to discover and discuss newly-developed models (in silico, in vitro, in vivo) and technologies that enhance our understanding of ion channels, from structure to physiological roles, and should foster the development of innovative therapeutic strategies.

Ion channels occupy a central role in current worldwide research and the French Ion Channel Association is proud of its 33 years of annual meetings and thankful to you for your participation, which reached this year the significant number of 117 registrations joined by more than 45 abstracts submissions.

This meeting wouldn't be possible without the support of our sponsors, as well as the resilience and hard work of all the members of the organizing committee: Valérie Coronas, Marie Demion, Ines Elbini, Adèle Faucherre, Alban Girault, Cécile Hilaire, Perrine Inquimbert, Claire Legendre, Loïc Lemonnier, Philippe Lory, Pietro Mesirca, Isabelle Rubera, Vincent Seutin, Jérôme Thireau and of course Arnaud Monteil and Caroline Strube, the guardians of the soul of this congress.

We hope that our scientific exchanges will be fertile and that the Mediterranean environment of the Lazaret domain will be the perfect setting to strengthen and promote new collaborations within our network.

Enjoy the meeting!

Perrine and Pietro

Presidents of the 33rd Ion Channels Meeting

PROGRAM

Sunday, September 8th 2024

16:00 – 19:00 Welcome of the meeting attendees

19:00 ***Welcome drink and Dinner***

21:00 Plenary lecture: Annette Dolphin (UK)
"Drugs and trafficking: lessons from calcium channels"

Monday, September 9th 2024

08:15 Opening session and 1-minute oral presentation for sponsors

08:30 Symposium 1: Techniques for studying ion channels and their environment
Organized by Jérôme Thireau (Montpellier, France)

Laetitia Mony (France)
"Combined approaches to reveal surprising role of ionic channels in the brain"

Scott Hansen (USA)
"Techniques to study membrane mediated mechanisms of disease. Role of lipids"

Lucie Bergdoll (France)
"Lipid composition of the membrane governs the oligomeric organization of VDAC1"

Selected speaker: Eleonora Centonze (Switzerland)
"Probing protonation-driven conformational changes in the ASIC1a β-turn domain through fluorescence measurements"

10:15 ***Coffee break***

10:40 Oral communication session 1: Ion Channels in Neuronal Function: From Molecular Mechanisms to Therapeutic Innovation
Organized by Cécile Hilaire (Montpellier, France)

Youssef Issa (France)
"Identification of specific molecular markers of ALS vulnerable motoneurons"

Laurent Ferron (Canada):
"Presynaptic remodeling of voltage-gated Ca^{2+} channels in central terminals of Trpv1 nociceptors during chronic pain"

Yousra El Ghaleb (Austria):
"Disease variants in the activation gate and VSDs of T-type calcium channel Cav3.3 linked to neurodevelopmental disorders and epilepsy"

Leos Cmarko (France):
"Pharmacological screen of new therapeutically relevant calcium channel modulators"

11:40 1-minute oral presentation for posters (even numbers)

12:30 Lunch

14:00 Poster session 1

15:45 Coffee break

16:00 Symposium 2: "Ion channels and cancer"
Organized by Loïc Lemonnier (Lille, France)

Sébastien Roger (France):
"Role of P2X4 receptor in aggressive properties of mammary cancer cells: invasiveness, autophagy and release of extracellular vesicles"

Barbara Ehrlich (USA):
"From Basic Science to Clinical Trials: Lessons from Chemotherapy"

Rainer Schindl (Austria):
"Structural analysis of patho-physiological STIM1 mutants"

Selected speaker: **Mathilde Fourgeaud (France):**
"Impact of TRPM7 channel on pancreatic stellate cell activation induced by cadmium exposure."

17:45 - 18:45 Oral communication session 2: "From toxicity to therapeutical involvement of ion channels "
Organized by Alban Girault (Amiens, France)

Sara Kaaki (France):
"Comparative effects of neonicotinoids and newly introduced insecticides on human α7 and mammalian α4β2 neuronal nicotinic acetylcholine receptors"

Chloë Radji (France) :
"How does benzalkonium chloride affect ion and water channels in human corneal epithelial cells?"

Marc Bohnet (Switzerland):
"Impact of calcium on the pain-sensing ion channel hasic3"

Romain BAUDAT (France)
"Mambalgin-ASIC1a, a novel complex to detect non-small cell lung cancer"

18:45 - 19:15 Sponsors presentations

19:30 Apéritif dinatoire Sétois

Tuesday, September 10th 2024

08:30 Symposium 3: "Channelopathies at preclinical - modeling channelopathies"
Organized by Adèle Faucherre (Montpellier, France)

Dominique Glauser (Switzerland)
"Computer-assisted behavioral phenotyping in C. elegans as a tool to study channel function and dysfunction"

Carol Ann Remme (the Netherlands):
"SCN5A channelopathies: arrhythmia, cardiomyopathy, and beyond the cardiomyocyte"

Nadine Ortner (Austria):
"Cav1.3 variants: from molecular mechanisms to therapeutic approaches"

Selected speaker: Shi-Bing Yang (Taiwan):
"Beyond Membrane Excitability: Gain-of-function KATP Channel Mutant Causes Permanent Neonatal Diabetes via Suppressing Beta Cell Proliferation"

10:15 Coffee break

10:30 - 11: 00 1-minute oral presentation for posters (odd numbers)

11:00 - 12:30 Poster Session 2

12:30 Lunch

13:30 Appointment for Social event

17:15 Symposium 4: "Channelopathies at molecular - from structure to pharmacology"
Organized by Philippe Lory (Montpellier, France)

Barbara Ribeiro (France):
"Functional characterization of KCNH2 genetic variants, encoding hERG potassium channel, as

a clinically-relevant information for type 2 LQTS syndrome"

Henry Colecraft (USA):
"Hacking the ubiquitin code to control ion channel expression for therapy"

Jean-François Desaphy (Italia):
"Mutation-driven precision medicine in non-dystrophic myotonias"

Selected speaker: Lamia Goual (France):
"Modelling the neurocardiac junction in Long QT Syndrome type 2"

19:00 Annual meeting of the Association

20:00 Dinner

22:00 Evening Party
Jean-Yves Le Guennec (France): "Bloody numbers"

Wednesday, September 11th 2024

09:30 Symposium 5: "Glutamate receptors: trafficking, turnover, plasticity, agonists"
Organized by Vincent Seutin (Liège, Belgium)

Aude Panatier (France):
"Astrocytic EphB3 receptors control NMDAR functions and memory

Mark Farrant (UK):
"AMPA receptor function and dysfunction: the role GluA2 subunits"

Nelson Rebola (France):
"Contribution of NMDA receptors to the functional diversity of neocortical interneurons"

Selected speaker: Sofian Ringlet (Belgium):
"Co-agonist glycine controls the occurrence of bursts by activating extrasynaptic NMDARs in nigral dopamine neurons**"**

11:15 Coffee break

11:40 Prizes and Meeting closure

12:00 Lunch

14:00 Airport shuttle departure

SYMPOSIA AND ORAL COMMUNICATION ABSTRACTS

Sunday, September 10th 2024

DRUGS AND TRAFFICKING: LESSONS FROM CALCIUM CHANNELS

Annette C Dolphin
University College London (UCL), UK

N-type voltage-gated calcium channels ($Ca_V2.2$) and their auxiliary subunits $\alpha_2\delta$-1 and β are important for presynaptic neurotransmitter release. We have particularly studied their role in primary afferent terminals in the pain pathway. The $\alpha_2\delta$ subunits increase trafficking and modulate the functional properties of calcium channels. I will discuss the mechanisms of action of $\alpha_2\delta$ proteins in this process. Although changes in $Ca_V2.2$ trafficking and function generally go hand-in-hand [1], we have observed this is not always the case, for example if $\alpha_2\delta$ pre-proteins are not proteolytically cleaved to form the mature $\alpha_2\delta$ protein, and for certain splice variant combinations of $Ca_V2.2$ [2]

Furthermore, $\alpha_2\delta$-1 is a drug target in the treatment of neuropathic pain [3], being the therapeutic binding site for gabapentinoid drugs. We have identified a key mechanism of action; by binding to $\alpha_2\delta$-1 (and $\alpha_2\delta$-2), they induce a reduction in forward trafficking to the cell surface of both $\alpha_2\delta$ and the Ca_V2 complex containing $\alpha_2\delta$ [4]. In vivo, pregabalin inhibits $\alpha_2\delta$-1 trafficking in the pain pathway [5]. Furthermore, the $\alpha_2\delta$-1 increase that occurs following neuropathic injury promotes $Ca_V2.2$ trafficking in specific neurons in the dorsal horn [6].

Interestingly, the binding of gabapentinoid drugs to $\alpha_2\delta$-1 can be disrupted by a mutation [R241A in $\alpha_2\delta$-1], which is part of a universal amino acid binding pocket, that is conserved in bacterial chemoreceptors, and into which gabapentinoid drugs bind [7]. The physiological role of the amino acid binding site in $\alpha_2\delta$ proteins is still unclear, but since the R to A mutations reduce $\alpha_2\delta$-1/2 function as calcium channel subunits; binding of endogenous amino acids may be important for their optimal function, possibly occupying the binding site as positive modulators. In contrast, gabapentinoids appear to be negative regulators of $\alpha_2\delta$ function.

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Monday, September 9th 2024

8h30: Symposium 1: Techniques for studying ion channels and their environment

Organized by Jérôme Thireau (Montpellier, France)

COMBINED APPROACHES TO REVEAL SURPRISING ROLE OF IONIC CHANNELS IN THE BRAIN

Laura Piot[1]; Christina Heroven[2]; Simon Bossi[1]; Joseph Zamith[1]; Doris Wennagel[1]; Tomas Malinauskas[3]; Cécile Charrier[1]; Radu Aricescu[2]; **Laetitia Mony** [1]; Pierre Paoletti[1];
1: Institut de Biologie de l'ENS (IBENS) ; Ecole Normale Supérieure, Université PSL, CNRS, INSERM, F-75005 Paris, France
2: MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge, CB2 0QH, United Kingdom
3: Division of Structural Biology, Wellcome Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford, OX3 7BN, UK

Fast synaptic neurotransmission in the vertebrate central nervous system relies primarily on ionotropic glutamate receptors (iGluRs), which drive neuronal excitation, and type A γ-aminobutyric acid receptors (GABAARs), responsible for neuronal inhibition. However, the GluD1 receptor, an iGluR family member, is present at both excitatory and inhibitory synapses. Whether and how GluD1 activation may impact inhibitory neurotransmission is unknown.
GluD1 receptors are part of the GluD receptor family that displays atypical features compared to "classical" iGluRs (Yuzaki & Aricescu, Trends Neurosci 2017). These receptors indeed bind D-serine and glycine instead of glutamate. Due to their lack of ionotropic activity following agonist binding, GluD1 receptors have so far escaped thorough pharmacological and biophysical characterization. To investigate GluD1 function in vitro, we developed two approaches based on (i) mutation of GluD1 pore to create constitutively open channels ("Lurcher" mutant), as was previously done for GluD2 receptors (Wollmuth et al., J. Neurosci. 2000; Naur et al., PNAS 2007); and (ii) voltage-clamp fluorometry, as fluorescence-based method allowing real-time monitoring of receptor conformational changes (Mannuzzu et al., Neuron 1996; Cha & Bezanilla, J. Gen. Physiol. 1998). By combining these functional approaches with biochemical and structural analyses on isolated GluD1 ligand-binding domains, we show that GluD1 binds the inhibitory neurotransmitter GABA, an unprecedented feature for iGluRs.
We furthermore show that GluD1 activation produces long-lasting enhancement of GABAergic synaptic currents in the adult mouse hippocampus through a non-ionotropic mechanism requiring GABA (but not D-serine) binding as well as trans-synaptic anchoring. Thanks to a combination of approaches ranging from electrophysiology, receptor engineering, fluorescence and biochemistry, we therefore uncovered a new form of inhibitory synaptic plasticity. In addition, the identification of GluD1 as a GABA receptor that controls inhibitory synaptic plasticity challenges the classical dichotomy between glutamatergic and GABAergic receptors.

TECHNIQUES TO STUDY MEMBRANE MEDIATED MECHANISMS OF DISEASE: ROLE OF LIPIDS

Scott Hansen
Department of Molecular Medicine, University of Florida

Ion channels are embedded within lipid membranes, and their activation and function are critically influenced by their surrounding lipid environment. However, the mechanisms by which lipids regulate ion channels in cellular membranes remain incompletely understood. A comprehensive understanding of the lipid environment is essential for effectively reconstituting channels in vitro and stabilizing them in specific states for structural studies. At the nanoscale, cell membranes exhibit compartmentalization, with regions composed of cholesterol-dependent gangliosides (GM1) forming thick domains, and cholesterol-independent polyunsaturated lipids forming thin domains. Our research demonstrates that ion channels transit between these compartments, with their functional states driven by the surrounding lipid environment.
In this presentation, I will introduce a super-resolution imaging technique that utilizes fluorescently labeled lipids to track the movement of a GFP-tagged ion channel (TREK-1) and immunolabeled GABA_A receptors (GABA_AR) between lipid compartments in response to various stimuli. Our findings reveal that polyunsaturated fatty acids (PUFAs) and shear forces displace TREK-1 channels from thick membrane domains, prompting their migration to thin membranes where they are activated by both exposure to PIP2 clusters and the thin membrane environment. Similarly, the application of the neurotransmitter GABA to GABA_ARs induces channel dissociation from ordered lipids, resulting in their movement to PIP2 clusters, which correlates with state-dependent changes in current and desensitization. In neurons, the movement of ion channels is regulated by astrocyte-derived cholesterol. This cholesterol is packaged into the lipid transport protein apolipoprotein E (apoE), which is then taken up by neurons to modulate the ion channels' nanoscopic environment. We have found that the application of exogenous apoE serves as an effective receptor-mediated tool for regulating the lipid environment of ion channels in cell culture. Loading neurons with cholesterol via apoE recapitulates an inflammatory state and induces hyperexcitability.

LIPID COMPOSITION OF THE MEMBRANE GOVERNS THE OLIGOMERIC ORGANIZATION OF VDAC1

Elodie Lafargue[1]; Jean-Pierre Duneau[2]; Varun Ravishankar[2]; Nicolas Buzhinsky[1]; Ignacio Casuso[1]; James Sturgis[2]; **Lucie Bergdoll**[2];
1: U1325 DyNaMo INSERM, Aix-Marseille Université, Turing Centre for Living System, Marseille, France
2: Laboratoire d'ingénierie des Système Macromoléculaires, LISM, University of Aix-Marseille, CNRS, UMR7255, Marseille, France.

Mitochondrial physiology is intricately linked to the oligomerization of voltage-dependent anion channels (VDAC), acting as gatekeepers to mitochondria. However, the molecular determinants of VDAC oligomerization remain poorly understood. In this study, we used atomic force microscopy to investigate the effects of three lipids of the Mitochondrial Outer

Membrane (MOM) on VDAC assemblies. We observed that VDAC forms lipid-sensitive clusters, termed honeycombs, and their compaction is regulated by cholesterol. Molecular dynamics simulations revealed VDAC's affinity for cholesterol and phosphatidylethanolamine, both of which affect the formation of these honeycombs. Although we identified honeycomb-like assemblies akin to those in the native MOM, deviations from the physiological lipid composition resulted in varying degrees of disruption of these native-like structures. This emphasizes the profound impact of the lipid environment on VDAC organization. These findings underscore the physiological significance of lipid heterogeneity and changes within biological membranes arising from membrane contacts or pathologies in modulating VDAC behavior.

PROBING PROTONATION-DRIVEN CONFORMATIONAL CHANGES IN THE ASIC1A B-TURN DOMAIN THROUGH FLUORESCENCE MEASUREMENTS

Eleonora Centonze; Stephan Kellenberger;
Université de Lausanne, Department of Biomedical Sciences

Acid-sensing ion channels (ASICs) are crucial components of the nervous system, playing pivotal roles in processes like learning, fear behaviors, pain sensation, and neurodegeneration following stroke. These voltage-independent Na+-selective channels are activated by extracellular acidification, leading to transient inward currents before entering a non-conducting desensitized state. Functional ASICs are trimers, comprised of three subunits, featuring a hand-like extracellular structure. The mechanisms of protonation leading to ASIC activation remain unclear. Protonation occurs at extracellular sites, yet induces conformational changes that control gate position, causing it to open or close. Understanding these alterations is crucial for comprehending ASIC function. ASIC1a, highly expressed in the central nervous system, is a promising drug target due to its involvement in various pathologies, highlighting the importance of understanding the molecular mechanisms driving its activation. Using voltage-clamp fluorometry (VCF), we investigate the involvement of amino acid residues within the β-turn in hASIC1a activation. This region connecting palm and thumb domains interacts with transmembrane domain TM1 and potentially influences channel gating. Our findings reveal conformational changes near the β-turn region, suggesting that protonation events may drive these alterations. An analysis of fluorescence signal kinetics was performed to corroborate the association between fluorescence changes and functional transitions, revealing potential conformational rearrangement sequences within ASIC1a's β-turn domain. Investigating activity-dependent conformational changes offers valuable insights into how protonation regulates ASIC activity, deepening our understanding of these channels.

10h40: Oral communication session 1 : Ion Channels in Neuronal Function: From Molecular Mechanisms to Therapeutic Innovation

Organized by Cécile Hilaire (Montpellier, France)

IDENTIFICATION OF SPECIFIC MOLECULAR MARKERS OF ALS VULNERABLE MOTONEURONS

Youssef Issa; Sara Marmolejo-Martínez-Artesero; Cédric Raoul; Frédérique Scamps; Cécile Hilaire;
The Institute for Neurosciences of Montpellier, INM, INSERM UMR1298, University of Montpellier, Montpellier, France

Modification of motoneurons electrical activity is a key factor in amyotrophic lateral sclerosis (ALS) disease progression. Experimental evidence has revealed a motoneuron-type vulnerability in ALS, beginning with the low excitability fast fatigable (FF) motoneurons, while the high excitability slow (S) motoneurons are preserved. These observations have led to the hypothesis that the high task demand of the FF motoneurons is responsible for their greatest vulnerability. To broaden our understanding of the role of excitability in selective degeneration and to improve the functional characterization of motoneurons types, we used the patch-seq method on FF and S motoneurons subtypes identified by patch-clamp electrophysiology.
The expression of voltage-gated channels was analyzed in six FF motoneuron RNA banks and six S motoneuron RNA banks. Cacna2d3, a gene encoding CaVα2δ3, a regulatory subunit of high voltage activated calcium channels, was significantly increased in the FF motoneurons. Due to its high expression in ALS vulnerable motoneurons, we are currently investigating its role in motoneuron physiology and under ALS pathological conditions.
In Cacna2d3-/- motoneurons, we show a drastic change in the subcellular localization of the P/Q type calcium channel CaV2.1, the major channel involved in spinal neurotransmission, suggesting a regulation of its function by the CaVα2δ3 subunit. Consistent with this results, behavioral studies show an increased endurance to locomotor tasks in the knock-out mice. The functional significance of Cacna2d3 in neurotransmission and firing properties of motoneurons will next be investigated by electrophysiological studies, as well as its impact on ALS progression by crossing the knock-out mice with a SOD1G93A mouse line.

PRESYNAPTIC REMODELING OF VOLTAGE-GATED CA2+ CHANNELS IN CENTRAL TERMINALS OF TRPV1 NOCICEPTORS DURING CHRONIC PAIN

Laurent Ferron; Erika K. Harding; Maria A. Gandini; Craig Brideau; Peter K. Stys; Gerald W. Zamponi;
University of Calgary - Department of Clinical Neurosciences, Hotchkiss Brain Institute, Calgary Cumming School of Medicine

Neuronal voltage-gated Ca2+ channels are the main source of Ca2+ influx into presynaptic neuronal compartments and are critical for neuronal excitability and synaptic transmission. In chronic pain context, the expression of some of these Ca2+ channels is altered in the soma of

primary sensory neurons. However, evidence that this somatic alteration has functional repercussions on the presynaptic terminals of primary sensory neurons in intact spinal cord is still lacking. In this study, we combined genetic tools with multi-photon Ca2+ imaging to explore the functional remodeling that occurs in central presynaptic terminals of dorsal root ganglion neurons during neuropathic pain. We crossed a Trpv1-cre mouse line with a cre-dependent GCaMP6s mouse line to express the genetically encoded Ca2+ indicator in nociceptors and monitored GCaMP6s fluorescence responses in an ex vivo spinal cord preparation. We show an increase in the Ca2+ transient amplitude in Trpv1 expressing DRG neuron central terminals following spared nerve injury. We also show that this increase is mediated by both N- and P/Q-type Ca2+ channels. We finally demonstrate that GABA B receptor-dependent inhibition of Ca2+ transient is potentiated in the superficial layer of the dorsal horn. Our results point towards a functional remodeling of presynaptic voltage-gated Ca2+ channels in Trpv1 expressing nociceptors in neuropathic pain conditions.

DISEASE VARIANTS IN THE ACTIVATION GATE AND VSDs OF T-TYPE CALCIUM CHANNEL CAV3.3 LINKED TO NEURODEVELOPMENTAL DISORDERS AND EPILEPSY

Yousra El Ghaleb[1]; Monica L. Fernandez-Quintero[2]; Petronel Tuluc[3]; Marta Campiglio[1]; Kerstin Kutsche[4]; Bernhard E. Flucher[1];
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4: University Medical Center Hamburg-Eppendorf, Institute of Human Genetics

The voltage-gated calcium channel family consists of ten isoforms, divided in high-voltage-activated and low-voltage-activated channels. CaV3.1-CaV3.3 are low-voltage-activated channels, also known as T-type channels. These channels activate and inactivate at potentials close to the resting membrane potential of neurons, making them perfectly equipped to regulate cellular excitability. Previously we identified the gene for CaV3.3 (CACNA1I) as a disease gene for neurodevelopmental disorder and epilepsy. Using a combination of structure modelling, site-directed mutagenesis, patch-clamp recording, and NEURON computer modelling, we characterized four gain-of-function disease variants located in the channel activation-gate. These variants show left-shifted voltage-dependence of activation and inactivation, and slower inactivation and deactivation kinetics, resulting in increased calcium influx during rest and activity. Our results indicated that these changes lead to hyper-excitability and calcium toxicity, underlying epilepsy and neurodevelopmental disorder in the patients. We are currently studying three new CACNA1I activation-gate variants linked to epilepsy and/or developmental disorders. Interestingly, two of these new variants represent different substitutions of the same amino acid residue, both leading to neurodevelopmental disorder. However, the two variants result in opposite effects on channel structure, one resulting in a gain and the other in a loss of channel function. Only the gain-of-function variant is linked to epilepsy. The propensity of the gain-of-function variants to generate epilepsy is supported by the increased excitability of neurons expressing this variant as predicted by the NEURON computer model, while the loss-of-function variant was predicted to decrease excitability compared to wild-type channels. For the first time, we also characterized two

putative disease variants that lead to a complete loss of channel function. Our preliminary data do not expose whether this results from a loss of membrane expression or a gating defect. Interestingly, these putative disease variants are the first identified in the voltage sensor domains of the CaV3.3 channel. Overall, our study of CaV3.3 disease variants reveals how substitutions in distant channel domains can lead to the same gating defects and similar disease phenotypes, while different substitutions of the same residue can result in opposite channel defects with a differential spectrum of neurological disease. *Funding: ESP-461-B to Y.E.G., P35618 to B.E.F.*

PHARMACOLOGICAL SCREEN OF NEW THERAPEUTICALLY RELEVANT CALCIUM CHANNEL MODULATORS

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Pharmacological modulation of T-type calcium channels (comprised by three distinct isoforms Cav3.1, Cav3.2 and Cav3.3) has become a promising avenue for the treatment of several neurological disorders such as chronic pain and epilepsy. However, the development of therapeutically relevant drugs has been hampered by the lack of T-type channel-selective modulators. In this work, we used automated patch-clamp electrophysiology as a high-throughput screening platform in order to screen for novel modulators of T-type calcium channels amongst a library of spider venom peptides. We screened a total of 30 spider venoms each separated into 60 primary sub-fractions by HPLC chromatography. The effects of these peptide fractions were tested on whole-cell T-type currents and based on the results, 'hit' fractions were subjected to a secondary separation. In a same fashion, these secondary fractions went through a round of testing and active peptide components from the effective fractions were isolated, purified and sequenced. This part of the work gave rise to three sequences of previously undescribed venom peptides with an inhibitory activity on T-type currents. Next, based on one of the resolved sequences, a synthetic peptide has been produced. In order to describe its complete pharmacological properties, we tested this peptide on both recombinant T-type channels as well as T-type and other channels in primary DRG neurons using both automated and manual patch-clamp electrophysiology. Altogether, we show that high-throughput electrophysiological screening is a viable approach for investigating new modulators of T-type calcium channels. As a result, we describe a novel peptide toxin which acts as an inhibitor of T-type channels with an IC50 < 100 nM.

16:00 Symposium 2: "Ion channels and cancer"

Organized by Loïc Lemonnier (Lille, France)

ROLE OF THE P2X4 RECEPTOR IN AGGRESSIVE PROPERTIES OF MAMMARY CANCER CELLS: INVASIVENESS, AUTOPHAGY AND RELEASE OF EXTRACELLULAR VESICLES

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3: School of Life Sciences, University of Sussex, Brighton, United Kinkdom

The P2X4 receptor belongs to the family of transmembrane ionotropic P2X receptors. While it acts as an ATP-gated non-selective cation channel, like other members of the P2X family, P2X4 differs from the other subtypes by its predominant localization in endosomal and lysosomal organelles of cells, rather than to the plasma membrane. We showed that the P2X4 receptor is significantly overexpressed in human breast cancer samples, compared to normal tissues. P2X4 was mainly targeted to acidic compartments of highly invasive mammary cancer cells and sustained invasive properties of cancer cells in vitro as well as mammary tumour growth and metastatic progression in vivo, in a syngeneic model of mammary cancer in immunocompetent mice. The pro-malignant roles of P2X4 were mediated by 1) the regulation of lysosome acidity and its fusion to the plasma membrane thus releasing lysosomal enzymes participating to extracellular matrix proteolysis, but also 2) in the promotion of autophagy and cell survival under metabolic challenges (hypoxia and nutrients deprivation). Moreover, the P2X4-dependent regulation of autophagic activity was associated with epithelial–to-mesenchymal transition (EMT). Pharmacological and gene silencing of P2X4 inhibited both autophagy and EMT, whereas its rescue in knocked-down cells led to the restoration of the aggressive phenotype. Furthermore, 3) we recently identified that P2X4 controls the release of extracellular vesicles (EVs) of nanometer size, this way modulating the invasive properties of recipient cancer cells. Collectively, our results provide compelling evidence supporting that P2X4 has a critical role in driving cancer cell aggressiveness in cells expressing it, but also through the communication between cancer cells. We propose these functions to be dependent on the role of P2X4 in regulating lysosomal functions and fate.

FROM BASIC SCIENCE TO CLINICAL TRIALS: LESSONS FROM CHEMOTHERAPY

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Alterations in calcium signaling are a major mechanism of chemotherapy-induced peripheral neuropathy (CIPN), a side effect of several chemotherapy regimens. Strategies to prevent off-target toxicities of these treatments has focused on the interaction between the inositol trisphosphate receptor (InsP3R) and Neuronal Calcium Sensor-1 (NCS-1). The InsP3R is a

calcium permeable channel in the membrane of the endoplasmic reticulum (ER). NCS1 is a highly conserved calcium-binding protein that helps maintain intracellular calcium homeostasis and regulates calcium-dependent signaling pathways. One of the ways that NCS1 exerts its effect of regulating calcium homeostasis is through a functional interaction with the InsP3R, enhancing the open probability of the InsP3R. NCS1 also is an off-target binding protein for chemotherapeutic agents that alter microtubule assemblies to kill cancer cells. It is this binding that leads to off-target toxicities. The addition of the chemotherapeutic drug and binding to NCS1 leads to increased calcium release from intracellular stores through the InsP3R. The enhanced calcium release activates calpain, a calcium activated protease, which initiates a neurodegenerative environment resulting in chemotherapy-induced peripheral neuropathy (CIPN) and cognitive impairment (CICI), or “chemo brain”. There are no approved, disease-modifying treatments for CIPN or CICI. We found compounds and peptides that prevent these chemotherapy-induced neuronal changes in cells and in mice. Our goal is to prevent the off-target effect of chemotherapy to protect nerve function in humans.

STRUCTURAL ANALYSIS OF PATHO-PHYSIOLOGICAL STIM1 MUTANTS

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The single-pass transmembrane protein Stromal Interaction Molecule 1 (STIM1), located in the endoplasmic reticulum (ER) membrane, has two primary functions: sensing ER-Ca2+ concentration and directly binding to the store-operated Ca2+ channel Orai1 to activate it when Ca2+ levels decrease. When the ER-Ca2+ concentration is high, the ER-luminal STIM1 domain remains monomeric. However, once Ca2+ stores are depleted, STIM1 undergoes di-/multimerization. This multimerization is crucial for exposing the C-terminal binding site of STIM1, which interacts with Orai1 channels. Mutations that destabilize the resting configuration of STIM1 can lead to tubular aggregate myopathy. Despite its importance, the structural basis of the luminal association sites has been elusive.
Here, we present our findings using a combined approach of molecular dynamics (MD) simulations, purified proteins, and live cell techniques. We identified two critical di-/multimerization segments: the α7 helix and the adjacent region near the α9 helix in the sterile alpha motif (SAM) domain. I will discuss how interactions between the SAM domains of STIM1 monomers are vital for the protein's multimerization and activation.

IMPACT OF TRPM7 CHANNEL ON PANCREATIC STELLATE CELL ACTIVATION INDUCED BY CADMIUM EXPOSURE.

Mathilde Fourgeaud[1]; Julie Auwercx[1]; Alexis Lalot[1]; Sylvie Brassart-Pasco[2]; Frédéric Hague[1]; Stéphanie Guénin[3]; Laurent Guttierez[3]; Nicolas Jonckheere[4]; Bertrand Brassart[2]; Mathieu Gautier[1];

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Introduction: Pancreatic stellate cells (PSCs) are quiescent in normal tissue but activates under stress conditions. Activated PSCs triggered desmoplasia which is a key feature of pancreatitis and pancreatic ductal adenocarcinoma (PDAC) whose incidence is increasing worldwide. Cadmium (Cd) is a pollutant present in the environment. Recent studies show a Cd contamination increased in humans and suggest a link with several cancers like PDAC. We previously showed that Cd exposure induced pancreatic epithelial cell transformation through Transient Receptor Potential Cation Channel Subfamily M Member 7 (TRPM7) channel expression which is a biomarker of PSC activation. We aim to characterize the effects of Cd exposure and the role of TRPM7 channels on human PSC activation.
Methods: PSCs were chronically exposed to 1µM CdCl2 during 30 weeks. Quiescent and activation marker expression were assessed by RT-qPCR and immunofluorescence, cell viability by MTT assays, cell migration in Boyden chambers, and protease secretions by zymography. PSC stimulation of pancreatic cancer cell (PCC) migration was assessed by using a model of PSC/PCC coculture. TRPM7 expression and activity were studied by RT-qPCR, western-blot, whole-cell patch-clamp and manganese quenching. Finally, TRPM7 involvement in PSC activation was studied by using pharmacological blocker (NS859, 25µM) or small interfering RNA (siRNA).
Results: Cd exposure induced a decrease of the glial fibrillary acidic protein (GFAP) quiescent marker expression and a loss of lipid droplets storing vitamin A. We observed also an increase of alpha smooth muscle actin (αSMA) staining organized into stress fibers. Cell viability was unchanged but migration was enhanced as well as protease secretions (MMP2 and Plasminogen Activator (uPA)) in Cd-exposed PSCs. All together, these results showed that Cd exposure transformed PSCs into a myofibroblastic phenotype. Moreover, Cd-exposed PSCs stimulated PCC migration to a greater extent than controls. We also noticed an increase of constitutive cation entries and an increase of both TRPM7 expression and activity. Finally, TRPM7 inhibition prevented PSC migration in control and Cd-exposed cells as well as PCC migration stimulated by PSC coculture.
Conclusion: In this study, we show that Cd exposure activates PSCs and induces a myofibroblastic phenotype. Moreover, we identify TRPM7 channels as important actors for PSC activation induced by Cd exposure.

17:45 - 18:45 Oral communication session 2: “From toxicity to therapeutical involvement of ion channels “

Organized by Alban Girault (Amiens, France)

COMPARATIVE EFFECTS OF NEONICOTINOIDS AND NEWLY INTRODUCED INSECTICIDES ON HUMAN A7 AND MAMMALIAN A4B2 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

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Neonicotinoid insecticides, widely recognized for their effectiveness in pest control, have been banned in the European Union due to concerns over their potential adverse effects on the environment and human health. Understanding the binding properties and pharmacological impacts of neonicotinoids and their derivatives on mammalian nicotinic acetylcholine receptors (nAChRs) remains crucial for developing safer and more targeted pest control strategies.
This study aims to compare the effects of two commonly used neonicotinoids, imidacloprid (IMI) and clothianidin (CLT), with the newly introduced pesticides sulfoximines (sulfoxaflor, SFX) and butenolides (flupyradifurone, FLU). Through electrophysiological recordings and site-directed mutagenesis, we investigated how these insecticides interact with human α7 and the two stoichiometries of the rat α4β2 neuronal nicotinic acetylcholine receptors (α4)3(β2)2 and (α4)2(β2)3.
Our results indicate that the tested insecticides act as weak agonists on the wild-type α7 and (α4)3(β2)2 receptors, and do not activate the (α4)2(β2)3 stoichiometry. Additionally, three mutations in the α7 receptor (E211N, E211P, and Q79K), which influence neonicotinoid binding, enhanced the effects of IMI, CLT, and FLU. Furthermore, the E226P mutation in the α4 subunit and the L273T mutation in the β2 subunit of the (α4)3(β2)2 receptor increased the effects induced by IMI, CLT, SFX, and FLU. Interestingly, both mutations were able restore the insecticide-induced responses in the (α4)2(β2)3 stoichiometry.
We succeeded in identifying residues involved in the binding characteristics of these insecticides to the receptors. These findings provide insights that could lead to the development of more selective and safer compounds.

HOW DOES BENZALKONIUM CHLORIDE AFFECT ION AND WATER CHANNELS IN HUMAN CORNEAL EPITHELIAL CELLS?

Chloë Radji [1]; Christine Barrault[1]; Léonie Briand[1]; Roxanne Flausse[2]; Nicolas Leveziel[2]; Catherine Bur[3]; Gaëtan Terrasse[3]; Anne Cantereau[4]; Frédéric Becq[1];
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The impact of eye drops and of their preservatives such as Benzalkonium chloride (BAK), on cells involved in tear film homeostasis remains insufficiently documented. In this study, we developed primary cultures of human corneal epithelial cell (hCEC) to identify ion and water channels, and investigated the impact of artificial tears and their constituents on transmembrane fluxes.
We obtained, isolated and cultured hCEC from 3 donors. Transmembrane ion fluxes were characterized by Ussing chambers to record short-circuit current (Isc). To study water transport, we recorded the optical pathway difference (OPD) by the quadriwave lateral shearing interferometry (QWLSI) method. We also evaluate the inflammatory response and cell death with Multiplex and MTT assays.
We identified an amiloride (100 µM)-sensitive ENaC Isc and a Chromanol 293B (100 µM)-sensitive KCNQ1 Isc. We recorded a CFTR-dependent Isc stimulated by forskolin (10µM) and by the CFTR potentiator Vx770 (10µM) and inhibited by the selective CFTR inhibitor CFTRinh172 (10µM). Finally, UTP (100 µM) stimulated a Ca2+-dependent Cl- Isc inhibited by the selective TMEM16a inhibitor Ani9 (10µM). All these responses were reduced after topical pretreatments with BAK and BAK-containing eye drops. We identified a forskolin-stimulated, HgCl2-inhibited, and BAK-inhibited concentration-dependent water flux. BAK and BAK-containing eye drops induce cell death without producing inflammatory cytokines such as IL-6 or IL-8.
To conclude, we developed novel hCEC models and identified ENaC, KCNQ1, CFTR and TMEM16a ion channels as well as a mercury-sensitive, cAMP-dependent water transport. We also found that BAK altered not only the Isc but also the water flux into the extracellular environment, not due to an inflammatory response.

IMPACT OF CALCIUM ON THE PAIN-SENSING ION CHANNEL hASIC3

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Acid-sensing ion channels (ASICs) are proton-gated sodium channels, distributed throughout the nervous system. In mammals, ASICs are encoded by 4 genes that splice into 8 isoforms. Among the different ASICs isoforms, ASIC3 is the most promising candidate for acidosis-induced pain, as it is predominantly expressed in the dorsal root ganglia. Furthermore, several animal studies have linked it to pain and inflammation and proposed it as a potential analgesic target. However most of the studies until now were performed with the rat isoform of ASIC3, which shares only 84% of protein similarity with the human one. Therefore, it is of particular interest to confirm those findings on the human isoform. In this project, the effect of calcium, a known ASIC modulator, is characterized on human ASIC3. To do so, measurements using two-electrode voltage-clamp on Xenopus oocytes and patch-clamp recordings on transfected cos7 cells were performed at different pH and various calcium concentrations.
The results obtained show that similarly to rat ASIC3, calcium has a regulatory effect on human ASIC3, shifting its activation and desensitization curve toward more acidic values. However, we observed distinct differences in the regulation of calcium between the two protein isoforms. These differences were particularly noticeable in terms of calcium potency and its

dependence on pH. Taken together, this improved understanding of human ASIC3 regulation could lead to more effective therapeutic strategies for targeting this pain-related ion channel.

MAMBALGIN-ASIC1a, A NOVEL COMPLEX TO DETECT NON-SMALL CELL LUNG CANCER

Romain BAUDAT [1]; Evelyne BENOIT[1]; Pascal KESSLER[1]; Benoit JEGO[2]; Mathilde KECK[1]; Vincent THOMAS DE MONTPREVILLE[3]; Charles TRUILLET[2]; Denis SERVENT[1];
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3: Service d'anatomie et de cytologie pathologiques, Hôpital Marie-Lannelongue, Le Plessis-Robinson

Non-small cell lung cancer (NSCLC) is a deadliest form of cancer detected in only 16% of cases at early stage. While current imaging approaches, such as radiography, computed tomography (CT) scans, and positron emission tomography (PET) scans, are commonly used for the anatomical characterization of lung cancer, these approaches have some limitations. Therefore, there is a critical need for the development of new imaging probes that are more specific to molecular targets for earlier diagnosis. Tumor progression is associated with various processes, notably the acidification of the microenvironment. This acidic environment disrupts the mechanisms controlling ion homeostasis, including pH-sensitive channels such as acid-sensing ion channels (ASICs) that are overexpressed and involved in tumor invasion, progression and metastasis. Our results show that the gene expression of ASIC1a was higher in the A549 lung cancer cell line compared to the BEAS-2B lung healthy cell line. Moreover, in 15 out of 24 patients with NSCLC, a genetic overexpression of ASIC1a was detected in tumor tissue compared to the surrounding one. This genetic overexpression occurred increasingly as the stage of NSCLC was higher. Therefore, we performed the chemical synthesis of Mambalgin-1 (Mamb-1), a peptide from the venom of Dendroaspis polylepis snake known to have high affinity for ASIC1a, that was then labeled with various fluorescent or radioactive probes using "click" chemistry. Electrophysiological studies showed that the affinity for ASIC1a of the chemically synthesized Mamb-1 labeled with cyanine 5.5 (Cy-5.5) was unchanged as compared to unlabeled toxin. In addition, quantitative microscopy imaging of biopsies from the 24 patients with NSCLC, labeled with Mamb-1-Cy5.5, revealed a significant fluorescence of the tumoral tissue while no labeling was detected from the surrounding tissue. The specificity of the Mamb-1-Cy5.5 labeling on the tumoral tissue was confirmed by its abolition following the addition of unlabeled Mamb-1 in excess. Altogether, these results strongly suggest that fluorescent or radioactive Mamb-1could be exploited as a promising tool to detect the increased expression of ASIC1a in NSCLC at different stages.

Tuesday, September 10th 2024

08:30 Symposium 3: “Channelopathies at preclinical - modeling channelopathies”

Organized by Adèle Faucherre (Montpellier, France)

COMPUTER-ASSISTED BEHAVIORAL PHENOTYPING IN C. ELEGANS AS A TOOL TO STUDY CHANNEL FUNCTION AND DYSFUNCTION

Dominique A. Glauser,
Dept. of Biology, University of Fribourg, Switzerland

Using mammalian models to study channel function in vivo and to model human channelopathies is often tedious, costly, and constrained by ethical concerns. The nematode C. elegans represents a complementary model with a large conservation of channel genes and the availability of powerful genetic tools for in vivo studies. The combination of genetic manipulations affecting channel genes with high throughput and high-content computer-assisted behavioral phenotyping represents an efficient way to reveal the biological roles of candidate channels and indirectly assess their activity in a physiologically relevant setting. I will illustrate how the model is used to study the molecular mechanisms controlling thermo-nociceptive behaviors and nociceptive plasticity. Furthermore, I will discuss more general strategies for the generation of worm-based models to study channel function and dysfunction.

SCN5A CHANNELOPATHY: ARRHYTHMIA, CARDIOMYOPATHY, AND BEYOND THE CARDIOMYOCYTE

Carol Ann Remme,
the Netherlands

The cardiac sodium channel Nav1.5, encoded by the *SCN5A* gene, is responsible for the fast upstroke of the action potential. Mutations in *SCN5A* may cause sodium channel dysfunction by decreasing peak sodium current, which slows conduction and facilitates reentry-based arrhythmias, and by enhancing late sodium current, which prolongs the action potential and sets the stage for early afterdepolarization and arrhythmias. Research in the last decade has uncovered the complex nature of Nav1.5 distribution, function, in particular within distinct subcellular subdomains of cardiomyocytes. Nav1.5-based channels furthermore display previously unrecognized non-electrogenic actions and may impact on cardiac structural integrity, leading to cardiomyopathy. Moreover, *SCN5A* and Nav1.5 are expressed in cell types other than cardiomyocytes as well as various extracardiac tissues, where their functional role

in, e.g. epilepsy, gastrointestinal motility, cancer and the innate immune response is increasingly investigated. Recent work from our lab furthermore uncovered altered firing frequency of intracardiac neurons isolated from *Scn5a* mutant mice, highlighting a potential novel (pro-arrhythmic) mechanism in sodium channelopathy.

CAV1.3 VARIANTS IN NEURODEVELOPMENTAL DISEASE: FROM MOLECULAR MECHANISMS TO THERAPEUTIC APPROACHES

Nadine J. Ortner; Horia C. Hermenean; Ferenc Török; Tamara Theiner; Petronel Tuluc; Jörg Striessnig;
Department of Pharmacology and Toxicology, Institute of Pharmacy, University of Innsbruck, Innsbruck, Austria

13 de novo missense variants of CACNA1D (Cav1.3) were found in the germline of 21 patients with a neurodevelopmental phenotype ranging from rather mild to very severe (± endocrine features). They cluster around functionally important regions, are absent in healthy controls and over 80 similar and/or identical somatic variants have been found in adrenal lesions causing hyperaldosteronism and treatment-resistant hypertension.
Using whole-cell patch clamp recordings in transiently transfected tsA201-cells, we could confirm variant-specific, complex gating changes with combined gain- and loss-of-function features. These included – for almost all variants – a shift of the voltage-dependence of activation to more negative potentials with or without additional effects on channel inactivation and deactivation. For the A749G variant we found similar gating changes in native cells isolated from our construct-valid Cav1.3AG knock-in mouse model, as well as altered excitability of endocrine and neuronal cells. Furthermore, Cav1.3AG mice reconstituted several aspects of the human disorder in a gene-dose dependent manner, including reduced bodyweight, hyperlocomotion, mild endocrine features and a social deficit. Almost all variants had preserved or even enhanced sensitivity to the LTCC inhibitor isradipine in vitro, however, therapeutically relevant plasma levels upon oral administration did not attenuate the hyperlocomotion in Cav1.3AG mice.
Together, we confirmed the pathogenicity of gating-modifying CACNA1D variants and the feasibility of Cav1.3AG mice to study molecular mechanisms and therapeutic approaches. Despite the low number of so far identified patients, it becomes evident that the shift in the voltage-dependent activation towards negative voltages is a key determinant of the disease severity. *FWF (P35087, P35722, DOC178)*

BEYOND MEMBRANE EXCITABILITY: GAIN-OF-FUNCTION KATP CHANNEL MUTANT CAUSES PERMANENT NEONATAL DIABETES VIA SUPPRESSING BETA CELL PROLIFERATION

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2: Institute of Biomedical Sciences, Academia Sinica

Ion channels play crucial roles in setting cell membrane excitability, and ion channel mutations often lead to diseases known as “channelopathies.” These mutations disrupt normal membrane excitability, making cells either more or less excitable, which leads to various human diseases. Gain-of-function mutations in KATP channels often cause neonatal diabetes mellitus (NDM), which is traditionally thought to inhibit insulin secretion from pancreatic beta cells. While most NDM patients respond to sulfonylureas, an insulin secretagogue that blocks the KATP channel, a subset remains resistant to sulfonylurea treatment and relies on insulin therapy. The pathophysiological mechanism behind this sulfonylurea resistance remains elusive. In this study, we identified a gain-of-function KATP channel mutation in a Taiwanese NDM patient resistant to sulfonylurea treatment. To understand the underlying mechanism of this resistance, we developed a humanized mouse model carrying the same KATP channel mutation. Contrary to in vitro predictions and the patient’s resistance to sulfonylurea, the mutant mice responded to clinical concentrations of sulfonylurea. We further identified that this gain-of-function mutation suppresses beta-cell proliferation, leading to NDM. Histological analysis demonstrated a substantial reduction in beta-cell mass in insulin-treated mutant mice, whereas glibenclamide treatment preserved beta-cell mass. Our findings suggest that the gain-of-function KATP channel mutation suppresses neonatal beta-cell proliferation during the critical developmental period. During the neonatal period, pancreatic beta cells exhibit significant expansion ability, characterized by low functional KATP currents, high excitability, and resistance to low glucose silencing, which supports their high proliferation capacity. This hyperexcitability gradually diminishes as beta cells mature and regain glucose responsiveness after the first postnatal week. This suppression of beta-cell expansion, rather than beta-cell loss due to glucotoxicity, underlies the failure of some patients to transition from insulin to sulfonylureas. This study provides new insights into the pathophysiology of NDM and highlights the novel role of KATP channels in regulating beta-cell proliferation.

17:15 Symposium 4: “Channelopathies at molecular - from structure to pharmacology”

Organized by Philippe Lory (Montpellier, France)

FUNCTIONAL CHARACTERIZATION OF *KCNH2* GENETIC VARIANTS, ENCODING HERG POTASSIUM CHANNEL, AS A CLINICALLY-RELEVANT INFORMATION FOR TYPE 2 LQTS SYNDROME

Barbara Ribeiro Oliveira-Mendes[1], Malak Alameh[1], Jérôme Montnach[1], Sylvain Feliciangeli[2], Thomas Gabillon[2], Frank Chatelain[2], Nawel Aoujaj[1], Béatrice Ollivier[1], Floriane Bibault[1], Vincent Probst[3], Julien Barc[1], Florence Kyndt[3], Isabelle Baró[1], Jean-Jacques Schott[1], Isabelle Denjoy[4], Florian Lesage[2], Gildas Loussouarn[1], Michel De Waard[1]

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Long QT syndrome type 2 (LQTS2) is a potentially fatal cardiac arrhythmia disorder, often resulting from rare loss-of-function variations in the *KCNH2* gene, encoding the hERG cardiac channel. The identification of hERG variants is essential for diagnosing LQTS2 and providing appropriate management strategies. To date, more than 3000 variants of the *KCNH2* gene have been identified in the ClinVar database. However, the potential pathogenicity of most of them remains unknown, they are classified as variants of uncertain significance or VUS. There remains an urgent clinical need for high-throughput functional characterization of VUS variants to allow optimal stratification of the rhythmic risk for each patient.

To meet this clinical need, in this work we have implemented a large-scale multiparametric evaluation of the 300 *KCNH2* variants identified within the French CARDIOGEN network. We have optimized and accelerated the entire process of functional characterization of hERG from low throughput to high throughput. (i) We increased the success rate of mutagenesis to 99% by implementing the Gibson assembly strategy, (ii) by using an electroporation system, we increased the efficiency of transient expression to 80%, (iii) we optimized a protocol for acquiring all the biophysical properties of hERG in less than 5 minutes, (iv) we switched to an automatic patch-clamp system that performs simultaneous recording of 384 cells, which allowed us to increase by 30 times the number of variants analyzed per month and improve the statistical value of the results obtained, (v) we also implemented automated data analysis in the R language. For the membrane trafficking studies, we used the pHluorin tag and confocal microscopy. To assess the structural impact of hERG variants, we used the structure resolved by Cryo-EM and UCSF Chimera software to perform a structural characterisation of the variants in their near-regional environment.

We have characterized 300 hERG variants, including 179 VUS. Our strategy confirmed the pathogenicity or benignity of the previously classified 121 variants. We observed that variants causing a significant decrease in current or presenting major defects in membrane localization also exhibited substantial structural perturbations. Combined, the trafficking approach and exhaustive biophysical protocols allow us to understand the mechanisms related to hERG loss or gain of function. Finally, we validated our approach for direct clinical application by reclassifying the 179 VUS variants.

In conclusion, functional studies, including electrophysiological assays, play a crucial role in assessing the pathogenicity of hERG variants. Our data will be used to develop a new database integrating functional, clinical, and genetic information, aimed at improving the pathogenic classification of each *KCNH2* variant. This database will serve as a diagnostic and prevention tool for clinicians ensuring appropriate management of affected individuals and their families.

HACKING THE UBIQUITIN CODE TO CONTROL ION CHANNEL EXPRESSION FOR THERAPY

Henry Colecraft

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Dozens of human diseases arise due to either too little or too much functional expression of particular proteins. In principle, the ubiquitin-proteasomal system holds the solution to all such diseases since ubiquitin is a major determinant of protein half-life, and, thus, the steady-state concentrations of virtually all proteins. However, this goal is confounded by the ubiquity of ubiquitin which sets up the challenge of how to selectively adjust the ubiquitination status of a chosen protein target, without disturbing global proteostasis. I will discuss protein engineering approaches we have developed for targeted protein stabilization and targeted protein destabilization, respectively, and the application of these tools to correct diverse ion channel-related diseases such as cardiac arrhythmias, cystic fibrosis, epilepsy, and pain.

MUTATION-DRIVEN PRECISION MEDICINE IN NON-DYSTROPHIC MYOTONIAS

Jean-François Desaphy

Dept. of Precision and Regenerative Medicine, School of Medicine, University of Bari Aldo Moro, Bari, Italy

Nondystrophic myotonias are genetic diseases due to mutations in the skeletal muscle Nav1.4 sodium channel or the ClC-1 chloride channel, which all cause sarcolemma hyperexcitability. The resulting delayed skeletal muscle relaxation may greatly affect the quality of life, while rare cases of life-threatening events have been reported in babies. Sodium channel blockers are used empirically in myotonia, regardless the culprit gene, because they reduce action potential firing in myofibers. Randomized clinical trials confirmed mexiletine effectiveness in myotonic patients. However, a number of patients complain of poor tolerability or unsatisfactory response to mexiletine. Thus, alternative treatments are warranted to address the unmet needs of myotonic patients.

Compared to symptomatic treatment, drugs targeting the molecular defect of channel mutants would allow significant improvement. Indeed, myotonic Nav1.4 mutations can modify channel sensitivity to mexiletine, due to alteration of binding site or channel gating. For instance, the Nav1.4 mutants displaying a negative shift of fast inactivation voltage dependence are less sensitive to mexiletine, whereas they maintain sensitivity to flecainide. Patients carrying such mutations who were little satisfied with mexiletine were successfully treated with flecainide, thereby demonstrating the translatability of in vitro pharmacological studies to humans. Regarding ClC-1, myotonic mutations induce loss of function mainly due

to gating defect or to impairment of plasma membrane expression. Direct ClC-1 channel activators are not currently available. Potent and reversible ClC-1 inhibitors represent potential tools for defining the binding sites and better understanding the effects on channel gating. They were used in proof-of-concept studies to verify the ability of pharmacological chaperones to restore sarcolemma expression of trafficking-deficient ClC-1 mutants. These studies define a strategy to address precision medicine in myotonic individuals. *Supported by Association Française contre les Myopathies, Italian Telethon Foundation, and University of Bari.*

MODELLING THE NEUROCARDIAC JUNCTION IN LONG QT SYNDROME TYPE 2

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Long QT syndrome (LQT, 1:2000 of live birth) is a potentially life-threatening cardiac arrhythmia characterized by delayed myocardial repolarization that produces QT prolongation and increased risk of Torsades de Pointes (TdP). This syndrome triggers syncopes, seizures, and sudden cardiac death in otherwise healthy young individuals with structurally normal hearts. LQT type 2 (LQT2) is a rare condition (30-40% of LQTS) caused by pathogenic variants in the KCNH2/HERG gene. HERG encodes the α-subunit of the human ether-à-go-go (hERG) channel, thus affecting the rapid component of the delayed rectifier K+ current (IKr) of the action potential. Associations between HERG, LQT2 and SUDEP (Sudden Unexpected Death in Epilepsy) suggest that HERG mutations confer a susceptibility to primary neuronal excitability defects. The main objective of our study is to model the neurocardiac axis using a human-derived neuro-cardiac junction from both a healthy individual and a LQT2 patient, aiming to elucidate the potential involvement of neurocardiac defects in LQT2. Using a 2D sandwich-based protocol, we differentiated hiPSC-derived ventricular cardiomyocytes (hiPSC-CM) and sympathetic neurons (hiPSC-NR), which we respectively characterized by sarcomeric (α-actinin and cardiac troponin I) and by sympathetic neuronal markers (β3-tubulin and tyrosine hydroxylase). We confirmed the expression of hERG in both cell types regardless of genotypes and investigated the functional properties of hiPSC-CM alone or innervated by hiPSC-NR, focusing on the intracellular Ca2+ handling and contractile properties. Our preliminary data show a retention of hERG in the perinuclear area in LQT2 hiPSC-CM. Moreover, LQT2 mutation seems to affect intracellular Ca2+ dynamics in the two cell types. Additionally, we developed an LQT2 organ-on-chip in microfluidic compartmentalized chambers enabling the neuronal axons to project towards cardiomyocytes. Innervated LQT2 hiPSC-CM exhibit a higher incidence of aberrant Ca2+ transients and contractions compared to LQT2 hiPSC-CM alone. We also confirmed that nicotinic activation of hiPSC-NR modulates hiPSC-CM contractile

functions through ‘synaptic junction’. Overall, our data indicate a loss of function of hERG in the LQT2 line due to impaired trafficking to the membrane, potentially compromising neurocardiac function. These technological and conceptual advancements should facilitate our understanding of the neurocardiac dysfunctions underlying the LQT2 syndrome.

Wednesday, September 11th 2024

09:30 Symposium 5: “Glutamate receptors: trafficking, turnover, plasticity, agonists”

Organized by Vincent Seutin (Liège, Belgium)

ASTROCYTIC EPHB3 RECEPTORS CONTROL NMDAR FUNCTIONS AND MEMORY

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The activation of classical NMDA receptors (NMDARs) requires the binding of glutamate and of a co-agonist. D-serine released from astrocytes is acting as such a co-agonist at several central synapses. In the hippocampus, while D-serine is the co-agonist of synaptic NMDARs, glycine is the one at extra-synaptic sites. The close apposition of astrocytic processes with synaptic neuronal elements could be an interesting signal for synaptic release of D-serine. Interestingly, it has been shown in astrocytic cultures that astrocytic EphB3 receptors play a role in the synthesis and release of D-serine. However, we do not know whether it could impact synaptic NMDAR activity. Here, we first established that the stimulation of EphB receptors by exogenous ephrinB3 led to an increase of D-serine availability at CA3-CA1 synapses, inducing an increase of NMDAR activity. Importantly, the inhibition of endogenous EphB3 receptors impaired NMDAR activity. These effects depended on astrocytes as EphB3 receptor activation by exogenous ephrinB3 had no impact on NMDAR activity under conditions where calcium activity was inhibited specifically in astrocytes. Finally, the knock down of EphB3 receptors specifically in astrocytes leads to LTP and novel object recognition memory impairment, both rescued by exogenous D-serine. Altogether our data indicate that astrocytic EphB3 receptors play a key role in synaptic NMDAR functions and memory.

AMPA RECEPTOR FUNCTION AND DYSFUNCTION: THE ROLE OF GLUA2 SUBUNITS

Mark Farrant
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AMPA-type glutamate receptors (AMPARs) are ligand-gated ion channels that play a crucial role in fast excitatory synaptic signalling in the central nervous system (CNS). These receptors are encoded by the genes GRIA1-4, and the four AMPAR subunit proteins (GluA1-4) can assemble as homo- or heterotetramers, creating a variety of receptor subtypes. Of the AMPAR subunits, GluA2 is particularly significant. Its presence determines key biophysical properties of the receptors. Specifically, the inclusion of GluA2 reduces the channel's conductance and transforms the receptor from being calcium-permeable (CP) to calcium-impermeable (CI). In

addition to the heterogeneity resulting from the inclusion of different pore forming subunits, AMPAR diversity is further extended by co-assembly with various auxiliary subunits. These proteins, such as the transmembrane AMPAR regulatory proteins (TARPs), influence AMPAR biogenesis, synaptic targeting, and function. I will describe some of our recent work on AMPARs, focusing on GluA2-lacking CP-AMPARs, on their regulation by auxiliary subunits, and on GRIA2 variants linked to neurodevelopmental disorders.

CONTRIBUTION OF NMDA RECEPTORS TO THE FUNCTIONAL DIVERSITY OF NEOCORTICAL INTERNEURONS.

Nelson Rebola
Institut Du Cerveau-Paris Brain Institute-ICM, Paris - FRANCE

Synaptic variability between neuronal connections is thought to contribute to brain's remarkable information processing capacities. The density as well as subunit composition of synaptic NMDA receptors (NMDARs) varies considerably between synapses and between neurons. Despite the well documented heterogeneity of NMDARs, the functional relevance of such diversity is poorly known. In the past, the functional relevance of NMDAR diversity has been mostly considered in the context of neuronal development and long-term synaptic plasticity. However, model simulations have also highlighted that dendritic computations are strongly influenced by synaptic NMDAR density and biophysical properties. Recently, we have explored how variability in NMDAR function affects the processing of sensory information in primary sensory cortices. We have observed that the diversity of NMDARs strongly influences the integrative properties of single neurons. In particular, we have identified that the density of synaptic NMDARs is quite different between somatostatin (SST) and parvalbumin (PV)-interneurons (INs). Experimental and theoretical analyses indicate that such differences in the levels of synaptic NMDAR have a significant impact in dendritic integration properties of cortical INs. Using two-photon glutamate uncaging we observed that SST-INs present NMDA receptor-dependent supralinear dendritic integration, while PV-INs express distance-dependent sublinear synaptic integration. Model simulations suggest that the distinct dendritic integration properties result in population-specific temporal activity dynamics in PV- and SST-INs. In addition, we have uncovered that SST-INs are equipped with the atypical glycine-binding NMDARs subunit, GluN3A. We found that GluN3A subunits selectively control SST-INs excitability via formation of atypical excitatory GluN1/GluN3A receptors. Recent findings suggest that GluN3A subunits also play a role in regulating the function of synaptic NMDARs, pointing to a previously unknown mechanism influencing the integrative properties of cortical INs. Overall, our results reveal that the diversity of NMDAR-dependent signaling is crucial for information processing within cortical networks.

CONTRIBUTION OF NMDA RECEPTORS TO THE FUNCTIONAL DIVERSITY OF NEOCORTICAL INTERNEURONS

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Introduction : Dopamine (DA) neurons in the substantia nigra are crucial for the control of movement. This vital function is encoded by the firing pattern of DA neurons which switches from spontaneous firing to bursts of action potentials. The latter produces an increase of DA release in targeted areas which is associated to locomotion initiation. The impairment of this signal, such as DA neurons degeneration, leads to the motor symptoms of Parkinson's disease. The activation of the N-methyl-D-aspartate receptors (NMDARs), by the simultaneous binding of glutamate and a co-agonist, is indispensable for the generation of this physiological cue. While both D-serine and glycine can potentially serve as co-agonist, the identity of the principal co-agonist is correlated to development, synapse specificity, NMDAR subunits and level of synaptic activity. However, in nigral DA neurons the identity of the endogenous co-agonist responsible for the production of bursting activity remains unknown.
Methods : Identification of the main co-agonist is determined by using glycine and D-serine specific degradation enzymes during whole-cell patch-clamp recordings in brain slices from wild-type Wistar rats (4-7 weeks old).
Results : We found that D-serine and glycine respectivelly binds synaptic and extrasynaptic NMDARs, even though both NMDARs populations are composed of the same subunits (GluN2B-D triheteromers). Interestingly, burst occurrence requires the same co-agonist as the one necessary for extrasynaptic NMDARs. In addition, the generation and general pattern of bursts are not affected by the specific blockade of synaptic NMDARs.
Conclusion : The co-agonist glycine through the activation of extrasynaptic NMDARs plays a key role in the generation of bursts responsible for the enhanced release of dopamine in postsynaptic areas initiating downstream motor-related behavior.

POSTER ABSTRACTS

1- CANCER-ASSOCIATED LOSS-OF-FUNCTION MUTATIONS OF KCNQ1 PROMOTES WNT/B-CATENIN PATHWAY ACTIVITY

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KCNQ1, a voltage-dependent potassium channel, exhibits varied electrophysiological properties based on its association with KCNE subunits family. This channel is crucial for cardiac repolarization when associated with KCNE1, and for potassium recycling in epithelial cells when associated with KCNE3. It is also involved in H+ secretion in the stomach when associated with KCNE2. Recent studies highlight the role of KCNQ1 as a tumor suppressor in various epithelial cancers, particularly colorectal cancer (CRC). KCNQ1 expression limits the activity of the Wnt/β-catenin pathway by retaining β-catenin at adherens junctions, while activation of this pathway inhibits KCNQ1 expression. Although the function of ion channels in the pathophysiology of cancers has been widely described, the effect of mutations in ion channels in this pathology remains underexplored.
In this study, we identified KCNQ1 mutations in several epithelial cancers using public databases. Most of the studied mutations result in a loss of function (LOF), significantly impacting channel properties and promoting Wnt/β-catenin pathway activity. The increased activity of the Wnt/β-catenin signaling induced by LOF KCNQ1 mutants is independent of the Wnt ligand and Fizzled/LRP6 activation. However, it requires ligand-independent c-MET activation. Additionally, LOF mutations reduce the expression of negative Wnt regulators such as DKK-1 and Wif-1, thereby also increasing ligand-dependent Wnt signaling. These results were confirmed in organoids derived from mouse colon crypts.
This study elucidates the significant impact of KCNQ1 mutations on the activity of the Wnt/β-catenin signaling pathway, highlighting the crucial role of ion channels in cancer biology.

2- THIK GROUP K2P CHANNELS INVOLVED IN ACUTE AND INFLAMMATORY PAIN

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Potassium channels play a crucial role in the nervous system, as they can affect resting membrane potential and modulate action potentials making them important targets for the search for new neuronal modulators. The K2P group of potassium channels are involved in various physiological functions mostly cardiac and neuronal. Recently, several K2P channels have been linked to the regulation of pain and mutation in K2P channels are associated with migraine and neurodevelopmental disorders. K2P channels are known to finely regulate neuronal excitability by hyperpolarizing their membrane.

Members of the Tandem pore-domain Halothane-Inhibited K+ channels subfamily (THIK1 and THIK2) are highly expressed in the Central and Peripheral Nervous System (CNS and PNS), but their role in the control of pain sensation has not been studied yet. Using in-situ hybridization technique (RNAscope), we have recently shown that THIK channels are co-expressed by non-peptidergic nociceptive neurons that express the Purinergic Receptor 2X3 (P2RX3) in PNS that are unmyelinated nociceptive neurons. These are known to be involved in the transmission of slow nociceptive messages in Dorsal Root Ganglia (DRG) such as inflammatory and chronic ones. Moreover, RNAseq data shows that THIK1 and THIK2 are the most highly expressed K2P channels in microglial cells of the CNS, in which THIK1 has been linked to inflammasome activation. This suggests that these channels might play a role in inflammatory pain.
We are now investigating their role in transmitting sensory and nociceptive messages and whether these channels function as homomers or heteromers. Initial studies have shown that THIK2 knockout mice exhibit allodynia and inflammatory hyperalgesia. We aim to further explore the functions of THIK1 and THIK2 in nociception and differentiate the roles of homomeric and heteromeric forms of the THIK channels. This distinction is crucial for the development of specific pharmacology and targeted therapy.

3- SINUS NODE DYSFUNCTION-RELATED CONDUCTION DEFECTS AND FIBROSIS ARE RESCUED BY GENETIC ABLATION OF G PROTEIN-GATED K+ CHANNELS (GIRK4)

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Introduction
Cardiac automaticity is generated in the sino-atrial node (SAN) tissue by specialised myocytes. Failure to generate a normal impulse in the SAN leads to sinus node dysfunction (SND). We have previously demonstrated that genetic ablation or pharmacological inhibition of G-protein-mediated GIRK4 K+ channels abrogates bradyarrhythmia in models of primary and secondary SND, but the mechanisms underlying cardiac rhythm rescue from the SND phenotype by GIRK4 inhibition are not fully elucidated. In this context, a decrease in intrinsic electrical coupling due to progressive tissue fibrosis has been proposed as a major factor in age-related SND, but its role in other forms of SND is currently unknown.
Objective
To test if genetic inhibition GIRK4 channels rescues cardiac conduction defects and fibrosis in mice lacking L-type Cav1.3 channels (Cav1.3-/-), model of primary SND.
Method
We performed in vivo and ex vivo electrocardiogram recordings in control, GIRK4-/-, Cav1.3-/- and GIRK4-/-/Cav1.3-/- mice. Pacemaker activity in isolated SAN tissue was investigated by optical and electrical mapping. Immunohistochemistry (IHC) staining was performed to study fibrosis in SAN tissues.

Results
ECG recordings in freely moving animals showed P-wave duration, PR and RR intervals significantly longer in Cav1.3-/- than in WT and GIRK4 indicative of conduction troubles and enhanced fibrotic tissue in the SAN of Cav1.3-/- animals. Inhibition of GIRK4 channels in Cav1.3-/- animals significantly shortened P-wave duration (in ms: 17.7±0.8 Cav1.3-/- vs 14.3±0.2** GIRK4-/-/Cav1.3-/-) and both PR (in ms: 59±1 Cav1.3-/- vs 44±1**** GIRK4-/-/Cav1.3-/-) and RR intervals (172±12 Cav1.3-/- vs 137±5* GIRK4-/-/Cav1.3-/-) to WT values. P-wave duration, PR and RR intervals recorded in GIRK4-/-/Cav1.3-/- isolated perfused heart was similar to that recorded in WT counterparts, suggesting recovery of SND phenotype. Genetic ablation of GIRK4 in SND tissue also restored the position of leading pacemaker site and abrogated defects of conduction recorded in Cav1.3-/- SAN. IHC experiments showed more 8% of fibrotic tissue in Cav1.3-/- than in WT SAN tissue. GIRK4-/-/Cav1.3-/- SAN tissue fibrosis was strongly ameliorated.
Conclusion
We show that, in a model of primary SND generated by Cav1.3 loss-of-function, concomitant genetic ablation of GIRK4 restores normal pacemaker activity by limiting fibrosis in SAN tissue.

4- GENERATION AND CHARACTERIZATION OF HIPSC-DERIVED SINOATRIAL NODE PACEMAKER CELLS (SAN-HIPSCS) FROM A HEALTHY INDIVIDUAL VERSUS CPVT BRADYCARDIA-ASSOCIATED PATIENTS

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Introduction
Human induced pluripotent stem cells derived-cardiomyocytes (hiPSC-CMs) are widely recognized as a model to study several cardiac disorders. In this perspective, differentiation of hiPSCs into sinoatrial node like cells (SAN-hiPSCs) may represent a significant advance in providing an in vitro platform for exploring SAN dysfunction (SND) and evaluate new therapeutic approaches. Recently, hiPSC-CMs derived from patient carrying novel mutation in the cardiac type 2 ryanodine receptor (RyR2-H29D) have been linked to catecholaminergic polymorphic ventricular tachycardia
(CPVT), in association with SND-dependent severe bradycardia. Here we propose a new protocol for differentiate hiPSCs from patient carrying RyR2-H29D in SAN-hiPSCs.
Objective
We aimed to obtain hiPSCs-derived cardiac pacemaker cells (SAN-hiPSCs) from RyR2-H29D mutated patient and characterize these cells at electrophysiological level.
Method
hiPSCs derived from patients with CPVT and healthy subjects were differentiated into SAN-hiPSCs using a 2D matrix sandwich method, modulating Wnt signaling. Moreover, cells were treated with triiodothyronine, dexamethasone and intracellular cyclic AMP (DTA) "cocktail" to

improve cell maturity, including intracellular Ca2+ handling. At day 40 post-differentiation, electrical activity was assessed by patch-clamp.
Results
Significant decrease in action potential (AP) frequency was observed in SAN-hiPSCs derived from CPVT patients compared to control (Ctrl) ones (Ctrl: 157.5±9.9 bpm; CPVT: 38.4±13.6 bpm). RyR2-H29D mutation did not affect pacemaker 'funny' current (If) (@-135mV Ctrl: -34.9±16.7 pA/pF; CPVT: -21.8±2.8 pA/pF).
Conclusion
SAN-hiPSCs derived from CPVT patient present a bradycardic phenotype compared to SAN-hiPSCs derived from healthy patient and consistent with patient clinical feature. Bradycardia is not related to alteration of If. Nevertheless, G protein-gated inwardly rectifying potassium current (IKACh), L- and T- type Ca2+ currents as well as intracellular Ca2+ handling, involved in cardiac automaticity, remain to be evaluated. Finally, recent studies have demonstrated that the genetic ablation or pharmacological inhibition of IKACh prevents SND-dependent bradycardia. In the future, this therapeutic strategy could be tested to rescue bradycardia in our model of SAN-hiPSCs derived from CPVT patient.

5- FUNCTIONAL EVALUATION OF KCNH2/HERG VARIANTS IN C. ELEGANS

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Congenital Long QT Syndrome (LQTS) is an inherited cardiac channelopathy that leads to a risk of sudden cardiac death in young patients. Variants of the KCNH2 gene account for 30-45% of LQTS cases. KCNH2 encodes the hERG subunit of the Kv11.1/IKr potassium channel involved in cardiomyocyte repolarization. Loss of channel function results in delayed repolarization of the ventricular action potential, exposing the myocardium to early afterdepolarizations that can lead to fatal ventricular arrhythmias.
Thanks to genomic sequencing, an ever-increasing number of variants have now been identified. Almost two out of five cases involve variants of uncertain significance (VUS). Functional validation studies are crucial in confirming or refuting the pathogenicity of a variant.
Existing in vitro and in vivo models have limitations, such as overexpression artifacts and high costs. To address these, we propose a novel in vivo model using the nematode C. elegans.
We selected 6 KCNH2 pathological variants, well-characterized in vitro. We introduced patient variants into the hERG ortholog, unc-103, using the CRISPR/Cas9 gene editing technique. By using a fluorophore-tagged UNC-103 reporter strain (wrmSC::unc-103), we localized UNC-103 at the neuromuscular junction (NMJ) through cholinergic presynaptic and postsynaptic

markers and in vulvar muscles. In the context of wrmSC::unc-103, we inserted the 6 variants. We quantified the impact of variant trafficking at the NMJ and observed a significant decrease in UNC-103 in this region, consistent with a trafficking defect. Functionally, the most straightforward phenotype is detected in the unlaid eggs phenotype. For all selected variants, we detected fewer retained eggs, consistent with hyperexcitability of the vulvar muscles implicated in the egg-laying phenotype. These data confirm that all tested variants exhibit a loss-of-function (LOF) phenotype.
This in vivo approach offers new perspectives for dissecting the molecular and cellular consequences of hERG mutations.

6- A NEW POTENT PEPTIDE BLOCKER OF THE HUMAN CAV1.2 CHANNEL SUBTYPE FROM POECILOTHERIA SUBFUSCA SPIDER

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Over the last two decades, animal venom toxins have been explored as an original source of new antinociceptive drugs targeting ion channel subtypes. Although the gold standard remains the time-consuming manual patch-clamp, automated patch-clamp platforms were developed to increase the number of positive hits. In the present study, we performed high-throughput screening of a collection of venom toxins through automated whole-cell patch-clamp experiments on human embryonic kidney (HEK)-293 cells overexpressing the genetically validated antinociceptive target Nav1.7 or the cardiac Nav1.5 human subtypes of voltage-gated sodium (hNav) channels. This first step aimed to identify bioactive peptides that were then purified, sequenced and chemically synthesized. The second step consisted in characterizing the synthetic peptide of interest on hNav, voltage-gated potassium (hKv) and calcium (hCav), as well as on inward-rectifier potassium (hKir), channel subtypes overexpressed in cells, and on the action potential and calcium currents of human induced pluripotent stem cell-derived cardiomyocytes. The key results are the identification of a new peptide, poecitoxin-1a, the first one isolated from the Poecilotheria subfusca spider venom, as a 35 amino acid toxin belonging to the inhibitor cystine knot (ICK) structural family. This

peptide inhibited hCav1.2 with very high affinity (24 nM producing 50% current inhibition) and selectivity against the other subtypes. In conclusion, poecitoxin-1a is the first ICK spider toxin specifically targeting hCav1.2. This peptide represents thus, at least, a valuable tool to study the Cav1.2 function and location and, at best, a valuable drug to treat some cardiomyopathies such as the long QT syndrome.

7- CALCIUM CHANNELOPATHY-ASSOCIATED CACNA1D (CAV1.3) VARIANTS LEAD TO COMPLEX ALTERATIONS OF CHANNEL GATING

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Ca2+ influx through voltage-gated Cav1.3 channels (encoded by the CACNA1D gene) is essential for several physiological processes. In humans, homozygous CACNA1D loss-of-function (LOF) causes bradycardia and deafness (SANDD syndrome) whereas de novo (heterozygous) variants causing complex activity-enhancing gating changes are associated with a neurodevelopmental disorder (NDD). Herein, we functionally characterized three missense mutations causing SANDD (inherited homozygous variant A376V, reported in 10 patients) or NDD (de novo variant V1447L and the premature STOP codon C1436X variant) and sought to establish if distinct gating changes could explain the diverse clinical phenotypes. Whole-cell voltage-clamp recordings (15 mM Ca2+) were performed in tsA201 cells transfected with C-terminally long/short wild-type (WTL or WTS, respectively), A376V, V1447L or C1436X human Cav1.3 α1-subunit co-transfected with auxiliary β3 and α2δ1. V1447L (alone/co-expressed with WTL) displayed typical NDD-associated hyperpolarising shifts in the voltage-dependent activation and inactivation by ~-11 mV and ~-6 mV, respectively. A376V, associated with clinical LOF, surprisingly showed robust Ca2+ currents combining loss & gain-of-function gating changes – similar to NDD variants. These included hyperpolarising shifts in the voltage-dependent activation/inactivation (by ~-6.5 mV and ~-8 mV, respectively) and faster inactivation kinetics. Lastly, C-terminally truncated C1436X alone or co-expressed with a membrane-tagged C-terminus gave no detectable currents. However, C1436X co-expressed with WTL shifted the voltage-dependent activation by ~-7 mV to more negative potentials and induced faster inactivation kinetics, while WTS co-expressed with C1436X did not gate differently from WTS alone. Our data allows the classification of V1447L as 'pathogenic' and revealed a dominant effect on WTL channels when expressed together. Similarly, we observed typical NDD-associated gating changes with C1436X when co-expressed with WTL. Our data further indicates that the effect of the C1436X variant requires full-length WTL channels. Surprisingly, A376V is the first complex gating-modifier leading to a clinical LOF phenotype (SANDD syndrome). Overall, our results show that CACNA1D variants induce distinct functional changes associated with broad symptomatologies, with a left-shifted activation as core feature. Whether the observed dominant effect also occurs in native tissues and by which mechanism still has to be investigated.

8- EFFECT OF THE GATING PORE BLOCKER 1-(2,4-XYLYL)GUANIDINIUM ON THE FIRING RATE OF ISOLATED MOUSE SINUS NODE CELLS

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Although the mechanisms underlying fast pacemaking are rather clear, the ones that give rise to slow pacemaking are more controversial, for example in sinus node cells of the heart. Previously, some of us (KJ and VS) have demonstrated that a drug known to block gating pore currents, 1-(2,4-xylyl)guanidinium (XG), completely blocks pacemaking of midbrain dopaminergic (DA) neurons in the high micromolar range (Jehasse et al., 2021). Given the similarities between ion channel species expressed by DA neurons and sinus node cells (e.g. both have large Ih and Cav1.3 currents), we tested whether XG also would affect these cells.
Experiments were performed on isolated mouse sinus node cells in the perforated patch clamp mode. In current-clamp recordings using a physiological solution (T° 37 °C), 300 µM XG inhibited the firing of sinus node cells by 85 ± 6 % (n = 11). The effect was weakly reversible and XG did not markedly hyperpolarize the cells, as had been seen in DA neurons. Additional experiments were performed in voltage clamp mode to test the possibility that XG affects important currents in these cells. Various solutions were used in order to isolate the relevant currents. We found that XG does not significantly affect Cav currents, Ih or the delayed rectifier. Thus, XG markedly inhibits the firing of sinus node cells without acting on some well-known targets that are present in these cells.
Taken together with the previous study, our results suggest that one or several ion channels producing a gating pore-type current, or a closely related protein plays a critical role in the spontaneous firing of some slow pacemakers in the brain and heart.

9- MODULATION OF CALCIUM HANDLING BY β2AR SIGNALLING AND PDE4S ACTIVITY UNDER MECHANICAL STRETCH

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The heart's adaptability to varying hemodynamic demands is crucial for maintaining cardiovascular stability, especially under conditions inducing myocardial stretching, such as hypertension or acute valve malfunctions. Key to this process are the transverse tubules (TT), which facilitate excitation-contraction coupling (ECC). The cardiac ECC involves critical components like L-type calcium channels (LTCC, CaV1.2), ryanodine receptors (RyR), and sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA2a), all pivotal in calcium handling and cardiac function. β-adrenoceptors (βARs), particularly β1 and β2 subtypes, modulate this calcium signaling network. Phosphodiesterase 4 (PDE4), especially subtypes B and D, regulates cAMP levels, illustrating the complex interplay of mechanisms governing cardiac responses to stress.

Hypo-osmotic solution was used to mimic stretch conditions for rat cardiomyocytes. Super-resolution Patch Clamp and Förster Resonance Energy Transfer (FRET) microscopy investigated LTCC and RyR/SERCA2a activity, while a Langendorff-optical mapping system assessed the effects of stretching and PDE4s on cardiac electro-excitability and calcium transients in the whole heart.
Hypo-osmotic stretching significantly affects cardiomyocyte structure and function. We observed a decrease in the Z-groove ratio, indicative of cardiomyocyte surface remodeling. This structural change correlates with reduced occurrence and altered functionality of LTCCs under stretch conditions. Under iso-osmotic conditions, there is tight regulation of the LTCC-β2AR complex, which can be lost by stretching or inhibiting PDE4B. Hypo-osmotic conditions led to diminished colocalization of PDE4B and CaV1.2, disrupting critical calcium signaling interactions. Functionally, this stretch enhances β2AR-induced phosphorylation of key calcium regulatory proteins SERCA2a and RyR, altering calcium dynamics essential for cardiac contraction and relaxation. At the whole heart level, stretching resulted in prolonged action potential durations and conduction velocity, particularly under β2AR stimulation.
In conclusion, stretching disrupts cardiomyocyte structure and function by altering the LTCC-β2AR complex, diminishing PDE4B-CaV1.2 colocalization, and enhancing β2AR-induced phosphorylation of calcium regulatory proteins, ultimately leading to prolonged action potential durations and conduction velocity at the whole heart level.

10- EFFECT OF HUWENTOXIN-IV AND IBERIOTOXIN ON NAV TTX-SENSITIVE AND KCA1.1 CHANNELS IN SINO-ATRIAL NODE MOUSE CELLS: PRELIMINARY RESULTS

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Introduction: Cardiac automaticity is defined by heart capacity to spontaneously generated action potentials (APs) in sino-atrial node mouse cells (SANCs). This capacity is due to diastolic depolarization (DD) which is a slow depolarizing phase developing during the diastole of the cardiac contraction cycle. DD is the results of a complex and not yet totally unraveled, interaction between intracellular calcium dynamic and sarcolemma ion channels. Among these ion channels, TTX-sensitive sodium channels (TTX-S Nav) and calcium activated potassium channels (KCa1.1) have been associated with intranodal conduction failure. Here, by using two different natural peptides (huwentoxin-IV (HWTX-IV) and iberiotoxin (IB)) inhibiting selectively TTX-S Nav and KCa1.1 ion channels, we investigated their implication in cardiac automaticity.
Method: SANCs were isolated from wild-type mouse and used for recording ion currents and APs in voltage-clamp and current-clamp configuration of patch-clamp technique, respectively. 100nM HWTX-IV was perfused on isolated SANCs alone or in combination with 50nM TTX (as positive control). Same protocol was applied for studying the effect of KCa1.1 inhibition by IB (100nM) alone or with 100nM Paxillin (as positive control). Multi-Electrode Array in isolated

hearts were performed in the same conditions to confirm the results in more integrated model.
Results: Inhibition of TTX-S Nav channels by 100nM HWTX-IV induced a significant reduction of the sodium current which is not enhanced by 50nM TTX, a dose knowing to block 100% of TTX-S Nav channels. Moreover, 100nM HWTX-IV showed a significant 12% reduction of spontaneous firing frequency in isolated SANCs. 50nM TTX did not further reduce the SANCs APs rate suggesting a complete inhibition of TTX-S Nav channels by 100nM HWTX-IV. IB at 100nM did not show any effect neither on KCa1.1 current density nor in APs firing rate. In isolated hearts, we confirmed that perfusion of 100nM HWTX-IV induced a significant reduction in heart rate without affecting cardiac conduction.
Conclusion: Inhibition of TTX-S Nav channels significantly reduced spontaneous APs frequency in SANCs suggesting an important role for Nav TTX-S channels in cardiac automaticity. However, methodological adjustments are required to evaluate the possible effect of IB on the KCa1.1 current and on cardiac automaticity.

11- ZEBRAFISH LOCOMOTION IS NOT AFFECTED BY SKELETAL MUSCLE NAV1.4 LOSS OF FUNCTION

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The voltage-gated Na+ channel Nav1.4 is mandatory for muscle contraction in mammals. Its loss of function (LoF) results in death at birth (null alleles) or in a subgroup of congenital myopathies (hypomorph alleles) that we call Sodium Channel Weakness (SCW). Zebrafish is recognized as an excellent preclinical model for human diseases and its neuromuscular system is described as similar to humans. We established and investigated zebrafish mutant lines for Nav1.4 in order to establish a preclinical animal model for SCW with the final objective to perform phenotypic-based drug screening.
Two Nav1.4 paralogs are expressed in zebrafish skeletal muscles. We developed LoF mutant lines for each paralog and generated "double mutant" lines by intercross. Surprisingly, we did not detect any motor phenotype in the double mutants compared to controls. Double mutants were obtained at the expected frequency at the adult ages. They did not present any deficit in the usual locomotor activity tests performed at the larval stages (touch evoked; white-light or sound induced-escape responses). We excluded any gene compensation resulting from muscle overexpression of Scn paralogs. Our data suggest that skeletal muscle fibers of zebrafish do not require Nav1.4 activity to efficiently contract, and question the relevance of this vertebrae model for drug-based therapeutic assays in the field of neuromuscular disorders as well as sodium channelopathies.

12- GRAPHENE NANODEVICES FOR ION CHANNEL DETECTION WITHIN LIVING CELLS

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Because this sensing method is less damaging for cell than patch-clamp, field-effect transistors have appeared as good candidates to probe biochemical signals at the cell membrane interface. Particularly, graphene field-effect transistors (G-FETs) benefit from the high graphene sensitivity and chemical stability in various reactive media (ionic solutions, physiological media). Moreover, graphene biocompatibility and bendability allow a good coupling with living cells and soft matters. G-FET sensitivity to ion concentration offered various applications in detecting analyte composition[1],[2] or pH[3],[4] changes but ion channel resolution has long been difficult to reach because of their size (nm) and low ionic currents (pA) involved. Recently, graphene topological defects have allowed to surpass this limit using a high sensitivity to very weak ion currents[5]. Here, we benefit from a high coupling between polycrystalline graphene and a Xenopus oocyte to read ion channel currents and transduce biological signals with ultra-high sensitivity. The sensitivity of our G-FET surpasses the limit imposed by the high charge screening in buffer solution and allows to detect on real-time extra-cellular potassium fluxes which are not detectable with fluorescent methods.
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13- CHLORPYRIFOS INCREASES VIABILITY AND MIGRATION OF NORMAL AND CANCER COLON EPITHELIAL CELLS THROUGH TRPA1 CHANNEL.

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Chlorpyrifos (CPF) is an organophosphorus insecticide found as residues in food. Chronic exposure to CPF triggers gut dysbiosis chronic inflammation [1], production of reactive oxygen species (ROS) [2] and an increase in intracellular calcium concentration ([Ca]i) [3]. These processes result in a higher risk of the development and progression of colorectal cancer (CRC) [4,5]. Among TRP channels, TRPA1 is sensitive to ROS and liposaccharides or endotoxins produced by gram-negative bacteria. TRPA1 is also overexpressed in the tissues of patients with CRC and constitutes a bad prognostic marker [6]. Our aim is to investigate whether

chronic CPF exposure alter normal and cancer colon epithelial cells hallmarks. CPF chronic treatment (10 µM) increased viability and migration from day 7 (16.0 ± 6.1%, 33.4 ± 10.0%, $p<0.01$), and basal [Ca]i at day 14 (25.4 ± 0.03%, $p < 0.001$) of normal cells. Similar effects were found in cancer cells, viability increased at day 7 and 14 (17.48 ± 3,1% and 13.3 ± 2.7%, $p<0.001$) and migration at 14 and 21 days (37.6 ± 12.5% and 63.2 ± 14.9%, $p<0.01$). Moreover, the perfusion of 10 µM CPF increased the [Ca]i, only in the presence of 2 mM extracellular calcium, that was completely suppressed by TRPA1 channel blocker (HC-030031, 10 µM) as well as cell viability and migration of both normal and cancer cells ($p<0.05$). In conclusion, by targeting TRPA1 channel, CPF may drive processes that control viability and migration of normal and cancer epithelial colon cells, eventually causing pre-cancerous lesions and increasing cancer aggressiveness.

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14- HOW CAN TRPM8 ENHANCE PAIN SENSITIVITY IN COLD WEATHER?

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Mild cold is often associated with enhanced sensitivity to generic pain stimuli. Interestingly, some medical conditions or treatment side effects can also trigger cold hypersensitivity, as observed in chemotherapy-induced peripheral neuropathy. However the underlying mechanisms remain poorely studied. Among multiple cold sensor proteins, TRPM8 was the first ion channel identified as playing an important role in cold sensation. It is activated at temperatures between 8°C and 26°C. While it is not considered as a pain sensor, recent evidence suggests that TRPM8 activity could be modulated in general pain sensation under reduced temperature, although specific mechanisms remain to be elucidated. Here we report that TRPM8 ion channel is a likely candidate involved in such regulation. We have observed that stimulation of the TRPM8 activity by specific agonists, such as menthol and icilin, produced a significant effect upon whole cell currents in the PC-12 cell line that is often used to model neuronal activity and is known to natively express TRPM8. However, and unlike the classical TRPM8 responses associated with direct cold sensation, TRPM8 activation in PC-12 cells led to a decrease of potassium currents. This suggests that instead of the classic TRPM8-mediated whole cell currents, TRPM8 activation produces an inhibitory effect upon voltage gated potassium channel activity. Inhibition of these potassium currents is commonly associated with neuronal depolarization and enhanced excitability, which could explain the enhanced sensitivity to mild pain stimuli in cold weather. Our ongoing work focuses on unraveling the details of this regulatory mechanism in PC-12 cells as well as characterizing it in the TRPM8-expressing DRG neurons.

15- POTASSIUM CHANNEL KV10.1 PROMOTES EPITHELIAL-MESENCHYMAL TRANSITION, MIGRATION AND INVASIVENESS OF BREAST CANCER CELLS IN BRAIN UNDER HYPOXIC CONDITIONS

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Breast cancer is the most frequently diagnosed cancer in women and remains a leading cause of cancer-related death, mainly due to metastases. Metastases, particularly to the brain, are more frequently found in triple-negative breast cancers. These metastases are partly regulated by ion channel expression and activity dysregulation. The potassium channel Kv10.1 is implicated in tumorigenesis and regulates HIF-1α expression in non-cancerous cells. However, its role in breast cancer under hypoxia is unclear. Epithelial-mesenchymal transition (EMT) and migration, both are enhanced by hypoxia and associated with metastasis. EMT favors the expression of mesenchymal markers (N-cadherin, vimentin, ZEB-1) and decreases epithelial ones (E-cadherin). Transcription factors YAP and TAZ, also linked to the hypoxic microenvironment, play a role in breast cancer cell invasiveness, enabling them to cross the blood-brain barrier (BBB).
Our study aims to identify the role of Kv10.1 in the development of breast cancer metastases by promoting EMT and migration under hypoxic conditions.
We used two breast cancer cell lines: MDA-MB-231 (triple-negative basal cells) and MCF-7 (luminal cells). Under severe hypoxia (1% O_2, 18h), both Kv10.1 and HIF-1α expression increased. Western blot analysis showed that Kv10.1 silencing reduced the expression of HIF-1α, N-cadherin, vimentin, and ZEB-1; while it increased E-cadherin expression. Hypoxia-induced migration significantly decreased in siKv10.1 cells or cells treated with astemizole (a Kv10.1 pharmacological inhibitor) which is associated with morphological changes. Kv10.1 silencing also reduced integrin β1 expression and FAK phosphorylation. Finally, by using an in vitro BBB model, we studied the impact of Kv10.1 on cell adhesion and transmigration and the phosphorylation of YAP/TAZ. Both siKv10.1 and astemizole reduced transmigration along with an increase in phosphorylated TAZ.
These results suggest that hypoxia increases Kv10.1 expression that enhances ZEB-1 expression leading to increase of EMT markers. Kv10.1 also regulates migration through integrin β1expression and FAK phosphorylation. Kv10.1 may contribute to brain metastasis by affecting cell adhesion and transmigration likely through YAP/TAZ signaling pathways.

16- GLOBAL REMODELLING OF THE AUTONOMIC NERVOUS SYSTEM IN A MOUSE MODEL OF CATECHOLAMINERGIC POLYMORPHIC VENTRICULAR TACHYCARDIA

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Background: Catecholaminergic polymorphic ventricular tachycardia (CPVT) is caused by mutations in RYR2 (encoding ryanodine receptor 2, RyR2). So far, investigation into the

underlying pro-arrhythmic mechanisms have focused on cardiomyocytes. However, RyR2 is also present in neuronal tissue, and patients often present with clinical signs of autonomic dysfunction.
Aim: Investigate the neuronal phenotype induced by RYR2 mutations, using a CPVT mouse model (RyR2-R2474S) to assess functional alterations as well as broader autonomic remodelling.
Results: Cardiac neuron innervation density was assessed by immunohistochemistry of mouse mid-ventricular sections for the sympathetic neuron marker tyrosine hydroxylase (TH), revealing an increase in the percentage of sympathetic neurons in ventricular tissue of CPVT mice (5.7% wild-type vs 7.2% RyR2-R2474S, *p=0.0253, N=6).
To explore the mechanism underlying this sympathetic hyper-innervation in CPVT mouse hearts, we investigated alterations in neuronal function.
Using immunocytochemistry, we show that RyR2 is abundantly expressed in mouse stellate ganglia neurons (SGN) - crucial autonomic (sympathetic) modulators of cardiac function that directly innervate the heart.
Functional investigation of calcium homeostasis using the fluorescent calcium indicator Fura Red revealed a smaller reduction of fluorescence intensity after caffeine exposure in CPVT SGNs (F/F0 0.45 wild-type vs 0.53 RyR2-R2474S, **p=0.0013, N=10-12), indicating the presence of calcium leak from the endoplasmic reticulum (known to increase neurite projection).
We additionally explored a potential impact of cardiomyocyte (dys)function on innervation and observed an increased abundance of nerve growth factor (known to increase neuronal innervation) in CPVT cardiomyocytes (212.4 wild-type vs 311.8 RyR2-R2474S, *p=0.0182, N=7).
Conclusions: The RyR2-R2474S mutation leads to significant alterations of calcium homeostasis in SGNs and sympathetic hyper-innervation of ventricular myocardium, which may have important consequences for neurotransmitter release, cardiac function and arrhythmogenesis. Our findings furthermore suggest that cardiomyocyte dysfunction in the setting of CPVT may reciprocally affect innervation of the myocardium.

17- TRPV6 CHANNEL TARGETING USING MONOCLONAL ANTIBODY INDUCES PROSTATE CANCER CELL APOPTOSIS AND TUMOR REGRESSION

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TRPV6 calcium channel is a prospective target in prostate cancer (PCa) since it is not expressed in healthy prostate while its expression increases during cancer progression. Despite the role of TRPV6 in PCa cell survival and apoptotic resistance has been already established, no reliable tool to target TRPV6 channel in vivo and thus to reduce tumor burden is known to date. Here we report the generation of mouse monoclonal antibody mAb82 raised against extracellular epitope of the pore region of the channel. mAb82 inhibited TRPV6 currents by 90% at 24 µg/ml in a dose-dependent manner while decreasing store-operated calcium entry to 56% at only 2.4 µg/ml. mAb82 decreased PCa survival rate in vitro by 71% at 12 µg/ml via inducing cell death through the apoptosis cascade via activation of the protease calpain, following bax activation, mitochondria enlargement, and loss of cristae, Cyt C release, pro-caspase 9 cleavage with the subsequent activation of caspases 3/7. In vivo, mice bearing either PC3Mtrpv6+/+ or PC3Mtrpv6-/-+pTRPV6 tumors were successfully treated with mAb82 at the dose as low as 100 µg/kg resulting in a significant reduction tumor growth by 31% and 90%, respectively. The survival rate was markedly improved by 3.5 times in mice treated with mAb82 in PC3Mtrpv6+/+ tumor group and completely restored in PC3Mtrpv6-/-+pTRPV6 tumor group. mAb82 showed a TRPV6- expression dependent organ distribution and virtually no toxicity in the same way as mAbAU1, a control antibody of the same Ig2a isotype. Overall, our data demonstrate for the first time the use of an anti-TRPV6 monoclonal antibody in vitro and in vivo in the treatment of the TRPV6-expressing PCa tumors.

18- BIOPHYSICAL PROPERTIES OF TWO-PORE DOMAIN POTASSIUM CHANNEL TWIK1 IN BLACK LIPID MEMBRANES

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Leak potassium channels (K2P) have two pore domains and are involved in many processes such as cell volume regulation and excitability. They are open at all membrane potentials and therefore define the resting potential of the cells.
The first K2P discovered was TWIK1 (Tandem of P domains in a Weak Inward rectifying K+ channel). The particular interest in this channel comes from its high expression in the brain, heart and kidney. However, its characterization has long been made difficult by the lack of functional activity observable in whole-cell patch-clamp. It was established that this was due to the channel being mainly localized in subcellular compartments such as recycling endosomes.
We therefore propose to study TWIK1 by inserting it into artificial lipid membranes made of a bilayer of phospholipids. This technique allows us to investigate the unitary biophysical properties of the channel such as its unitary conductance and its open probability according to different physicochemical parameters, such as pH. Indeed, a particularity of TWIK1 is that its selectivity changes when the pH becomes acidic and the channel then becomes permeable to sodium ions. The physiological relevance of this permeability switch is yet to be determined. Our findings show that TWIK1 has a very low open probability (≃1%) compared to TWIK1-I293-294A-K274E, a known membrane-localized functional mutant with a greater open probability (≃15%). The latter, contrary to the wild type, produces strong currents and

depolarizes the reverse potential (Erev≃-70mV) near to the K+ equilibrium potential in CHO cells, in the whole cell configuration.
In addition to its fast internalization, the poor open probability of TWIK1 could explain its low functional activity in mammalian cells.

19- CONTRIBUTION OF THE LRRC8/VRAC ANION CHANNEL TO MICROCRYSTAL-MEDIATED JOINT INFLAMMATION

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Gout and chondrocalcinosis (CC) induce inflammation flares respectively by the deposition in the joints of monosodium urate (MSU) and calcium pyrophosphate (CPP) crystals. Resident macrophages initiate the inflammatory response through NLRP3 inflammasome activation and the release of cytokines including IL-1β. Crystal-mediated NLRP3 inflammasome activation mechanism is still under investigation.
Measurements in synovial fluid (SF) of patients diagnosed with gout or CC (cohort - Hôpital Lariboisière, Paris) showed a decrease in SF osmotic pressure as compared to SFs of non-inflammatory osteoarthritis patients and a negative correlation with SF IL-1β concentration.
Knowing that the ubiquitously expressed volume regulated anion channel LRRC8/VRAC is osmo-sensitive, we suspected that this anion channel might contribute to crystal-mediated inflammation in the context of gout and CC.
At the cellular level, in THP-1 macrophages, MSU or CPP crystals-induced NLRP3 inflammasome activation and IL-1β release were abolished by pharmacological inhibition of LRRC8/VRAC with DCPIB or by knocking down the expression of LRRC8A, the mandatory subunit of VRAC channels. By combining different approaches (patch-clamp, fluorescence measurement…) we showed that this channel is crucial in crystal-mediated ATP release necessary for inflammasome activation.
In vivo, in murine air pouch model (mimicking a joint cavity), crystal-induced inflammation was inhibited by increasing plasmatic osmolarity (i.p. mannitol injection) in WT mice or by tissue specific invalidation of LRRC8A in macrophages using Cx3cr1CreERT2/Lrrc8Aflox/flox mice.
In conclusion, our findings provide valuable insights into the role of LRRC8 channels in joint inflammation, particularly in the context of gout and chondrocalcinosis. By elucidating the mechanisms of NLRP3 inflammasome activation and the impact of osmolarity on inflammation, the study opens new avenues for therapeutic interventions such as osmotherapy or by targeting LRRC8 channels.

20- IDENTIFYING NEW PH-SENSING RESIDUES INVOLVED IN THE GATING OF ACID-SENSING ION CHANNEL 1A (ASIC1A)

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Acid-sensing ion channels (ASICs) are Na+-permeable channels that are activated by extracellular acidification. While rapid acidification occurs in synapses during neuronal activity, slower and sustained acidification develops in inflammation or ischemia. ASICs are widely expressed in the nervous system and have many physiological and pathological functions, such as pain sensation, fear sensing and neurodegeneration after ischemia. ASIC1a is the most H+-sensitive subunit expressed in the central nervous system. Acidification leads to protonation of a number of extracellular residues, inducing conformational changes that lead to channel activation. Identifying the protonation sites is essential to better understand the activation mechanism of ASIC channels. Combined mutation of a large number of titratable residues in specific regions affected the pH dependence but did not abolish the ability of the channel to open in response to acidification. This suggests the existence of additional pH-sensing residues. Here, we predicted new pH-sensing residues of ASIC1a by a computational approach, which calculates on the basis of the crystal structures the pKa values of titratable amino acid residues of the membrane-inserted and hydrated protein. Predicted residues were mutated to different amino acids to identify pH-sensors and determine which biochemical side chain properties are important for their function. This analysis identified several glutamate, aspartate and some histidine and lysine residues as part of H+-sensors in different key regions of ASIC1a. The interactions of these residues were also investigated by pairing mutations of residues that are in close proximity to each other, identifying three intersubunit interactions and two intrasubunit interactions which contribute to ASIC1a gating.

21- MOLECULAR MECHANISMS OF ASIC1A ACTIVATION: INSIGHTS INTO PROTONATION AND CONFORMATIONAL CHANGES

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Acid-sensing ion channels (ASICs) are widely expressed throughout the body, acting as primary sensors of extracellular acidosis in various physiological and pathological conditions. Activated by extracellular protons, ASICs undergo conformational changes that open the channel, allowing ions such as sodium and calcium to pass through. Prolonged low pH exposure leads to a non-conducting desensitised state of the channel. There are at least six mammalian ASIC subunits (ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4) which are encoded by four genes (ASIC1, ASIC2, ASIC3, and ASIC4). The structural arrangement of a single subunit resembles a clenched hand holding a ball, with defined domains named the wrist, palm, finger, knuckle, thumb, and β-ball. Functional ASICs form as trimers, either homomeric or heteromeric, with distinct biophysical and pharmacological properties.

Pharmacological inhibition of ASIC1a has been shown to be neuroprotective and cardioprotective in several currently poorly treated conditions such as stroke, multiple sclerosis, and myocardial infarctions. This makes ASIC1a a promising new therapeutic target for drug development. However, the molecular mechanism driving ASIC1a activation remains poorly understood. To enhance our understanding of ASIC activation, we investigated the protonation of specific human ASIC1 residues and the resulting conformational changes during channel activation. Using a combination of electrophysiological studies, site-directed mutagenesis, voltage-clamp fluorometry, and molecular dynamics simulations, we identified protonation sites in the finger and knuckle domains of human ASIC1a which seem to be important for channel activation. Our findings provide valuable insights into the protonation events and structural changes underlying ASIC1a activation.

22- EFFECTS OF BETA-CARDIOTOXIN FROM KING COBRA (Ophiophagus hannah) ON FUNNY CURRENT (If) AND MITOCHONDRIAL BIOLOGY: PRELIMINARY RESULTS

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Activities of ion channels in the Sino-atrial node (SAN) cells, particularly the Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, are crucial for regulating heartbeats. In addition, these processes are also influenced by the relationship between the dynamics of intracellular calcium and the calcium storage organelles including mitochondria. Isolated Beta-cardiotoxin (β-CTX) from the King cobra's venom has previously been shown to have a negative chronotropic effect on rat 'hearts. However, in the cardiomyocytes, calcium transients were not attenuated by β-CTX unless they were challenged by isoproterenol. Nevertheless, the effects of β-CTX on ion channels involved in heart rate regulation and mitochondrial biology have never been investigated. Therefore, this preliminary study aims to elucidate its mechanism of action on HCN channel and mitochondrial biology. HCN current (funny current) was recorded in isolated mouse SAN cells using the whole-cell voltage-clamp configuration of the patch-clamp technique. Three concentrations of β-CTX (0.03µM, 0.3µM and 1µM) were applied on isolated SAN cells. Interestingly, the results showed that 1µM β-CTX, as well as 0.3µM, reduced significantly funny current density of 40%. 0.03µM did not have any statistical effect on If density. Three male Sprague Dawley rats were used for mitochondrial isolation. Three concentrations of β-CTX (0.14µM, 1.4µM and 14µM) were used to evaluate isolated mitochondrial biology, including mitochondrial swelling, mitochondrial ROS production, mitochondrial membrane potential, and mitochondrial Ca2+ uptake. The

results show that at the lowest dose of β-CTX, all mitochondrial biological parameters were not different from the control group. However, β-CTX at 14µM altered mitochondrial biology. In conclusion, β-CTX at doses showed not to alter mitochondrial biology, causes a significant reduction in the funny current, and maybe the underlying mechanism of negative chronotropic effect previously showed in heart.

23- TOWARD A PREDICTIVE ASSAY FOR TORSADOGENIC EFFECTS OF DRUGS USING NEURO-CARDIAC ORGAN-ON-CHIP

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Long QT syndrome is a primary arrhythmia syndrome with high risk of sudden cardiac death with genetic or iatrogenic etiology. For the drugs known to induce LQT, the consequence is the blocking of the IKr current, encoded by the human ether-a-go-go-related gene (hERG), inducing an abnormal lengthening of ventricular repolarization and finally Torsades de Pointes (TdPs). Consequently, regulatory evaluations assessing the potential hERG channel-blocking effects of new drugs are imperative for ensuring their safety. The objective is to pioneer a novel predictive assay for detecting drug-induced torsadogenic effects, which involves neurocardiac organ-on-chip composed of patient-derived autonomic neurons (NRs) innervating cardiomyocytes (CMs), all derived from human induced pluripotent stem cells (hiPSC).
To achieve this, hiPSC-CMs and NRs were differentiated and characterized by expressing hERG expression, sarcomeric cardiac markers for CMs (α-actinin, cardiac-troponin-I), and neuronal markers (β3-tubulin), sympathetic markers (tyrosine-hydroxylase, dopamine-β-hydroxylase) for NRs .
Our data show that CMs respond to adrenergic stimulation by increasing intracellular Ca2+ transient (CaT) and contraction frequency. CMs with hERG blockers exhibit aberrant CaT, longer decay time and increased repolarization time. Microelectrode-array on CMs suggest modulation of the field potential duration in presence of adrenergic agonist or hERG modulators. NRs respond to nicotinic stimulation by increasing CaT frequency and modulate CaT properties when exposed to hERG modulators.
Experiments are ongoing to built-up a neurocardiac organ-on-chip to investigate neurocardiac dysregulations associated with TdPs and establish a patient-specific testing platform to enhance the evaluation of new pharmacological compounds for safety and efficacy, taking into account effect of drugs on both NRs and CMs. Compartmentalized microfluidic chambers are designed to allow the establishment of a neurocardiac network: cardiomyocytes innervated from afar by sympathetic neurons. Preliminary data show the formation of a neurocardiac junction, with changes in intracellular Ca2+ cycling and contractility of NRs-innervated-CMs, which provides evidence of a functional human neurocardiac OOC.

24- LOSS OF FUNCTION OF HCN2 CAUSE SEVERE NEURODEVELOPMENTAL DISORDER

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Hyperpolarization activated Cyclic Nucleotide (HCN) gated channels are essential for various neurophysiological functions, and HCN malfunction is responsible for brain disorders. To date, only six pathogenic HCN2 variants have been described, causing mild epilepsy in few individuals. Thanks to GeneMatcher, we recruited 21 individuals with HCN2 variants from 15 unrelated families, carrying 13 HCN2 variants. Here, we report three novel inherited biallelic missense HCN2 variants (p.(Leu377His), p.(Pro493Leu) and p.(Gly587Asp)) in individuals who exhibit severe developmental delay and intellectual deficiency, hypotonia or hypertonia and locomotor disorders, but not all of them have epilepsy. These mutations were introduced into the wild-type HCN2 sequence for expression in Xenopus laevis oocytes and subsequent electrophysiological characterization. These variants rendered HCN2 electrophysiologically silent in Xenopus oocytes. Indeed, the (p.(Leu377His) variant elicited leak currents in Xenopus laevis oocytes. By expressing p.(Leu377His), p.(Pro493Leu) and p.(Gly587Asp) variants in HEK cells and confocal imaging, we found that they impaired membrane trafficking, explaining complete loss of HCN2 elicited currents in Xenopus oocytes. We also examined the structural impacts of these three mutations. We first built 3D models of HCN2 in both close and open states by homology using the cryoEM HCN1 structures. Pro493 and Gly587 are in C-linker of HCN2. We found that Pro493 stabilizes the kink between the A' and B' anti-parallel helices. Gly587 is located at a central turn of the CNBD β core, in the vicinity of Lys187 of a neighbour subunit. Thereby, the structural 3D-analysis in depolarized and hyperpolarized states of HCN2 channels, revealed that the three pathogenic variants p.(Leu377His), p.(Pro493Leu) and p.(Gly587Asp) strongly alter molecular interactions, that in turn could alter HCN2 function. In conclusion, our data broadens the clinical spectrum associated with HCN2 variants, and strongly suggest that HCN2 loss of function induces severe neurodevelopmental disorders with or without epilepsy.

25- MODELLING THE NEUROCARDIAC JUNCTION IN LONG QT SYNDROME TYPE 2

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Long QT syndrome (LQT, 1:2000 of live birth) is a potentially life-threatening cardiac arrhythmia characterized by delayed myocardial repolarization that produces QT prolongation and increased risk of Torsades de Pointes (TdP). This syndrome triggers syncopes, seizures, and sudden cardiac death in otherwise healthy young individuals with structurally normal hearts. LQT type 2 (LQT2) is a rare condition (30-40% of LQTS) caused by pathogenic variants in the KCNH2/HERG gene. HERG encodes the α-subunit of the human ether-à-go-go (hERG) channel, thus affecting the rapid component of the delayed rectifier K+ current (IKr) of the action potential. Associations between HERG, LQT2 and SUDEP (Sudden Unexpected Death in Epilepsy) suggest that HERG mutations confer a susceptibility to primary neuronal excitability defects. The main objective of our study is to model the neurocardiac axis using a human-derived neuro-cardiac junction from both a healthy individual and a LQT2 patient, aiming to elucidate the potential involvement of neurocardiac defects in LQT2. Using a 2D sandwich-based protocol, we differentiated hiPSC-derived ventricular cardiomyocytes (hiPSC-CM) and sympathetic neurons (hiPSC-NR), which we respectively characterized by sarcomeric (α-actinin and cardiac troponin I) and by sympathetic neuronal markers (β3-tubulin and tyrosine hydroxylase). We confirmed the expression of hERG in both cell types regardless of genotypes and investigated the functional properties of hiPSC-CM alone or innervated by hiPSC-NR, focusing on the intracellular Ca2+ handling and contractile properties. Our preliminary data show a retention of hERG in the perinuclear area in LQT2 hiPSC-CM. Moreover, LQT2 mutation seems to affect intracellular Ca2+ dynamics in the two cell types. Additionally, we developed an LQT2 organ-on-chip in microfluidic compartmentalized chambers enabling the neuronal axons to project towards cardiomyocytes. Innervated LQT2 hiPSC-CM exhibit a higher incidence of aberrant Ca2+ transients and contractions compared to LQT2 hiPSC-CM alone. We also confirmed that nicotinic activation of hiPSC-NR modulates hiPSC-CM contractile functions through 'synaptic junction'. Overall, our data indicate a loss of function of hERG in the LQT2 line due to impaired trafficking to the membrane, potentially compromising neurocardiac function. These technological and conceptual advancements should facilitate our understanding of the neurocardiac dysfunctions underlying the LQT2 syndrome.

26- A NEW KV1.3 CHANNEL BLOCKER FROM THE VENOM OF THE ANT TETRAMORIUM BICARINATUM.

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Venoms of many species are extensively studied for human health applications such as the development of anti-venoms or the identification of new pharmacological agents and therapeutic molecules. Nevertheless, ant venoms have been little studied due to the difficulty of access and the small quantities available, even though over 11000 venomous ant species have been reported, making ant venom a major source of bioactive molecules. Our research group has determined the venom composition of several ant species and focuses on proteinaceous ant venoms. Recently, we identified the peptide Tb11a from the ant

Tetramorium bicarinatum. This 34-amino acid peptide features a compact helix ring structure stabilized by one disulphide bridge. Tb11a is non-cytotoxic on insect cells and exhibits paralytic activities in vivo on insects (Barassé et al., 2023). Preliminary membrane potential perturbation assays and the presence in Tb11a of a functional dyad (Lys-Tyr) found in many voltage-gated potassium channel (Kv) blockers derived from venoms suggest that Tb11a may interact with Kv channels.
To test this hypothesis, human potassium channels were heterologously expressed in HEK293T and patch clamp technique (voltage clamp, whole cell configuration) was used to assess the effect of Tb11a on channel activity. We found that Tb11a decreases Kv1.3 maximum current by 50%. Further experiments are ongoing to test if other Kv channels are modulated by Tb11a. Since some of these channels are involved in cell cycle regulation and could be linked to carcinogenesis, we also aimed to evaluate the effects of Tb11a on different cancer processes. Breast cancer cells (MDA-MB-231) were used to test the effect of Tb11a on cell migration by wound healing and on cell proliferation by cell counting. While no effect on cell migration was detected, Tb11a had a slight but significant inhibitory effect on cell proliferation, without showing any cytotoxicity at high concentration.
We now plan to test Tb11a analogues found in other ants to initiate a structure-function relationship study and better characterize the interaction between the peptide and the voltage-dependant potassium channel. The identification and characterization of new pharmacological agents targeting Kv channels could be of great interest in cancer research and therapy.

27- MOUSE DORSAL RAPHE BRAIN SLICE CULTURES AS A MODEL TO STUDY SK CHANNEL PHYSIOLOGY: A CHARACTERIZATION.

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In the context of depression, the dorsal raphe (DR) nucleus plays a central role. It is generally believed that DR serotoninergic neurones are hypofunctional in depressive disorders or, alternatively, that a boost of this system helps relieve depressive symptoms. It is known that small-conductance calcium activated potassium (SK) channels regulate the firing frequency of DR serotonin neurones and that their overactivity leads to depressive symptoms in mice. The classical way to investigate the role of SK channels is the use of chemical blockers. Unfortunately, these molecules do not allow to discriminate between the different SK channels subtypes (SK1, SK2 and SK3) and keep us from understanding precisely the SK subtype(s) that is(are) critical in the regulation of their firing frequency. In that context we aim to develop a CRISPRi (Clustered Regularly Interspaced Short Palindromic Repeats inhibitory) based approach for precisely inhibiting the expression of the SK2 or/and SK3 subtypes in the mouse dorsal raphe. For that purpose, we adapted the organotypic brain slice culture method for obtaining P1 mice pups brain slice cultures, which we keep up to two weeks in vitro. The interest of such slices is to apply and test the CRISPRi machinery and proceed to electrophysiology recordings after an incubation period. To verify the accuracy of this model

we are characterizing the cultured slices at different timepoints and comparing them to acute slices obtained at the same timepoints.
We will present the dorsal raphe slice culture procedure, the electrophysiological characterization of the cultured slices at different timepoints in culture and the comparison with acute slices. We will also show the effects of pharmacologically induced SK channels blockade in cultured slices.

28- UNRAVELING THE ROLE OF NALCN IN NEURAL CIRCUIT DEVELOPMENT

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The sodium (Na+)-leak channel (NALCN) is a non-selective ion channel essential for maintaining the neuronal resting membrane potential by conducting a background sodium leak current. Pathogenic mutations in the NALCN channelosome complex (comprising NALCN, UNC80, and UNC79) are associated with rare developmental and epileptic encephalopathies. Despite the frequent occurrence of cognitive deficits in NALCN-related disorders, the specific role of NALCN in brain development remains unclear. To address this gap, we investigated the role of Nalcn in cortical neural circuit formation using mouse models. We utilized publicly available single-cell RNA sequencing datasets along with Nalcn-Gfp reporter mice, immunohistochemistry, and electron microscopy to map Nalcn expression across various neural cell types, revealing specific enrichment in certain neuron types and subcellular compartments. Additionally, neuroanatomical and molecular characterizations of mouse models with Nalcn loss- and gain-of-function during corticogenesis demonstrated that Nalcn gain, but not loss-of-function, is necessary for generating a normal density of deep layer pyramidal neurons. Behavioral assessments of mouse models with Nalcn dysfunction in the cerebral cortex showed distinct sex-specific deficits. Males with Nalcn gain-of-function exhibited impaired spatial memory, while females showed reduced nest-building behavior compared to controls. Conversely, Nalcn loss-of-function in the cerebral cortex resulted in reduced survival rates beyond postnatal day 50, along with nest-building, anxiolytic-like behavior, and increased sociability in both males and females during early juvenile stages. In conclusion, our study illuminates the critical role of Nalcn in regulating the development of cortical glutamatergic circuits and provides insights into the pathophysiological mechanisms underlying NALCN channelopathies. These findings enhance our understanding of NALCN's

function in cortical circuit formation and suggest its potential as a therapeutic target during crucial periods of cortical glutamatergic circuit development.

29- NALCN EXPRESSION ACROSS DEVELOPMENTAL STAGES AND CELLULAR IDENTITIES IN THE MOUSE CEREBRAL CORTEX

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Our goal is to characterize the spatiotemporal expression of the sodium leak channel non-selective (Nalcn) in the mammalian cerebral cortex. Using publicly available single-cell RNA-sequencing datasets, we explore gene expression of Nalcn at different time points of development and adulthood and in different brain cell types from the mouse cerebral cortex. Our analysis shows a significant upregulation of Nalcn transcripts in particular cortical neurons when compared to non-neuronal cells. Moreover, when examining Nalcn expression across developmental stages, we observed a noteworthy abundance of Nalcn transcripts at specific time points. In particular, early embryonic stages display higher counts of Nalcn transcripts for specific neuronal types. To further validate this in silico analysis, we determined the protein levels of NALCN in mouse cortical tissue at different developmental stages using SDS-PAGE and Western blot. We observed a notably abundance of NALCN during early developmental stages in mouse cortical tissue, gradually decreasing in postnatal and adult stages. These insights provide valuable knowledge of the expression of Nalcn during development and highlight its potential role in neurodevelopmental processes. The comprehensive approach combining both single-cell RNA-sequencing and molecular analysis offers novel perspectives on the complex regulation of NALCN expression in the brain, opening the door to future investigations about its role in neural development and function.

30- IDENTIFICATION OF A BINDING SITE FOR SMALL MOLECULE INHIBITORS TARGETING HUMAN TRPM4

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Transient receptor potential (TRP) melastatin 4 (TRPM4) protein is a calcium-activated monovalent cation channel associated with various genetic and cardiovascular disorders. The anthranilic acid derivative NBA is a potent and specific TRPM4 inhibitor, but its binding site in TRPM4 has been unknown, although this information is crucial for drug development targeting TRPM4. We determined three cryo-EM structures of full-length human TRPM4 embedded in

native lipid nanodiscs without inhibitor, bound to NBA, and a new anthranilic acid derivative, IBA. We found that the small molecules NBA and IBA were bound in a pocket formed between the S3, S4, and TRP helices and the S4-S5 linker of TRPM4. Our structural data and results from patch clamp experiments enable validation of a binding site for small molecule inhibitors, paving the way for further drug development targeting TRPM4.

31- OPTIMIZATION OF HUMAN ACID-SENSING ION CHANNEL 1A (hASIC1a) FOR ION CHANNEL-BASED BIOSENSOR DEVELOPMENT

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The biosensor development industry has witnessed a significant growth in recent years due to the increasing demand for implantable, biodegradable biosensors capable of real-time monitoring of physiological parameters. Our work focuses on optimizing the human acid-sensing ion channel ASIC1a, which is a proton-gated, transiently activated Na+-channel, for the development of an ion channel-based biosensor for pH. ASICs are expressed in the central and peripheral nervous system and play crucial roles in physiology such as synaptic transmission and fear conditioning, as well as in pathology since activation of ASICs after ischemia contributes to neuronal cell death. Four genes encode at least six subunits in rodent ASIC1a/b, ASIC2a/b, ASIC3, and ASIC4. ASIC1a is the most pH-sensitive subunit in the CNS, while ASIC3 is the most pH-sensitive in the PNS. Our objective was to identify mutants with four specific properties: high current, substantial sustained current (Isustained/Ipeak ratio), an alkaline shift of the pH dependence of activation, pH50, and acidic shift of desensitization pH dependence, pHD50. Thirty-seven mutants were tested with electrophysiology. The screening resulted in 25 mutants with substantial sustained current, six mutants showing an alkaline shift of the pH50 and five mutants with an acidic shift of the pHD50. L415K and Q276R were selected for further development due to their stable sustained current at varying pHs, suggesting their potential for long-term pH sensing without desensitization. Subsequently, the WT and L415K mutant were expressed in CHO cells as fusion proteins with a histidine tag and GFP. Our future work will involve reconstituting the purified channels in liposomes with different lipid compositions, to identify the lipid composition that ensures optimized channel activity, potentially enhancing the stability of the biosensor. In conclusion, our findings underscore the potential of mutations in the palm domain of ASICs to shift the pHD50 towards the acidic range and generate substantial sustained currents. Nevertheless, further optimization is necessary to shift the pH50 of activation to a more alkaline range, representing a critical step in advancing our biosensor development efforts.

32- CONDITIONAL ABLATION OF L-TYPE CAV1.3 CHANNELS IN THE CARDIAC CONDUCTION SYSTEM INDUCES BRADYCARDIA AND LOSS OF POSITIVE CHRONOTROPIC EFFECT OF CATECHOLAMINES UPON SIMULTANEOUS INHIBITION OF THE FUNNY (IF) CURRENT

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Background: The spontaneous activity of pacemaker cells in the sinoatrial node (SAN) controls the heart rhythm under physiological conditions. Among the wide array of ionic channels that SAN expresses, pacemaker cells constitute a unique cardiac cell type, as they co-express two functionally distinct isoforms of L-type Ca2+ channels: the cardiac Cav1.2 (Cacna1c) isoform and the Cav1.3 (Cacna1d) isoform. Mice lacking Cav1.3 channels globally show slow pacemaker activity but also an increased susceptibility to atrial fibrillation, potentially due to loss of Cav1.3 in the atria. We aim to evaluate the effects of Cav1.3 inactivation in the conduction system.
Material and Methods: We generate a conditional floxed Cav1.3 allele (Tamoxifen activated) in the channel pore Cacna1d-eGFPflex (Cav1.3Flex), selective for HCN4 expressing cells by crossing the Cav1.3Flex line with the HCN4CreERT2 one.
Results: Tamoxifen-treated Cav1.3Flex-HCN4CreERT2 mice displayed significant bradycardia compared to untreated group. We observed moderate rate reduction in isolated heart and SAN cells from Tamoxifen-treated Cav1.3Flex-HCN4CreERT2 mice. Isolated hearts and SAN cells from Tamoxifen-treated Cav1.3Flex-HCN4CreERT2 mice, showed increased pacemaking after perfusion of isoproterenol (ISO), similar to the unfloxed Cav1.3Flex counterparts. However, perfusion of ivabradine abrogated the chronotropic response to ISO in Cav1.3Flex-HCN4CreERT2, but not in unfloxed Cav1.3Flex hearts. Cav1.3 mediated current (ICav1.3) was strongly reduced in Tamoxifen-treated Cav1.3Flex-HCN4CreERT2 mice compared to Ctrl ones. Selective blockade of Cav1.2 by calciseptine, showed that deletion of Cav1.3 did not affect ICav1.2 in SAN cells.
Conclusions: Our data demonstrate that selective deletion of Cav1.3 in the conduction system induces sinus bradycardia and reduces SAN pacemaker activity. In addition, the Cav1.3Flex-HCN4CreERT2 mouse demonstrates that when If is inhibited, the positive chronotropic effect of catecholamines is reliant on Cav1.3 channels, underscoring the fundament role of this SAN L-type isoform in pacemaking.
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33- INVESTIGATING THE PATHOPHYSIOLOGY OF VARIOUS NAV1.4 MUTATIONS IN PARAMYOTONIA CONGENITA AND THEIR SENSITIVITY TO DIFFERENT NEGATIVE MODULATORS

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Paramyotonia congenita (PMC) is a rare neuromuscular disorder characterized by muscle stiffness that is exacerbated during exercise and by cold temperatures. This condition results from dominant mutations in the SCN4A gene, encoding the main subunit of the Nav1.4 sodium channel which is crucial for muscle action potential propagation. Nav1.4 channels transition through three states: closed, open (activated), and inactivated. Mutations in Nav1.4 disrupt these transitions, usually leading to impaired inactivation, increased repetitive firing, and delayed relaxation.
In our study, we characterized the functional impact of Nav1.4 mutations, focusing on the wild-type, the L1436P mutant, of which a cluster of patients was found in the Liège area by FCW, and had never been characterized so far, and R1448H variants. We conducted whole-cell patch clamp recordings using transiently transfected HEK293 with either wild-type or mutant Nav1.4 α subunits alongside β1 subunits. Our findings indicated that different mutations have different effects on channel characteristics
We further investigated the effects of temperature on these mutations, examining inactivation defects at room temperature and under cold (15 °C) conditions. Our experiments reproduced the cold sensitivity observed in PMC patients, with defects becoming more severe at lower temperatures.
Additionally, we tested the efficacy of Nav1.4 channel negative modulators, flecainide, and lidocaine, on normal and mutant channels. We confirmed that these two compounds act very differently on the channels.
By understanding how each mutation is affected by different drugs, we aim to develop treatments that might be tailored to the various mutations underlying PMC, potentially advancing these findings to clinical applications.

34- INVOLVEMENT OF CAMKK PATHWAY IN THE MODULATION OF A -BUNGAROTOXIN-INSENSITIVE NICOTINIC ACETYLCHOLINE RECEPTORS IN INSECT NEUROSECRETORY CELLS

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Neonicotinoids are very effective systemic insecticides that target insects' nicotinic acetylcholine receptors (nAChRs). Due to their high efficacy and selectivity, they were the most widely used insecticides in agriculture. However, because of the extensive and uncontrolled use, many insects' species developed high levels of resistance, posing significant challenges to pest management strategies. Therefore, understanding the resistance mechanisms to neonicotinoids in insects is of great importance. In our project, we aim to further explore how nAChRs regulation through intracellular calcium (Ca2+) pathways can

alter their sensitivity to neonicotinoids. These pathways include various kinases and phosphatases that transduce intracellular Ca2+ signals into changes in nAChRs activity.
Recent electrophysiological studies performed on insect neurosecretory cells found that calcium/calmodulin-dependent protein kinases (CaMKs) family can modulate nAChRs phosphorylation state and change their sensitivity to neonicotinoids. For example, in a previous study, we demonstrated that CaMKII inhibition can reduce nAChRs respond to the neonicotinoid, clothianidin. To explore more how can intracellular calcium pathways affect nAChRs activity, we used cockroaches' Periplaneta americana dorsal unpaired median (DUM) neurons. These neurons are a class of insect neurosecretory cells that express two different α-bungarotoxin- insensitive nAChRs subtypes, nAChR1 and nAChR2, that have different pharmacological properties and sensitivity to neonicotinoid.
Using whole-cell patch-clamp technique, we found that a distinct CaMKK/AMPK pathway is involved in the intracellular regulation of nAChRs. Our results show that the selective inhibition of CaMKK reduced nicotinic currents amplitude through nAChR2. Additionally, both AMPK selective activation and inhibition had a significant effect on nicotine-induced currents through nAChR2.
We concluded that a potential distinct CaMKK/AMPK pathway could be involved in the intracellular regulation of nAChR2, and these findings provide insights into how calcium signaling can influence receptor sensitivity, unraveling the mechanisms of neonicotinoid resistance.

35- ROLE OF NON-VOLTAGE-DEPENDENT CALCIUM CHANNELS IN GLIOBLASTOMA STEM CELLS.

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Glioblastoma (GBM) is the most common and most aggressive primary brain tumor. Despite treatment by radiotherapy, chemotherapy with temolozomide (TMZ) and surgery, median survival is poor, at around 15 months, mainly due to tumor recurrence in almost all patients. Tumor recurrence has been attributed to the presence of cancer stem cells (GSCs). This small subpopulation of tumor cells shares proliferative and self-renewal properties with stem cells, and is resistant to current treatments. Transcriptomic studies disclosed an enrichment of calcium signaling transcripts in GSCs. In non-excitable cells, store-operated channels (SOCs) represent a major route of calcium influx. As we have previously observed that SOCs regulate the self-renewal of adult neural stem cells which are possible cells of origin of GSC, we investigated the possible roles of SOCs in GSCs.
We analyzed the roles of SOCs in cultures of GSCs derived from five different glioblastoma surgical specimens. Our data showed that GSCs express two core SOC proteins (Orai1 and TRPC1) that support store-operated calcium entries (SOCEs). Pharmacological inhibition of SOCEs decreased proliferation, impaired self-renewal, and reduced expression of the stem cell marker SOX2 in GSCs. A previous multicenter phase IB clinical trial showed that

carboxyamidotriazole (CAI), an oral non-voltage-dependent calcium channel blocker, in combination with TMZ is safe and can increase median progression-free survival by up to 28 months in newly diagnosed patients. Our ongoing studies indicate that CAI decreases SOCEs. Pharmacological inhibition of the main store operated channel Orai1 reduces SOCEs and no further effect was observed when an Orai1 inhibitor was combined with CAI, suggesting that CAI acts via SOCE pathways. Exposure of GSCs to CAI reduces proliferation and self-renewal capacities and enhances the antitumoral properties of TMZ.
As calcium signaling is involved in tumor progression and stem cell properties, and as CAI can be used therapeutically, our work showing an involvement of non-voltage-dependent calcium channels in GSCs, which are responsible for tumor growth and relapse, provides a better understanding of the mechanisms that control GSCs and could contribute to improving the therapeutic management of GBM.

36- NEURONAL KATP CHANNELS ARE DISPENSABLE FOR GLUCOSE HOMEOSTASIS IN MICE

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The ATP-sensitive potassium (KATP) channels consisting of Kir6.2 and SUR1 subunits are crucially involved in the tight regulation of glucose homeostasis. Without functional KATP channels, pancreatic β cells fail to secrete insulin properly, but the impacts on central nervous system-mediated aspects, though having been implied, remain elusive due to the difficulties to precisely target neural populations. In this study, we use a conditional Kir6.2 knockout mouse strain to show that neuronal KATP channels are not required for the regulation of these glucose homeostatic aspects. However, the loss of functional pancreatic KATP channels induces hyperglycemia and glucose intolerance as the circulating glucose levels become unstable under different physiological states, and these aberrations can be reversed upon the exclusive resuscitation of pancreatic KATP channels. Pancreatic KATP channels are thus sufficient to maintain the stability of circulating glucose levels and should be treated as the primary target for therapeutic intervention to rescue glucose dyshomeostasis.

37- CHANNELOME: TOWARDS A PARADIGM SHIFT IN DRUG SCREENING

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Voltage-gated ion channels (VGICs) are specialized proteins crucial for generating and regulating electrical signals across cell membranes. They play an essential role in the proper functioning of various cells and organs, making them important targets for research in cell physiology, drug discovery, and quality control. Traditionally, the manual patch-clamp method

has been the gold standard for studying the biophysical properties of ion channels. However, this technique is limited by its capacity to investigate only a small number of ion channels simultaneously, which poses challenges for comprehensive research and drug screening across the entire spectrum of VGICs.
The introduction of automated patch-clamp systems has significantly advanced the field by enabling high-throughput recordings from thousands of cells daily. Nevertheless, many contemporary studies still rely on conclusions derived from older, more limited experiments. For example, compounds tested on a limited number of ion channels are often labeled as "specific blockers," and drugs are sometimes prematurely deemed non-interacting after being screened against only a subset of ion channels. Given the critical role of VGICs in drug safety and efficacy, it is imperative to conduct thorough and comprehensive testing. However, even major pharmaceutical companies frequently assess multiple drug candidates against only a select group of ion channels rather than conducting exhaustive testing against all VGICs.
In this study, we developed a novel ChatGPT-based AI tool to screen the published effects of drugs on all VGICs, providing a first-of-its-kind comprehensive analysis of a drug's reported impact on VGICs. Alongside this, we employed an in-house generated library of ion channel-expressing stable CHO cell lines in a Ready-to-Record format and utilized a high-throughput automated patch-clamp robot for drug screening. Our findings demonstrate that it is now feasible to screen multiple drugs across all major VGICs within a week. We illustrate this capability through the screening data of 10 different drugs, highlighting the potential for expansive and holistic drug testing.

38- COMPARATIVE EFFECTS OF NEONICOTINOIDS AND NEWLY INTRODUCED INSECTICIDES ON HUMAN A7 AND MAMMALIAN A4B2 NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS

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Neonicotinoid insecticides, widely recognized for their effectiveness in pest control, have been banned in the European Union due to concerns over their potential adverse effects on the environment and human health. Understanding the binding properties and pharmacological impacts of neonicotinoids and their derivatives on mammalian nicotinic acetylcholine receptors (nAChRs) remains crucial for developing safer and more targeted pest control strategies.
This study aims to compare the effects of two commonly used neonicotinoids, imidacloprid (IMI) and clothianidin (CLT), with the newly introduced pesticides sulfoximines (sulfoxaflor, SFX) and butenolides (flupyradifurone, FLU). Through electrophysiological recordings and site-directed mutagenesis, we investigated how these insecticides interact with human α7 and the two stoichiometries of the rat α4β2 neuronal nicotinic acetylcholine receptors (α4)3(β2)2 and (α4)2(β2)3.
Our results indicate that the tested insecticides act as weak agonists on the wild-type α7 and (α4)3(β2)2 receptors, and do not activate the (α4)2(β2)3 stoichiometry. Additionally, three mutations in the α7 receptor (E211N, E211P, and Q79K), which influence neonicotinoid binding, enhanced the effects of IMI, CLT, and FLU. Furthermore, the E226P mutation in the α4 subunit and the L273T mutation in the β2 subunit of the (α4)3(β2)2 receptor increased the

effects induced by IMI, CLT, SFX, and FLU. Interestingly, both mutations were able restore the insecticide-induced responses in the (α4)2(β2)3 stoichiometry.
We succeeded in identifying residues involved in the binding characteristics of these insecticides to the receptors. These findings provide insights that could lead to the development of more selective and safer compounds.

39- PROBING PROTONATION-DRIVEN CONFORMATIONAL CHANGES IN THE ASIC1A B-TURN DOMAIN THROUGH FLUORESCENCE MEASUREMENTS

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Acid-sensing ion channels (ASICs) are crucial components of the nervous system, playing pivotal roles in processes like learning, fear behaviors, pain sensation, and neurodegeneration following stroke. These voltage-independent Na+-selective channels are activated by extracellular acidification, leading to transient inward currents before entering a non-conducting desensitized state. Functional ASICs are trimers, comprised of three subunits, featuring a hand-like extracellular structure. The mechanisms of protonation leading to ASIC activation remain unclear. Protonation occurs at extracellular sites, yet induces conformational changes that control gate position, causing it to open or close. Understanding these alterations is crucial for comprehending ASIC function. ASIC1a, highly expressed in the central nervous system, is a promising drug target due to its involvement in various pathologies, highlighting the importance of understanding the molecular mechanisms driving its activation. Using voltage-clamp fluorometry (VCF), we investigate the involvement of amino acid residues within the β-turn in hASIC1a activation. This region connecting palm and thumb domains interacts with transmembrane domain TM1 and potentially influences channel gating. Our findings reveal conformational changes near the β-turn region, suggesting that protonation events may drive these alterations. An analysis of fluorescence signal kinetics was performed to corroborate the association between fluorescence changes and functional transitions, revealing potential conformational rearrangement sequences within ASIC1a's β-turn domain. Investigating activity-dependent conformational changes offers valuable insights into how protonation regulates ASIC activity, deepening our understanding of these channels.

40- INVESTIGATION OF INTRACELLULAR DOMAINS OF NAV1.5 BY TANDEM PROTEIN TRANS-SPLICING

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Voltage-gated sodium channels (Nav1.5) are transmembrane proteins mainly expressed in cardiomyocytes. They mediate the initial fast sodium influx that follows membrane depolarization, thus shaping the cardiac action potential. Hundreds of variants of these channels have been linked to life-threatening cardiac arrhythmias. Numerous of these variants

act by impairing inactivation, i.e. the rapid closing of the Nav1.5 ionic pore that normally prevents hyperexcitability in cardiomyocytes. Thus, inactivation is of tremendous physiological importance. Previous functional studies and recent Cryo-EM structures have revealed that intracellular domains of Nav1.5 are involved in the occlusion of the ionic pore during inactivation. However, the precise molecular mechanisms of this fast inactivation process remain unclear.
Nav1.5 intracellular domains are subject to extensive post-translational modifications, such as phosphorylation, which are suggested to regulate channel function. However, studying the functional effects of phosphorylation is difficult because the presence of both kinases and phosphatases present in a cellular environment makes it impossible to predict a desired degree of phosphorylation or lack thereof.
To overcome these challenges, we use tandem Protein Trans-Splicing, which allows us to express an engineered Nav1.5 channel at the membrane of a living oocyte. To this end, we attach two orthogonal split intein pairs to synthetic peptides containing either non hydrolysable phosphorylations or fluorescent probes within sequences corresponding to intracellular linkers of Nav1.5. We then inject these peptides into oocytes expressing the remaining Nav1.5 fragments and study the resulting full-length channels by optical and electrophysiological techniques.
This strategy aims to gain insight into functional effect of phosphorylation, its putative impact on protein-protein interactions and into the molecular motions that occur in the intracellular domains of Nav1.5 channels.

41 - INVESTIGATION OF THE EFFECT OF THE NaV1.5/R219H VARIANT ON THE DEVELOPMENT OF DILATED CARDIOMYOPATHY IN MICE

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Keywords: Dilated cardiomyopathy, SCN5A, gating pore current

Dilated cardiomyopathy (DCM) is a myocardial dysfunction characterized by an enlargement of the left ventricle leading to impaired contraction function and a decrease in systolic ejection fraction. Numerous mutations have been identified on genes encoding proteins of the contractile apparatus in patients with DCM. However, several mutations have also been detected in the SCN5A gene, which encodes the voltage-dependent sodium channel SCN5A ($Na_V1.5$), a protein responsible for the depolarization phase of the cardiac action potential. Recently, it has been discovered that the R219H mutation, located in the voltage-sensing domain of the $Na_V1.5$ channel, leads to the development of complex arrhythmias associated with DCM. In cardiomyocytes derived from induced pluripotent stem cells, this mutation generates a proton leak current at rest (omega current) which would be responsible for the deregulation of numerous intracellular processes that are still poorly understood. After inducing the $Na_V1.5$/R219H mutation in a knock-in mouse model, our objective was to investigate the development of dilated cardiomyopathy and its deregulated associated mechanisms across male and female wild-type, heterozygous, and homozygous phenotypes. To accomplish this, we employed in vivo approaches including electrocardiogram and echocardiogram recordings. We examined sarcomere organization through immunocytofluorescence and conducted measurements of action

potentials, sodium, and omega currents via patch-clamp on isolated ventricular cardiomyocytes. In parallel, we conducted mRNA-sequencing to identify dysregulated intracellular pathways. Preliminary findings confirmed the presence of a dilated left ventricle in elderly male mice, accompanied by electrical conduction abnormalities. Proton leak current was observed in heterozygous and homozygous mice, while minimal differences were detected in action potential and sodium channel biophysical properties. The mRNA-sequencing analysis enabled us to formulate hypotheses to explain the development of dilated cardiomyopathy in $Na_V1.5$/R219H mutant mice.

42 IDENTIFICATION OF A NEW TYPE OF PREPULSE FACILITATION IN HONEYBEE $C_{aV}4$ Ca^{2+} CHANNEL:

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We have recently identified a honeybee homologue of DSC1, the *Drosophila* channel 1 displaying sequence similarity to the voltage-gated sodium channel (Na_V), identified over twenty years ago. This channel exhibits strict selectivity for Ca^{2+}, an unprecedented type of inactivation, which depends on both an IFM motif, like Na_V, and on the permeating divalent cation, like CaV1.2, and defines a new family of Ca^{2+} channels, called Ca_V4. We show that Ca_V4 exhibits a prepulse-dependent current facilitation that develops during depolarisations of small amplitudes. Increasing the duration of the depolarisations decreases current facilitation. Analysis at the single channel level demonstrate that neither the single current amplitude nor the mean channel open-time, the short closed-time or the number of opening per burst are affected. This current–dependent facilitation is primarily due to an increase in the open probability at -20 mV. Our study underscores the unique profile of the Ca_V4 Ca^{2+} channel and defines this channel as a novel class of voltage-gated Ca^{2+} channel.

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USEFUL INFORMATION

The attendees are expected on Sunday, between 4PM and 7PM. During this period, they would have the time to proceed to their check-in and meet each other before a welcome drink and the diner. The congress will begin on sunday evening with the plenary lecture and finish on Wednesday after lunch.

The conference will take place at Centre de vacances du Lazaret, a leisure center at Sète on the Mediterranean coast of France, close to the city of Montpellier.

A forum has been setup if you want to share a taxi or a car (personal or rental one).

Directions to "Le Lazaret"

Le Lazaret
La Corniche
223 Rue Pasteur Benoît
34200 Sète

Tel: +33 (0)4 67 53 22 47
Fax: +33 (0)4 67 53 36 13
Web: www.lazaret-sete.com
Mail: le-lazaret@capfrance.com
GPS coordinates:
43°23'40.01 N
003°40'26.60 E

Arriving

By Air:
Montpellier Méditerranée Airport is the closest airport. At you arrival you will find taxi. We strongly encourage you to share a taxi using the forum.
If you want to book a taxi in advance you can use
Taxi Valenti, +33 611 57 18 05, taxi.valenti@sfr.fr

By train:
In Sète, there is a SNCF railway station, which is covered by TGV. At your arrival you can find taxis and public transportation. For city bus you can take a bus of the n°3 line operating to the Centre Malraux and stop at the halt "Plan de la Corniche". Alternatively, you can take a bus of the n°9 line operating to Marseillan Plage and stop at the halt "Le Lazaret". You can check for timetables, directions and prices at the following web address: https://www.mobilite.agglopole.fr/

By Car :

Free parking are available at the center of "le Lazaret".


Le Lazaret,223 Rue Pasteur Benoît, 34200 Sète
Sète
Port de Sète
Gare de Sète
Quai d'Alger
Quai de Bosc
Avenue Victor Hugo
Avenue Gilbert Martelli
Route de Cayenne
Rue de Bruxelles
Rue de Copenhague
Rue de Madrid
Avenue des Eaux Blanches
Michelot Hervé
Pointe Longue
Grande Rue Haute
Le Môle Saint-Louis
Eglise Saint Joseph
OPEN SPACE Galerie
Centre Régional d'Art Contemporain
Musée Paul Valéry
Musée De La Mer
Rue de la Révolution
Rue Paul Bousquet
Rue Jean Moulin
Rue Prévot d'Augier
Rue de la Caraussane
Rue de Belfort
Chemin de Saint-Clair
Rue Jean Vilar
Prom. Maréchal Leclerc
Crique de l'Anau (la Conque)
Rue Robespierre
Boulevard Chevalier de Clerville
Chemin du Glacis
Chemin des Pierres Blanches
Combe d'Aubagnac
Chemin de la Pipe
Rue d'Artois
Casino de Sète
Corniche de Neuburg
Plage du Lazaret
Chemin des Hirondelles
Rue des Cormorans
Rue des Goélands
Rue du Football
Avenue de la Source
Rue des Loriots
Rue des Roitelets
Auchan Sète
Espace Georges Brassens
Boulevard Jean Mathieu Grangent
Montée des Pierres Blanches
Rue d'Aquitaine
Chemin des Quilles
Plage de la Corniche
Cimetière de Py
Canal des Quilles
Chemin Château de Villeroy
Route des Quatre Chansons
Avenue du Ponant
Plage des Quilles
Le Dôme
Tanger, MA - Sète, FR
Nador, MA - Sète, FR
Plan
Satellite
Google
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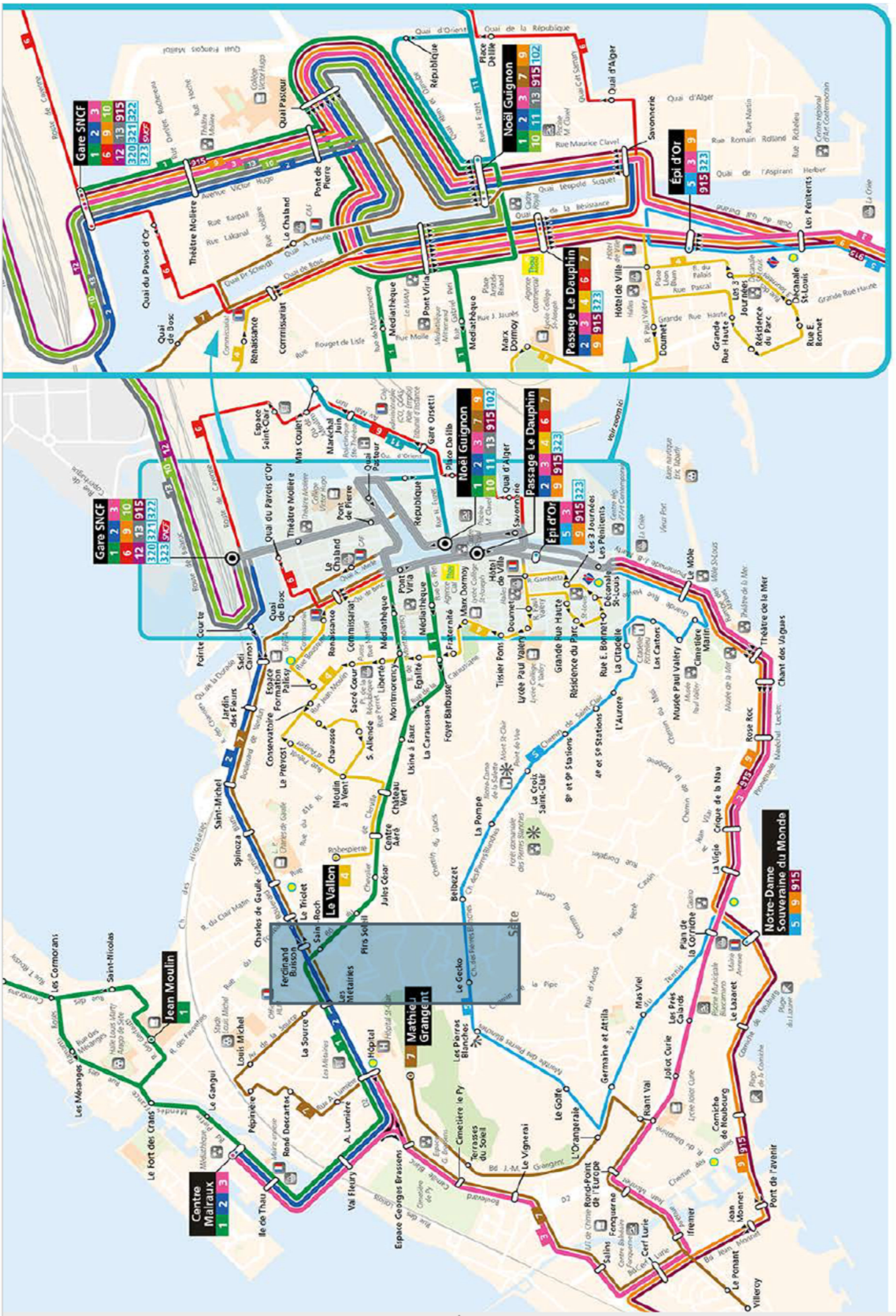
Gare SNCF
Noël Guignon
Passage Le Dauphin
Épi d'Or
Centre Malraux
Jean Moulin
Mathieu Grangent
Le Vallon
Notre-Dame Souveraine du Monde

MISCELLANEOUS

Lazaret Holiday Village

www.lazaretsete.com

Rue du Pasteur Lucien Benoît, 34200 Sète Téléphone : 04 67 53 22 47

Tourist office

www.tourisme-sete.com/

60, Grande Rue Mario Roustan, 34200 Sète, 04 99 04 71 71

Public bus

http://mobilite.thau-agglo.fr/eng, 04 67 53 01 01

The direct bus line between the Sète SNCF railway station and the Lazaret are the line 3 and 9

Espace Georges Brassens

http://www.espace-brassens.fr/

67 Boulevard Camille Blanc, 34200 Sète, France 04 99 04 76 26

Musée Paul Valéry

http://www.museepaulvalery-sete.fr/

Rue François Desnoyer, 34200 Sète, France 04 99 04 76 16

Others activities

https://www.tripadvisor.fr/Attractions-g660465-Activities-Sete_Herault_Occitanie.html#ATTRACTION_LIST