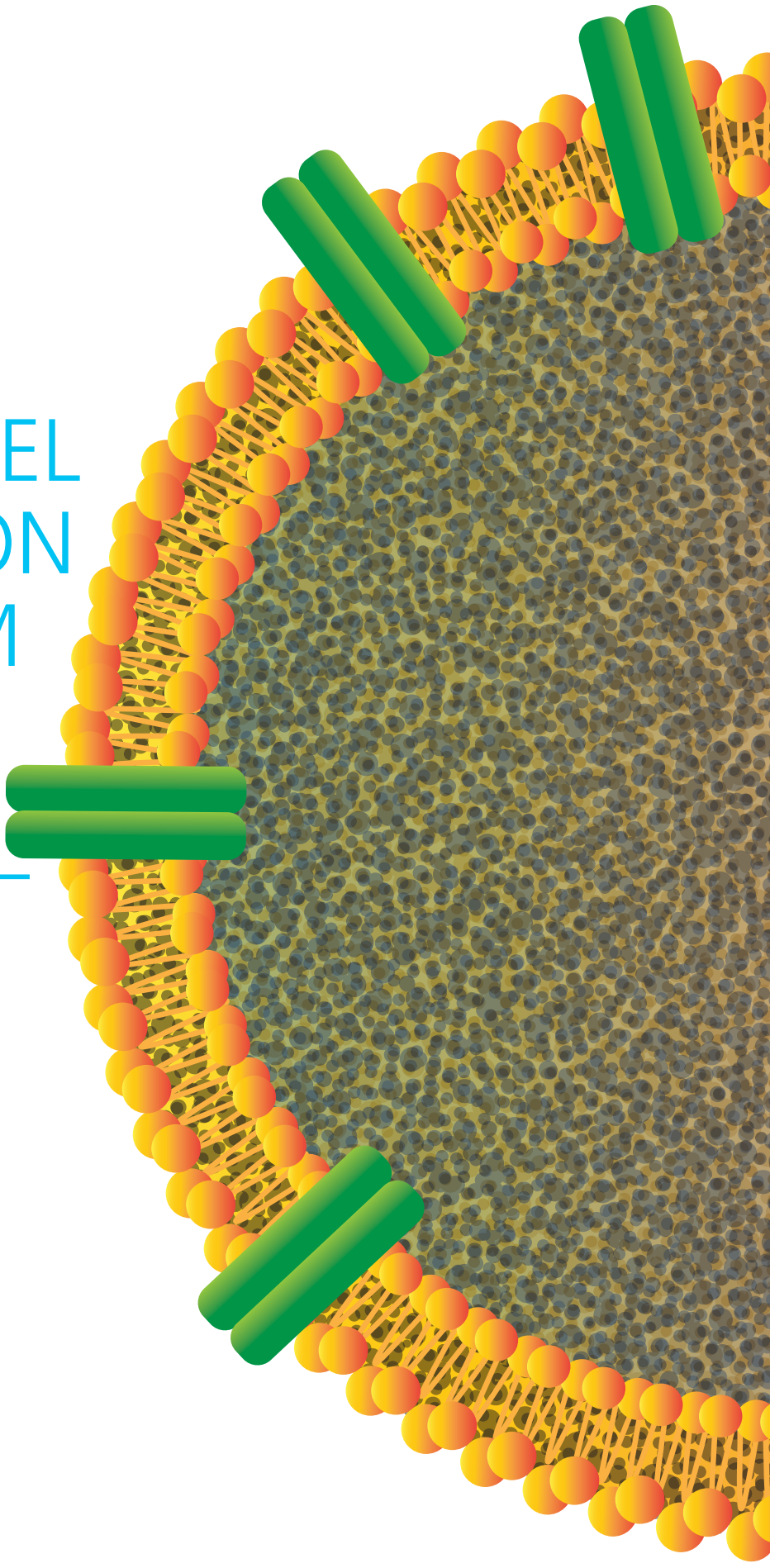
ION CHANNEL MODULATION SYMPOSIUM 2022



November 5 - 6, 2022

Venue:
NYU Grossman School of Medicine
Science Building
The Schwartz Lecture Hall and Lobby

Co-hosted by:

SOPHION BIOSCIENCE

NYU Grossman School of Medicine

Index:

Sponsors:

metrion biosciences

ION BIOSCIENCES

Agenda - November 5, 2022

08.00 AM	Sign-in - Tea/Coffee
09.00 AM	Welcome Remarks - Thomas Binzer - Sophion Bioscience
	Session 1 - Chair: Stefan Feske - New York University, Grossman School of Medicine
09.15 AM	Merritt Maduke - Stanford University *Developing pharmacological tools to selectively target CLC chloride-channel*
09.40 AM	Dianne Lipscombe - Brown University *Forms and Functions of Voltage-Gated Calcium Ion Channels Critical for Adaptive and Maladaptive Pain*
10.05 AM	Rajesh Khanna - New York University, Pain Research Center *Answers to $Na_V1.7$ Analgesic Failures: Post Translational Targeting for Pain Reduction*
10.30 AM	Tea/Coffee, Exhibits and Posters Session
	Session 2 - Chair: Stefan Feske - New York University, Grossman School of Medicine
11.15 AM	Heike Wulff - University of California, Davis *Kv1.3 and KCa3.1 as Targets for Neuroinflammation*
11.40 AM	Geoffrey Abbott - University of California, Irvine *Potassium channel-targeted small molecule discovery from plants*
12.05 PM	Anjali Rajadhyaksha - Weill Cornell Medicine *L-type calcium channel mechanisms and neuropsychiatric disorders*
12.30 PM	Janina Sörmann - Evotec SE *Defective X-gating of TASK-1 in a novel channelopathy associated with sleep apnea*
12.40 PM	Lunch - Exhibits and Posters
	Session 3 - Chair: William Coetzee - New York University, Grossman School of Medicine
02.00 PM	David Clapham - Howard Hughes Medical Institute *The primary cilia is a short circuit for receptor signaling to the nucleus*
02.25 PM	Bhama Ramkhelawon - New York University, Langone Health *Immunoprobing Piezo-1 signals in the vasculature*
02.50 PM	Daniel Sauter - Sophion Bioscience *A Journey through Patch-Clamp, Pain, and Passion*
03.15 PM	Tea/Coffee, Exhibits and Posters Session
	Session 4 - Chair: William Coetzee - New York University, Grossman School of Medicine
04.00 PM	Alexandra Pinggera - Metrion Biosciences *Advantages of the novel dynamic action potential patch clamp technique to characterise Nav1.2 disease mutations*
04.25 PM	David Stokes - New York University, Langone Health *How to put a channel to work: the potassium transport system KdpFABC*
04.50 PM	Crina Nimigean - Cornell University *Calcium gating and ball-and-chain inactivation in potassium channels*
05.15 PM	Wrap up - Duncan Jarman - Sophion Bioscience
05.30 PM	Group Photo and reception

Agenda - November 6, 2022

08.00 AM	Tea/Coffee
09.00 AM	Welcome Remarks - Duncan Jarman - Sophion Bioscience
	Session 5 - Chair: Rajesh Khanna - New York University, Pain Research Center
09.05 AM	Mark Estacion - Yale University *Pharmacogenomics of hNa$_V$1.7 variants to Lacosamide from responsive and non-responsive SFN patients*
09.30 AM	Theanne Griffith - University of California, Davis *Illuminating new roles for sodium channels in sensory neurons*
09.55 AM	Stefan Feske - New York University *Novel Ion Channels and Transporters in Immunity*
10.20 AM	Tea/Coffee, Exhibits and Poster Session
	Session 6 - Chair: Rajesh Khanna - New York University, Pain Research Center
11.20 AM	Gerald Zamponi - University of Calgary *Post-translational modification of T-type calcium channels as a molecular target for pain*
11.45 AM	Colin Nicols - Washington University *Personalized therapies for KATP channel diseases*
12.10 PM	Vera Moiseenkova-Bell - University of Pennsylvania *Structural Insights into TRPV Channel Gating*
12.35 PM	Steven Marx - Columbia University *Rad regulation of Ca$_V$1.2 channels controls cardiac fight-or-flight response*
01.00 PM	Lunch - Exhibits and Poster Session
	Session 7 - Chair: Steven Marx - Columbia University
02.00 PM	William Coetzee - NYU Grossman School of Medicine *A novel Kir6.2 specific KATP channel opener*
02.25 PM	Zhaozhu Qiu - Johns Hopkins Medicine, Baltimore *From SWELL to PAC: discovery of novel chloride channels*
02.50 PM	Anissa Bara - Sophion Bioscience *Electrophysiological characterization of hiPSC-derived cardiomyocytes using automated patch clamp*
03.15 PM	Tea/Coffee
	Session 8 - Chair: William Coetzee - New York University, Grossman School of Medicine
03.45 PM	Amanda Klein - University of Minnesota *KATP channel involvement in pain, opioid efficacy, and morphine tolerance depends on subtype and cellular location*
04.10 PM	Murali Prakriya - Northwestern University *TStore-operated calcium channels in the brain: roles in gliotransmitter release, neuroinflammation, and control of depression behaviors*
04.35 PM	Henry Colecraft - Columbia University *Removing voltage-gated calcium channels by targeted ubiquitination to treat pain*
05.00 PM	Wrap up - Thomas Binzer - Sophion Bioscience

Biographies - Speakers

Geoffrey Abbott - University of California, Irvine

Dr. Geoffrey W. Abbott earned his Ph.D. in Biochemistry from University of London in 1997, after which he pursued his postdoctoral studies on ion channel function and disease at Yale University, supported by a Wellcome Trust Fellowship. He was made a tenured full professor at Well Cornell Medical School in 2011 after ten years on the faculty. Soon afterwards he moved to take up his present position as Professor of Physiology and Biophysics in the School of Medicine at Universityof California, Irvine, where since 2020 he has also been Vice Dean for Basic Science Research and Senior Associate Dean for Academic Personnel. Funded by multiple NIH institutes, Dr. Abbott currently studies voltage-gated potassium channels, especially their interaction with and modulation by ancillary subunits, solute transporters, neurotransmitters, metabolites, and new and ancient medicines. In 2022 he was awarded a Samueli Scholarship for his molecular mechanistic research in botanical ethnomedicine.

Anissa Bara - Sophion Bioscience

Anissa Bara is a neuroscientist who recently joined Sophion Bioscience as an application scientist, where she contributes to the development of electrophysiological assays on automated patch clamp systems and customer support. She obtained her PhD in Neuroscience at the Mediterranean Institute of Neurobiology (Marseille, France) in the laboratory of Dr. Olivier Manzoni, where she studied how environmental stressors (cannabis, malnutrition) during early life cricital periods can remodel synaptic networks using *ex-vivo* electrophysiology. In 2018, she joined the group of Dr. Yasmin Hurd at the Icahn School of Medicine at Mount Sinai (New York, NY) as a postdoctoral researcher, where she investigated the consequences of prenatal cannabis exposure on epigenetic regulation, neural networks and behavior.

David E. Clapham - Howard Hughes Medical Institute

David E. Clapham, MD, PhD, is a senior group leader at HHMI's Janelia Research Campus. Dr. Clapham interests are in the physiology of cells, in particular the discovery and characterization of new ion channels. Past work included the discovery of the G protein βϒubunits' activation of cardiac potassium channels, intracellular calcium waves, the structure and function of novel ion channels such as spermatozoan CatSpers1-4, the mitochondrial MCU calcium channel, bacterial voltage-gated sodium channels, lysosomal ion channels, TRP channels, and ciliary ion channels. Dr. Clapham received his degree in Electrical Engineering at the Georgia Institute of Technology. He earned his Ph.D. and M.D. degree at Emory University and completed residency at the Brigham and Women's Hospital in Boston. He was a Fulbright Fellow in the laboratory of Dr. Erwin Neher at the Max Planck Institute for Biophysical Chemistry where he worked alongside Fred Sigworth. In 1997 he was selected as an Investigator of the Howard Hughes Medical Institute, and in 2001 was appointed the Aldo R. Castañeda Professor of Cardiovascular Research and director of the basic cardiovascular research laboratories at Boston Children's Hospital. From 2016 to 2022 he was the Chief Scientific Officer of the Howard Hughes Medical Institute. Dr. Clapham received the Cole Award of the Biophysical Society, the Basic Science Award of the American Heart Association, and the Bristol Myers Squibb Award for Distinguished Achievement in Cardiovascular Research. Dr. Clapham was elected to the American Academy of Arts and Sciences, National Academy of Sciences, National Academy of Medicine, and as a Fellow of the American Association for the Advancement of Science. He received the William Silen Lifetime Achievement in Mentoring Award at Harvard Medical School.

William A. Coetzee, DSc - New York University, Grossman School of Medicine

Dr. Coetzee is a tenured Professor in the Department of Pathology at New York University School of Medicine, with joint appointments as Professor in the Department of Physiology and Neuroscience and Professor the Department of Biochemistry and Molecular Pharmacology. With funding from the American Heart Association, the National Institutes of Health, National Institute of Justice and several foundations, Dr. Coetzee's research over the years has focused on ion channel biology in neurosciences and the cardiovascular system. Current research includes investigations of mechanisms that regulate KATP channel trafficking and function, genetic variation of ion channel genes in cases of sudden infant death syndrome, and several studies in collaboration with other NYU investigators to investigate the roles of ion channels in cancer and immune cells.

Biographies - Speakers

Henry Colecraft - Columbia University

Henry M. Colecraft, Ph.D. is the John C. Dalton Professorand Associate Vice Chair in the Department of Physiology and Cellular Biophysics at Columbia University Irving Medical Center. Dr. Colecraft is an international leader in the molecular physiology of ion channel proteins that underlie signaling in nerve cells and the heart. His research group has contributed seminal advances to understanding molecularmechanisms underlying regulation of voltage-dependent Ca^{2+} and K^{+} channels by accessory subunits, posttranslational modifications, and signaling molecules. His group also studies how inherited mutations in ion channels lead to devastating diseases (known as ion channelopathies) that span the cardiovascular, neurological, and respiratory systems, and devising new therapies for them.

Mark Estacion - Yale University

Mark has been working in the group of Dr. Stephen Waxman at Yale University since 2007 focusing on the role that voltage-gated sodium channels play in modulating sensory neuron excitability. Before that he has worked at the Metro Health Medical Center in Cleveland focusing on mammalian Trp channels and at UC Irvine studying FGF-receptor induced calcium influx. My current research interests are focused on detailed characterization of the role voltage-gated sodium channels play in neurological syndromes such as inflammatory and neuropathic pain. I employ patch-clamp electrophysiology and live-cell optical imaging techniques to monitor ion fluxes that are involved in signal transduction in various cell culture model systems. Specifically, I have been studying voltage-gated sodium channel mutants expressed in both HEK cells as well as primary neurons. A focus of this research is to understand the role of specific Na_V subtypes in pain sensation.

Stefan Feske - New York University, Grossman School of Medicine

Dr. Feske is the Jeffrey Bergstein Professor of Medicine, Vice Chair for Research in the Department of Pathology and director of the Ion Channels and Transporters in Immunity (ICTI) program at NYU Grossman School of Medicine. He is also a scientific co-founder of CalciMedica. He graduated summa cum laude with a research thesis and M.D. from the University of Freiburg, Germany, where he did a residency in rheumatology. He conducted his postdoctoral studies at the Max-Planck-Institute for Immunobiology and Harvard Medical School, where he made essential contributions to the discovery of the CRAC channel protein ORAI1 and identified the first patients with mutations in ORAI1 and STIM1. Research in his lab at NYU is focused on understanding how CRAC channels regulate immune responses in health and diease and on identifying novel ion channels that control immune responses to infection, tumors and in autoimmunity.

Biographies - Speakers

Theanne Griffith - University of California, Davis

Dr. Theanne Griffith received her undergraduate degrees in neuroscience and Spanish from Smith College and earned her doctorate in neuroscience from Northwestern University. As a graduate student, she combined electrophysiology and molecular biology to investigate the structure and function relationship between ionotropic glutamate receptors and their auxiliary subunits. This work was the first study to identify regions within kainate receptors targeted for modulation by auxiliary subunits and was selected as an Editor's Pick in the Journal of Physiology. As a postdoctoral fellow at Columbia University, Dr. Griffith harnessed her knowledge of ion channel function to investigate the molecular mechanisms governing excitability of mammalian sensory neurons. This project found an unexpected role for the voltage-gated sodium channel, $Na_V1.1$, in mediating action potential firing in a subpopulation of cold-sensing neurons and was featured on the cover of the Journal of Neuroscience.Dr. Griffith is currently an Assistant Professor in the Department of Physiology and Membrane Biology at The University of California Davis, where her lab investigates the cellular and molecular mechanisms of somatosensory transmission in health and disease, with current projects focusing on voltage-gated sodium channels in proprioception and pain. Her lab uses a combination of electrophysiology, transgenic mouse models, behavior, imaging, and molecular profiling. Dr. Griffith is a UC Davis Center for Advancing Multicultural Perspectives on Science (CAMPOS) Scholar, and a 2022-2023 UC Davis Public Scholarship Faculty Fellow. In addition to her research, Dr. Griffith is a children's book author of the science adventure chapter book series, The Magnificent Makers, which is published by Random House Children's Books. She also co-writes the non-fiction science series, Ada Twist, Scientist: The Why Files, which accompanies the Netflix show of the same name. Her work on promoting diversity in STEM and inclusive representation in children's media has earned her invitations to consult for NPR science podcasts for children and to deliver keynote addresses to organizations such as the Michigan Science Teachers Association and the National Math and Science Initiative.

Rajesh Khanna - New York University, Pain Research Center

Rajesh Khanna, PhD, MSc, an internationally known expert in ion channels and pain research, was named director of the NYU Pain Research Center, effective January 1, 2022. Prior to joining NYU, Dr. Khanna was the associate director of the University of Arizona Health Sciences Comprehensive Pain and Addiction Center, a professor of pharmacology at the University of Arizona College of Medicine –Tucson, and a member of the BIO5 Institute. Dr. Khanna earned a PhD in physiology from the University of Toronto and completed a NSERC postdoctoral fellowship in the Department of Physiology and Cellular and Molecular Neuroscience at the University of California, Los Angeles. He has held faculty positions at the Toronto Western Research Institute, Indiana University—Purdue University Indianapolis, and the University of Arizona. He has authored more than 185 peer reviewed publications, is the recipient of multiple national and international patents, has directed the dissertations of five PhD candidates, and trained 16 postdoctoral fellows.

Amanda Klein - University of Minnesota

Amanda H. Klein, received her Ph.D. from the Molecular Cellular and Integrative Physiology program at the University of California Davis. During her graduate school career she has published manuscripts in the field of chemesthesis, looking at sensations of irritation, pain, and itch, in the oral cavity and skin by chemical mediators. During her postdoctoral training, at Johns Hopkins, Dr. Klein investigated the mechanisms of pain and itch in primate and rodent models. The current focus of Dr. Klein's research revolves around opioid signaling pathways including potassium channels, with the ultimate goal to reduce tolerance, and decrease withdrawal symptoms.

Biographies - Speakers

Diane Lipscombe - Brown University

Diane Lipscombe studies ion channel expression, modulation, and function with interest in the molecular and cellular mechanisms that underlie neurological and psychiatric diseases. Her research team recently discovered a new mechanism that controls cell-specific pre-mRNA splicing of a voltage-gated calcium ion channel important for the transmission of noxious stimuli and the development of hyperalgesia in nociceptors.

Merrit Maduke - Stanford University

Merritt Madukeis an Associate Professor of Molecular and Cellular Physiologyat Stanford University Medical School. She received a B.S. degree in Chemistry summa cum laude from Wheaton College, ILand a Ph.D. in Chemistry and Biochemistry from USCD. She did her postdoctoral training studying with Professor Chris Miller at Brandeis University. At Stanford, research in the Maduke laboratory centers on understanding the molecular mechanisms of ion channels and transporters, with an emphasis on the chloride-selective CLC family. CLC proteins are expressed ubiquitously and perform diverse physiological functions in cardiovascular, neuronal, muscular, and epithelial function. We use an array of biophysical methods to determine CLC molecular structure, dynamics, and mechanisms. In addition, we apply our expertise in ion-transport mechanisms in collaborations to develop novel chemical tools and to understand the mechanism by which ultrasound modulates ion-transport to effect neuromodulation. Our research is funded by the National Institutes of Health (NIGMS, NINDS, and NIDDK).

Steven Marx - Columbia University

I have broad scientific background in biochemistry, cellular electrophysiology (ion channels), and animal cardiovascular physiology. I have recruited an outstanding team of investigators, with expertise in biochemistry, cellular electrophysiology, and animal-based research. I am board certified in Cardiology and Clinical Electrophysiology and am an Attending Physician at one of the leading Cardiology programs in the nation. I am very committed to training the next generation of cardiovascular investigators. In addition to my role in mentoring pre-doctoral students and post-doctoral fellows, I am also the Director of the Cardiovascular Fellowship Program. I am the PI of a T32, recruiting postdoctoral fellows and mentoring our cardiology and surgical trainees. My research has: (1) Established the scientific basis for the development of the FDA-approved rapamycin-eluting coronary stents. Via aseries of seminal papers, demonstrating rapamycin as a potent inhibitor of smooth muscle proliferation and migration, we focused attention on rapamycin, enabling it to "evolve" froma failed antibiotic with no apparent therapeutic utility to itscurrent status as the only therapeutic for drug-eluting stents. (2) Identified dysfunctional ryanodine receptor activity as a molecular basis for arrhythmias and heart failure. This work was published in Science and Cell. (3) Identified the molecular basis for adrenergic modulation of the human cardiac action potential duration through regulation of the slowly activating delayed rectifier. This work was published in Science. (4) Studies of atrial fibrillation, the most common human arrhythmia, have been hindered by the lack of a mouse model that accurately recapitulates human disease. This work, published in Journal of Clinical Investigation and JCI-Insight, introduced a transgenic mouse model of atrial fibrillation that nearly precisely phenocopies human atrial fibrillation, and enabled the identification of a new therapeutic approach to treat atrial fibrillation. This work utilized telemetry to record arrhythmias *in vivo*. (5) Identified that cardiac lipid overload (lipotoxicity) causes sudden cardiac death from spontaneous ventricular tachycardia, using a transgenic mouse model. For this study published in Circulation, we used implanted ECG telemeters to record PVC and fatal ventricular arrhythmias. (6) Elucidated the mechanisms underlying regulation of the cardiac calcium channel in health and disease. Despite the efforts of many leading scientists, the mechanisms responsible for adrenergic regulation of the calcium influx in the hearthave been controversial and unsolved for ~40 years. Using innovative approaches including proximity proteomics in mice, we identified the mechanism of adrenergic regulation of calcium influxin heart. This work was recently published in Nature, with a follow-up study in Circulation Research.

Biographies - Speakers

Vera Moiseenkova-Bell - University of Pennsylvania

Dr. Moiseenkova-Bell is a membrane protein biochemist and a structural biologist with expertise in cryo-electron microscopy (cryo-EM). Her research is focused on structure-function analysis of Transient Receptor Potential (TRP) channels and their interaction with agonists/antagonists to enhance our understanding of their function at the molecular level. In addition, her laboratory research program seeks to understand how TRP channels regulate cellular functionsand the role of their dysregulation in human disease. After obtaining a M.S. degree in Physics from Moscow State University in 1999, Dr. Moiseenkova-Bell switched to a biological research area and received Ph.D. in Cellular Physiology and Molecular Biophysics from the University of Texas Medical Branch in 2004. During her graduate work, she was the first to develop a methodology for overexpression and purification of functional TRP channels for structural studies. As a postdoctoral fellow, Dr. Moiseenkova-Bell continued her work on TRP channels at Baylor College of Medicine (BCM). During her training at BCM, she was the first researcher to solve and report the structure of a TRPV1 channel using cryo-EM. Because of this achievement, she received the Ruth McLean Bowman Bowers Excellence in Research Award from BCM. In 2009, Dr. Moiseenkova-Bell joined Department of Pharmacology at Case Western Reserve University (CWRU) as a tenure-track Assistant Professor and was promoted to Associate Professor with tenure in 2016. Dr. Moiseenkova-Bell moved to University of Pennsylvania in 2018, where she is a Full Professor and she is continuing her work on understanding molecular mechanisms of TRP channel activation, inhibition and desensitization using cryo-EM at the Department of Systems Pharmacology and Translational Therapeutics. She is also a Faculty Director of the Beckman Center for Cryo-EM and Electron Microscopy Resource Laboratoryat the University of Pennsylvania. In the past twelve years, Dr. Moiseenkova-Bell established herself as an independent scientist and as an expert in the field of TRP channels, focused on structural and functional analysis. She has published papers in Journal of Biological Chemistry, Journal of General Physiology, Molecular and Cellular Biology, Structure, Cell Reports, Nature Communications, Nature Structural and Molecular Biology. She has given numerous invited seminars and presentations both at the national and international levels. Dr. Moiseenkova-Bell has secured funding for her research from American Lung Association, American Heart Association, Mt. Sinai Foundation, Pfizer and NIH.

Colin Nichols - Washington University

Colin Nichols FRS is the Carl Cori Professor, and Director of the Center forthe Investigation of Membrane Excitability Diseases, at Washington University School of Medicine (WUSM) in Saint Louis. He obtained his PhD at Leeds University in the UK and was a post-doctoral fellow at the University of Maryland at Baltimore and Baylor College of Medicine in Houston, Texas, before joining the faculty of the Department of Cell Biology and Physiology at WUSM in 1991. His work on inward rectifier and ATP-senstive potassium channels has led to his elucidation of the fundamental causes of inward rectification, and of congenital hyperinsulinism, neonatal diabetes, and Cantu Syndrome. His animal models predicted human neonatal diabetes, insight that enabled patients to switch from insulin injections to oral drug therapy, and his ongoing work continues to seek new understanding of K channel function at the molecular level, as well as to develop novel understanding, and novel therapeutic approaches to treatment of, various K channel diseases.

Biographies - Speakers

Crina Nimigean - Weill Cornell Medical College

Crina Nimigeanhas B.S./M.S. degree in Physics from Bucharest University, Romania, after which she moved to the USA and obtained a Ph.D. in Physiology and Biophysics from the University of Miami, under the mentorship of Karl Magleby. After postdoctoral training in Chris Miller's lab at Brandeis University, Crina's first faculty position as Assistant Professor was in the Department of Physiology and Membrane Biology at the University of California at Davis. She moved her laboratory in 2008 to New York City at the Weill Cornell Medical College where she currently is a Professor jointly in the departments of Anesthesiology, and Physiology of Biophysics. Crina's research is geared toward understanding how ion channel protein structure and mechanism interrelate at the molecular level to allow channels to elaborate various biological properties. The main focus of the lab is to elucidate gating, selectivity, ligand modulation, and lipid/membrane modulation in ion channels using a wide range of biological and biophysical techniques including molecular biology, biochemistry, electrophysiology, single-particle cryo-electron microscopy, stopped-flow fluorescence assays,and X-ray crystallography. In addition, established collaborations with expertise in NMR spectroscopy, AFM, native mass spectrometry, and molecular dynamics simulations complement our toolbox. Over the years, Crina and her colleagues have contributed to the understanding of selectivity for potassium against sodium in potassium channels, proposed specific mechanismsfor calcium-gating and pH-gating in potassium channels, provided a framework for understanding how ligands, enzymes, and lipids modulate cyclic nucleotide-gated channels, and for how potassium channels inactivate.

Alexandra Pinggera - Metrion Biosciences

Alex completed her undergrade degree in Pharmacy at the University of Innsbruck (Austria) in 2011. She then continued her studies in Innsbruck, obtaining her PhD in 2016, which included a 6-month placement at UC Davies (US) in the lab of Prof. Johannes Hell. During her PhD and a subsequent one year post-doc in the lab of Prof. Joerg Striessnig, Alex investigated L-type voltage gated Ca^{2+} channel physiology and pharmacology, predominantly using whole-cell patch-clamp electrophysiology. One major aspect of this work was the biophysical characterisation of human Ca^{2+} channel mutations associated with autism spectrum disorders and epilepsy.Subsequently, Alex obtained a position as a post-doc at the Laboratory of Molecular Biology in Cambridge (UK). During her 4.5 years in the lab of Dr. Ingo Greger, she investigated various aspects of AMPA receptor signalling at hippocampal synapses using a complementary approach involving super-resolution imaging and different electrophysiological techniques. The primary focusof this post-doctoral research was on the molecular mechanisms of AMPA receptor positioning at the synapse. In September 2021 Alex joined Metrion Biosciences at Senior Scientist I level, where she continues to work on different types of voltage-gated and ligand-gated ion channels.

Murali Prakriya - Northwestern University

Murali Prakriya is the Magerstadt Professor of Pharmacology, Feinberg School of Medicine at Northwestern University in Chicago. Prakriya's research examines the molecular physiology of Orai channels including the structural mechanisms of Orai channel gating and their physiological roles in neurons, glia, and immune cells. He received his graduate training in the laboratory of Dr. Christopher Lingle, Washington University and this postdoctoral training with Richard Lewis, Stanford University. Prakriya's talk will discuss recent discoveries in his laboratory on role of Orai1 channels for two aspects of glial functions in the brain: the control of gliotransmitter release from astrocytes to regulate synaptic circuits, and glial regulation of neuroinflammation to control depression behaviors.These discoveries illustrate the expanding roles of Orai channels in the brain.

Biographies - Speakers

Zhaozhu Qiu - Johns Hopkins University, School of Medicine

Dr. Zhaozhu Qiu is an Associate Professor of Physiology and Neuroscience at Johns Hopkins University School of Medicine. He received his PhD from Columbia University and the postdoctoral training in the Laboratory of Dr. Ardem Patapoutian at the Scripps Research Institute/HHMI before joining Johns Hopkins Faculty in 2016. Chloride is the most abundant free anion in animal cells. Cl– channels play fundamental roles in physiology and their dysfunctions cause many human diseases. However, they are relatively under-studied compared to cation channels as elucidation of their molecular identity has lagged behind. Dr. Qiu's laboratory has been on the forefront of identifying the molecular identity of novel Cl– channels, understanding their regulatory mechanisms, and elucidating their role in physiology and disease. Using innovative high-throughput assays and unbiased RNAi screens, Dr. Qiu and his team have recently discovered novel membrane proteins SWELL1 (LRRC8) forming the long sought-after Volume-Regulated Anion Channel (VRAC) and PAC (TMEM206) as the Proton-Activated Cl– (PAC) channel. Both channel activities have been observed in a wide range of tissues for nearly 30 years, but their identities were mysteries. These discoveries open up the field and create unprecedented opportunities for studying the regulatory mechanisms, physiological function and disease involvement of these ubiquitous ion channels.

Anjali Rajadhyaksha - Cornell University

Anjali Rajadhyaksha, Ph.D., is professor of neuroscience at Weill Cornell Medicine, Cornell University in New York City, New Yorkand associate dean of the Weill Cornell Graduate School. Her research focuses on deciphering the molecular mechanisms underlying addiction-and neuropsychiatric-related behaviors, with a particular focus on L-type Ca^{2+} channels. She uses genetic, viral, calcium imaging and molecular techniques in animal models to link genes to disease-associated behavior. Dr. Rajadhyaksha received her undergraduate training in chemistry at Bombay University, India and obtained her Ph.D. degree in molecular biology from Purdue University, West Lafayette, Indiana. She then completed her postdoctoral training in neuroscience at Massachusetts General Hospital, Harvard Medical School and thereafter joined Weill Cornell Medicine as a faculty member.

Bhama Ramkhelawon - New York University, Grossman School of Medicine

Dr Ramkhelawon is an Assistant Professor of Surgery and Cell Biology at NYU Grossman School of Medicine. She graduated from the University of Paris, in France followed by a post-doctoral fellowship at NYU Grossman School of Medicine. Her research is focused on the study of inflammatory vascular diseases and has recently discovered pathological traits of mechano-sensation in the cardiovascular system.

Daniel Sauter - Sophion Bioscience

Daniel Sauter received his Ph.D. in molecular biomedicine from the University of Copenhagen, where he investigated the roleof various ion channels in pancreatic cancer. After graduating, he worked as Research Scientist in a start-up company, Acesion Pharma, where he was involved in the primary screening using automated electrophysiology. Daniel joined Sophion Bioscience in 2016 as an Application Scientist and relocated from the Danish headquarters to the US in 2018 to manage the lab in Woburn, MA, and support customers in the North American market. In his current role, Daniel oversees a team of Application Scientists and Field Service Engineers.

Biographies - Speakers

Janina Sörmann - Evotec SE

Janina Sörmann is a scientist dedicated to the investigation of ion channels and channelopathies, which she studies with the help of electrophysiological and biophysical methods. She recently joined Evotec SE as a research associate in the *In Vitro* Pharmacology group, where she contributes to the the discovery of novel therapeutic approaches. After her Bachelor's and Master's studies in Biophysics at Goethe University in Frankfurt, she obtained her PhD in Biochemistry at Goethe University in association with the Max-Planck Institute of Biophysics in the group of Prof Bamberg, where she studied microbial rhodopsins and contributed to the development of optogentic tools. She then joined the group of Prof Stephen Tucker as a postdoctoral research associate in the Department of Physics and the Kavli Institute for Nanoscience Discovery at the University of Oxford, where she studied potassium ion channels and connected sleep apnea to an impaired gating mechanism in a K2P channel.

David Stokes - New York University, Langone Health

Dr. Stokes is a Professor of Cell Biology at NYU School of Medicine and received training in cryo-electron microscopy and image analysis during his PhD work at Brandeis University in Boston and postdoctoral work at MRC Laboratory of Molecular Biology in Cambridge, UK. Much of his work has addressed ion transport by members of the superfamily of P-type ATPases. Early on, he elucidated conformational changes of SERCA, the calcium pump from sarcoplasmic reticulum and then moved on to solve early structures of Na,K-ATPase and CopA, a bacterial copper pump, using cryo-EM. More recently, the focus has been on Kdp, a unique potassium transport system from bacteria that is a hybrid between a P-type ATPase and a potassium channel.Although the initial structure was solved by X-ray crystallography, cryo-EM was later used to characterize conformational changes that accompany the transport cycle. Dr. Stokes has other interests in zinc transport by members of the Cation Diffusion Facilitator superfamily and has used electron tomography to produce 3D images of a variety of complex cellular assemblies.

Heike Wulff - University of California, Davis

Heike Wulff obtained her PhD in Medicinal Chemistry at the University of Kiel and then joined the laboratory of Dr. K. George Chandy at UC Irvine as a postdoctoral researcher in 1999. After training in molecular biology, electrophysiology and immunology, Dr. Wulff now is a Professor of Pharmacology at UC Davis. Her research is focused on potassium channel pharmacology and the design of new ion channel modulating drugs and tool compounds. She has authored 190 peer-reviewed publications on voltage-and Ca^{2+}-activated $^{K+}$ channels and is ranked in the top 1% of highly cited researchers in her field.

Gerald Zamponi - University of Calgary

Dr. Gerald Zamponi is Senior Associate Dean for Research and Full Professor in the Cumming School of Medicine at the University of Calgary where he commenced his faculty position in 1997. He previously served as the Head of the Department of Physiology and Pharmacology. He received his undergraduate training in Engineering Physics from the Johannes Kepler University in Austria, followed by a PhD in Neuroscience at the University of Calgary and post doctoral work at the University of British Columbia in Canada. Dr. Zamponi's research addresses how ion channels and receptors contribute to neurological disorders such as chronic pain, with the goal of developing strategies to regulate ion channel function for therapeutic intervention. His work also deciphers the functional connectomics of brain circuits that process pain signals. The translational impact of his work is evident from his co-founding of NeuroMed Pharmaceuticals and Zymedyne Therapeutics, and inventorship on 12 issued US patents pertaining to new pain therapeutics. He has published over 320 articles, has given more than 250 invited lectures across the globe and has attracted in excess of $25 Million in research support to his lab. Dr. Zamponi is the recipient of numerous prestigious awards. In addition to having been an Alberta Innovates-Health Solutions Scientist and Canadian Institutes of Health Research Investigator, he is currently a Canada Research Chair in Molecular Neurobiology. He is an elected Fellow of both the Royal Society of Canada, and the Canadian Academy of Health Sciences, which represents top honors for those in the basic and medical science, respectively.

Poster Abstracts

Identification of a novel neuropilin 1 inhibitor that blocks CRMP2 phosphorylation and reverses mechanical allodynia and thermal hyperalgesia in a rodent model of neuropathic pain

Paz Duran
New York University, Department of Molecular Pathobiology, College of Dentistry

Poster board 01

Chronic pain is a major societal burden with few therapies that are both efficacious and safe. We previously showed that blockade of the interaction between the cell surface receptor Neuropilin 1 (Nrp1) and vascular endothelial growth factor A (VEGF-A), with either the small molecule inhibitor EG00229 or the Spike protein from SARS-CoV-2, produces antinociception in a rodent model of neuropathic pain. Here, we focused on developing a class of Nrp1 inhibiting compounds with the potential to alleviate pain. Using an in silico docking approach, we screened a library of ~480K small molecules for binding to the extracellular b1b2 pocket of Nrp1 and identified nine chemical series with lead- or drug-like physico-chemical properties. Using an ELISA, we demonstrated that six compounds disrupted the VEGF-A-NRP-1 binding more effectively than EG00229. Compound Nrp1-4 was selected for further evaluation. Synaptic fractionation of rat spinal cord tissue revealed that treatment with NRP1-4 significantly attenuated the phosphorylation of the cytoplasmic phosphoprotein collapsin response mediator protein 2 (CRMP2) at Ser522 without affecting total CRMP2 expression levels. Furthermore, NRP1-4 did not affect the synaptic membrane localization of the pain relevant ion channels $Na_V1.7$ or $Ca_V2.2$, both of which are regulated by CRMP2. Whole cell sodium currents in cultured primary sensory neurons were potentiated by treatment with 1nM VEGF-A, but blocked by co-treatment with 12.5 µM of NRP1-4. A similar effect was observed with N-type calcium currents, wherein co-treatment with NRP1-4 blocked the VEGF-A mediated increase in whole cell current density. *Ex vivo* evaluation of synaptic activity showed that NRP1-4 reduced VEGF-A-mediated increases in both the frequency and amplitude of spontaneous excitatory post synaptic currents (sEPSCs). In rats with spared nerve injury induced neuropathic pain, intrathecal administration of NRP1-4 significantly attenuated mechanical allodynia. Importantly, when given intravenously, there was a potent reversal of both mechanical allodynia and thermal hyperalgesia in animals with spinal nerve ligation induced neuropathic pain. Together, our findings show that NRP1-4 is a first-in-class compound targeting the VEGF-A/NRP1 interaction and its action mechanism involve signaling upstream of CRMP2 to control ion channel activities and curb chronic pain syndromes.

Elucidating the role of ion channels and transporters in B cells

Anthony Tao
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Poster board 02

B cells are critical components of the adaptive immune response and major contributors to a variety of autoimmune diseases. A crucial facet of B cell biology is their ability to differentiate into antibody-producing plasma cells (PCs). Despite the importance of B cells, the repertoire of pharmacologies modulating B cell function is limited. In this regard, ion channels and transporters (ICTs) represent a promising class of therapeutic targets, though their roles in B cells are underexplored. Interestingly, B cell subsets express a different ICT profile compared to other immune cells, suggesting that B cells may functionally require a unique array of ICTs. However, an unbiased exploration of ICTs in B cells has not been conducted. Here, I performed transcriptomic and CRISPR screens to identify 2 ICTs of interest: a Ca^{2+} pump (CaP) and a pH regulator (pHR). CaP is involved in regulating ER Ca^{2+} homeostasis. CaP deletion results in increased cytosolic Ca^{2+}, impaired PC differentiation, and elevated apoptosis. These effects are rescued by calcineurin inhibition, implicating the NFAT pathway in these phenotypes. pHR, on the other hand, is an HCO3-transporter. pHRdeletion in B cells selectively impairs PC differentiation. Furthermore, pHR-deficient cells exhibit increased intracellular pH, reduced lysosomal pH and reduced mTOR activity, implicating the lysosome-mTOR axis in these PC phenotypes.

ATP-releasing SWELL1 channel in spinal microglia contributes to neuropathic pain

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Poster board 03

Following peripheral nerve injury, extracellular ATP-mediated purinergic signaling is crucial for microglia activation in the spinal cord and the development of neuropathic pain. However, the mechanisms of ATP release remain poorly understood. Here, we show that the volume-regulated anion channel (VRAC) is an ATP-releasing channel. It is expressed in microglia and activated by various stimuli, including inflammatory mediator sphingosine-1-phosphate (S1P). Mice with microglia-specific deletion of Swell1, the only essential subunit of VRAC, had reduced peripheral nerve injury-induced increase in extracellular ATP in the spinal cord. The mutant mice also exhibited decreased spinal microgliosis, dorsal horn neuronal hyperexcitability, and both evoked and spontaneous neuropathic pain-like behaviors. We performed a high-throughput screen and identified an FDA-approved drug dicumarol as a novel and potent VRAC channel inhibitor. Importantly, intrathecal administration of dicumarol in mice alleviated nerve injury-induced mechanical allodynia. Our findings suggest that ATP-releasing VRAC in microglia is a key spinal cord determinant of neuropathic pain, and reveal that dicumarol is a promising starting point for future development as a novel treatment of this debilitating disease.

Poster Abstracts

Mapping the Binding Site of NS309, a Superagonistic Positive Gating Modulatorfor the Calcium-activated Potassium Channel KCa3.1

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Poster board 04

The intermediate-conductance Ca^{2+}-activated K^+channel KCa3.1 is expressedin erythrocytes, vascular endothelial cells, and immune cells. KCa3.1 is involvedin a wide variety of disease pathologies, suggesting that both positive and negative gating modulators would be of interest as pharmacological tools and potential drugs. NS309 is the most potent positive gating modulator for KCa3.1, with an EC_{50} of 75nM, and interestingly, it is capable of increasing channel activity even at saturating calcium concentrations. However, its binding site is currently unknown. Previous X-ray crystallographic and solution-state NMR studies suggested that NS309 was binding between calmodulin's N-lobeand the C-terminal region of the calmodulin binding domain and interacting with residues M51 and R372. Unfortunately, the crystal dimer used in these studies was later found to be an artifact and disproved when the full-length crystal structure of KCa3.1 was published in 2018. This full-length structure suggested that the "real"binding site for positive gating modulators was located within the interface between calmodulin's N-lobeand the S4-S5 linker, a hypothesis later confirmed by our group for the benzothiazole SKA-111. NS309 could potentially be binding in this same site. However, computational modeling with Glide, SiteMap (Schroedinger) and Rosetta Ligand suggests a novel site located just above the benzothiazole pocket, between the S4-S5 linker and the S6 transmembrane helix, as an alternate possibility. To determine in which site NS309 is binding, we have mutated a series of residues from each pocket (S181, L185, N201, and R287). Using whole-cell patch clamp, we will be generating a concentration response curve for NS309 on each mutant and comparing it to that of the wild-type KCa3.1 channel. Any shift in concentration response curve found to be significant via Extra Sum of Squares F-test will be taken as evidence of NS309 binding in the respective pocket.

Repurposing the KCa3.1 blocker Senicapoc for ischemic stroke

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Poster board 05

Senicapoc, a small molecule inhibitor of the calcium-activated potassium channel KCa3.1, was evaluated in clinical trials for sickle cell anemia and asthma and found to be safe and well tolerated. We previously reported proof-of-concept data suggesting that both pharmacological inhibition and genetic deletion of KCa3.1 reduces infarction and improves neurologic recovery in rodents by attenuating neuroinflammation. Here we evaluated the potential of repurposing senicapoc for acute ischemic stroke. In activated, cultured microglia senicapoc inhibited KCa3.1 currents with an IC_{50} of 7 nM, reduced Ca^{2+} signaling induced by the purinergic agonist ATP, suppressed expression of pro-inflammatory cytokines and enzymes (iNOS and COX-2), and prevented induction of the inflammasome component NLRP3. When transient middle cerebral artery occlusion (tMCAO, 60-min) was induced in male C57BL/6J mice, twice daily administration of senicapoc at 10 and 40 mg/kg starting 12 h after reperfusion, dose-dependently reduced infarct area determined by T2-weighted magnetic resonance imaging (MRI) and improved neurological deficit on day-8. Ultra-high-performance liquid chromatography/mass spectrometry analysis of total and free brain concentrations demonstrated sufficient KCa3.1 target engagement. Senicapoc treatment significantly reduced microglia/macrophage and T-cell infiltration and activation in the infarcted hemisphere. Quantification of NeuN staining confirmed the MRI results and showed that senicapoc treatment attenuated tMCAO-induced neuronal death. Lastly, we demonstrated that senicapoc does not impair the proteolytic activity of tissue plasminogen activator (tPA) ***in-vitro***. Based on these findings we suggest that senicapoc could be repurposed as an adjunctive immunocytoprotective agent for combination with reperfusion therapy for ischemic stroke.

Poster Abstracts

Targeting aberrant mGluR1-TRPC3 signalling in Spinocerebellar Ataxias

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Poster board 06

The dominantly inherited spinocerebellar ataxias (SCAs) are a group of rare degenerative ataxia disorders characterised by a progressive loss of motor coordination and degeneration of the cerebellum. Over 40 subtypes of this disorder exist, each arising from mutations in different underlying genes, resulting in a wide array of symptoms and variable clinical outcomes amongst patients. Unfortunately, no effective treatment is yet availablefor any subtype. TRPC3 is a non-selective, calcium-permeable cation channel highly expressed at the Purkinje cell (PC) postsynaptic membrane; it facilitates mGluR1-dependent excitation, which is essential for healthy development of PCs, they themselves vital for cerebellar function. Increased, aberrant activation of these proteins is associated with several SCAs, including SCA41 and SCA44 (identified by our lab to arise from mutations in TRPC3 and mGluR1, respectively), as well as SCAs with unrelated genetic backgrounds such as the more commonly seen SCA1 and SCA2.TRPC3 integrates multiple signalling inputs at the plasma membrane, and, as such, presents apromising target for pharmacological modulation of signalling at the PC synapse. Here, we have used docking and molecular dynamic simulations,coupled with cell-based functional assays, to propose and validate a potential binding site and mechanism of a novel TRPC3 inhibitor. This may help to elucidate fundamental mechanisms of the channel and guide further drug development for the treatment of spinocerebellar ataxias.

A rare missense mutation causing functional alterations of the potassium ion-channel TREK2 is nominally associated with pain

Sam Bourne
LifeArc

Poster board 07

TREK2 is a member of the two-pore domain potassium (K2P) ion-channel family, that regulates the resting membrane potential of nociceptive sensory neurons. These promising therapeutic targets for the treatment of pain have yet to be exploited, however poor clinical translation of novel analgesics, with many failing at late stages, can impede therapeutic advances. One way to accelerate and improve drug translation is to use human functional genomics and naturally occurring genetic variations to validate targets. We highlight a TREK2 variant, Thr254Met, identified from the UK and Finnish Biobank GWAS results, which has a nominal association ($P < 0.05$) with 7 pain phenotypes (precordial, hip, throat, chest, temporomandibular, mononeuropathy and drug induced migraine pain). We cloned this mutant, located in the 3rd transmembrane domain of TREK2, into pcDNA5™/FRT and transiently expressed it in human tsA201 and CHO cells. Whole-cell voltage-clamp recordings from TREK2 Thr254Met expressed in tsA201 cells (n33) gave outward currents that were significantly reduced ($P < 0.001$, one way ANOVA Fishers LSD) compared to WT (n13). Reduced basal activity was also observed in CHO cells using a high-throughput thallium flux assay on the FLIPR tetra (n3, $P < 0.001$, one way ANOVA Fishers LSD). However, current passing through the Thr254Met variant could be rescued using small molecule agonists. To profile the pharmacology further, we screened 10 literature and in-house small molecule candidates in duplicate using a 10-point half-log serial dilution in thallium flux. There was no significant difference between Thr254Met and WT TREK2 responses in terms of potency (n3, logEC50 +/-0.5, nonlinear regression), however the percentage enhancement of current for some compounds was greater for Thr254Met ($P < 0.05$, two-way ANOVA Fishers LSD). We confirmed that functional differences were not due to changes in mRNA expression using TaqMan RT-qPCR on CHO cell lysates (n=3, one way ANOVA Fishers LSD) but likely due to disrupted hydrogen bonding within transmembrane domain 3, confirmed by probing the available homology models for TREK2 (PDB: 4BW5). Here we demonstrate a novel clinical Thr254Met variant associated with pain phenotypes that reduces TREK2 function, and which may impinge upon nociceptive signalling through altering neuronal firing. Furthermore, this data provides functional translational validation of TREK2 as a promising pain therapeutic target and highlights the potential of small molecule agonists to rescue function and provide therapeutic benefit.

Repurposing the KCa3.1 blocker Senicapoc for ischemic stroke

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Poster board 08

Complexes formed with α5-integrins and the voltage-gated potassium (K^+) channel KCNB1 (Kv2.1), known as IKCs, transduce the electrical activity at the plasma membrane into biochemical events that impinge on cytoskeletal remodeling, cell differentiation, and migration. However, when cells are subject to stress of oxidative nature IKCs turn toxic and cause inflammation and death. Here, biochemical, pharmacological, and cell viability evidence demonstrates that in response to oxidative insults, IKCs activate an apoptotic Mitogen-activated protein kinase/extracellular signal-regulated kinase (Ras-MAPK) signaling pathway. Simultaneously, wild-type (WT) KCNB1 channels sequester protein kinase B (Akt) causing dephosphorylation of BCL2-associated agonist of cell death (BAD), a major sentinel of apoptosis progression. In contrast, IKCs formed with C73A KCNB1 variant that does not induce apoptosis (IKCC73A), do not sequester Akt and thus are able to engage cell survival mechanisms. Taken together, these data suggest that apoptotic and survival forces co-exist in IKCs. Integrins send death signals through Ras-MAPK and KCNB1 channels simultaneously sabotage survival mechanisms. Thus, the combined action of integrins and KCNB1 channels advances life or death.

Poster Abstracts

Thallium Flux Assay Adaption for Multi-Instrument Compatibility

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ION Biosciences

Poster board 09

Over the past 15+ years, fluorescence-based measures of Tl^+ flux have brought about the discovery of small-molecule modulators of a host of ion channels, transporters, GPCRs and other targets of interest for both drug discovery and basic research. Here,we introduce ION's Brilliant Thallium Snapshot Assay Kit, which provides a new way to conduct thallium flux assays. Brilliant Thallium Snapshot is designed for multi-well plate-based, high-throughput measurements of Tl^+ flux through K^+, Na^+, non-selective cation channels, and some Na^+ or K^+ transporters. Unlike traditional thallium flux assays, the patent-pending Snapshot assay format generates a long-lasting signal, so ion channel or transporter activity can easily be detected and changes can be quantified on most fluorescence-capable instruments. As a result, whether usinga FLIPR® (HTS), a fluorescence microscope, a standard fluorescence plate reader, a high-content imager (HCS), or a flow cytometer – functional screens can be run on viable cells using thallium flux, enabling entirely new possibilities.

Instrument compatibility is demonstrated using a CHO GIRK1/2 (CHO G12) expressing cell line. GIRK channels are modulated using known activators, ML297or VU0466551 (VU551). Comparable EC_{50} values are obtained using data acquired from three commonly available instruments - a fluorescence microscope, standard fluorescence plate reader, and Flexstation®. Thallium flux paired with microscopy enables monitoring of specific cells within a diverse population - an ideal solution for measuring cell-specific ion channel activity in complex co-culture systems. Other applications of this technology could include clonal selection using FACS and identifying target expressing cells within dissociated tissue samples.

Automated high throughput patch clamp studies of voltage gated ion channels in hiPSC derived neurons

David Nagy
Sophion Bioscience

Poster board 10

Human induced pluripotent stem cell (hiPSC) derived neurons express native complements of human ion channels and their accessory proteins providing enhanced translatability from early in vitrostudies to patient biology. Despite this promise, few studies have examined the suitability of hiPSC neurons for automated patch clamp studies. Here, we establish the feasibility of recording voltage gated channel activity from hiPSC-derived excitatory neurons in 384 well format with the Sophion Qube automated patch clamp system.

hiPSC-derived neurons were generated by overexpression of the transcription factor NGN2 driven from a stably integrated cassette in the AAVS1 locus. We first optimized dissociation procedures with 2-3 weeks *in vitro* NGN2 neurons by assessing the percentage of cells with voltage gated Sodium (Na_V) and potassium (K_V) currents on the Qube system. Recordings following optimized dissociation found that ~30% of single cells had Na_V currents >200 pA, leading to recordings of >100 cells in parallel. Minimal reduction of experimental throughput was observed with recordings following culture up to four weeks. Isolation of Na_V currents with Cesium internal solution showed expected Na_V activation and inactivation curves with mean Na_V currents >1 nA. Exchange of intracellular solution from cesium to potassium-based reversed block of K_V channels and did not significantly impact recording success rate. In multi-cell recording configurations we attained success rates of ~80%, sufficient to examine dozens of experimental conditions simultaneously.

These results suggest that key hiPSC NGN2 neuronal properties, Na_V and K_V activity, are retained in conditions that support high throughput patch clamp studies. Furthermore, we show that the automated patch clamp drastically increases experimental throughput for hiPSC neuron neurophysiology. Future studies will examine properties of other hiPSC derived neuronal types and establish the diversity of ion channels amendable to automated recordings.

Poster Abstracts

Distinctive regulation of $Ca_V3.1$ and $Ca_V3.2$ low-voltage-activated calcium channels by NEDD4 Family E3 Ubiquitin Ligases

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Poster board 11

$Ca_V3.1$and $Ca_V3.2$ calcium channels serve critical roles in the cardiovascular and nervous systems. Ca_V3 dysregulation underlies diverse diseases, including chronic pain. Interventions that tune the functional expression and gating of Ca_V3 channels represent powerful mechanisms to regulate physiology, and opportunities for developing new therapeutics. Ubiquitination is a prevalent post-translational modification that regulatesdifferent aspects of protein lifecycles, including subcellular localization and stability. However, how distinct Ca_V3 channels are regulated by ubiquitination is largely unknown.Here, we investigated mechanisms of regulation of $Ca_V3.1$ and $Ca_V3.2$ channels by members of the NEDD4 E3 ubiquitin ligase family in dorsal rootganglia (DRG) neurons and HEK293T cells. Using a comprehensive array of methods,we demonstrate that Smurf1 is abona fide regulator of both low and high voltage-activated calcium channels, thus unveiling a new class of substrates for Smurf1. Whole-cell currents of $Ca_V3.1$ channels reconstituted in HEK293T cells were markedly suppressed by Smurf1, but not NEDD4L, with minimal changes in the voltage-dependence of channel activation and steady-state inactivation. By contrast, $Ca_V3.2$ was significantly inhibited by both NEDD4L and Smurf1, with NEDD4L also causing a depolarizing shift in the voltage-dependence of activation. Although both NEDD4L and Smurf1 markedly decreased $Ca_V3.1$ and $Ca_V3.2$ stability, immunoprecipitation of the channels did not suggest an increase in the overall ubiquitination status of the channels. Using a flow cytometry fluorescence resonance energy transfer approach, we identified binding sites for NEDD4L and Smurf1 on discrete intracellular loops of $Ca_V3.1$ and $Ca_V3.2$. Directing the catalytic HECT domain of Smurf1 to YFP-tagged $Ca_V3.1$ and $Ca_V3.2$ using a YFP-targeting nanobody recapitulated a ubiquitination-dependent decrease in current amplitude. NanoNEDD4L inhibited both $Ca_V3.1$ and $C_V3.2$ currents, but shifted activation gating of only $Ca_V3.2$. Overall, our studies reveal differential and unconventional regulation of $Ca_V3.1$ and $Ca_V3.2$ by NEDD4L and Smurf1, and suggest new avenues for regulating functional expression of Ca_V3 channels that may be exploited for therapy.

Distinct roles of ORAI1 in T cell–mediated allergicairway inflammation and immunity to influenza A virus infection

Lucile Noyer
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Poster board 12

T cell activation and function depend on Ca^{2+} signals mediated by store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release–activated Ca^{2+} (CRAC) channels formed by ORAI1 proteins. We here investigated how SOCE controls T cell function in pulmonary inflammation during a T helper 1 (T_H1) cell–mediated response to influenza A virus (IAV) infection and T_H2 cell–mediated allergic airway inflammation. T cell–specific deletion of *Orai1* did not exacerbate pulmonary inflammation and viral burdens following IAV infection but protected mice from house dust mite–induced allergic airway inflammation. ORAI1 controlled the expression of genes including p53 and E2F transcription factors that regulate the cell cycle in T_H2 cells in response to allergen stimulation and the expression of transcription factors and cytokines that regulate T_H2 cell function. Systemic application of a CRAC channel blocker suppressed allergic airway inflammation without compromising immunity to IAV infection, suggesting that inhibition of SOCE is a potential treatment for allergic airway disease.

Preclinical development of TRPC3 inhibitors to treat spinocerebellar ataxia

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Poster board 13

Spinocerebellar Ataxias (SCAs) are autosomal dominant neurodegenerative disorders affecting the cerebellum, a key region of the brain responsible for movement and coordination. Progressive dysfunction of the cerebellum leads to severely impaired quality of life and for many patients is fatal. In addition to problems with balance, speechand movement, patients often display cognitive disabilities, Parkinsonism and seizures. There is an unmet need for therapeutics to slow progression of the disorders. Over 40 pathogenic variants are associated with SCAs, but common mechanisms are thought to underlie different genetic forms. One such mechanism is dysregulated Ca^{2+} homeostasis in cerebellar Purkinje cells. The TRPC3 channel is essential for Ca^{2+}-signalling and excitability downstream of mGluR1 at the postsynaptic membrane of Purkinje cells. Gain-of-function mutations in TRPC3 have been shown to cause SCA phenotypes both in a patient with SCA41 and the *Mwk* mouse model. Notably, dysregulated TRPC3 signalling has also been identified a number of other SCAs, including SCA1 and SCA2. Inhibition of TRPC3 may therefore be a promising therapeutic strategy to treat multiple SCA subtypes. In collaboration with medicinal chemists, we are generating and optimising drug leads to target TRPC3 channels. Compounds have been screened initially using an immunofluorescence-based assay in Neuro-2A cells which has yielded a number of active inhibitors. Positive hits have been further validated using whole-cell electrophysiology to assess inhibition of TRPC3 channels overexpressed in HEK293-T cells. Promising compounds will be tested using *ex vivo* and *in vivo* SCA preclinical models. We are initially focusing on SCA2, one of the most common SCAs, since dysregulated mGluR1-TRPC3 signalling is well-described in mouse models of this subtype. Ultimately, we hope to translate these drugs into clinical testing in SCA patients.

Poster Abstracts

The role of proton-activated chloride channel PAC inmacrophage host defenseimmunity against bacterial infection

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Poster board 14

The newly identified Proton-Activated Chloride (PAC) channel traffics from the cell surface to several intracellular organelles in the endocytic pathway, where it mediates pH-dependent chloride transport and serves in important housekeeping roles, including regulation of endosome acidification and macropinosome shrinkage. However, its function in macrophages and host defense immunity is unknown. Here, we show that PAC expression is downregulated upon macrophage activation.This response is required for macrophage activation-induced phagosome acidification and increase in protease activity, which facilitates the bacterial clearance. In a peritoneal infection model, macrophage-specific PAC conditional knockout (cKO) mice exhibited enhanced bacterial clearance capacity and alleviated peritoneal infection disease with increased survival. Mechanistically, PAC mediates phagosome microenvironment in peritoneal macrophages, specifically in large peritoneal macrophage (LPM), but not small peritoneal macrophage (SPM). Furthermore, the facilitated bacterial clearance is important for the subsequent immune cell infiltration and dynamics, contributing to the disease resolution. In summary, our findings suggest that the PAC channel is crucial for phagosome acidification and plays an important role in macrophage host defense immunity against the bacterial infection.

Pentacyclic triterpenoids inhibit N- and T-type voltage-gated calcium channels to attenuate nerve-injury associated neuropathic pain

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Poster board 15

Background and Aims: Natural products usage has increased in the last three decades almost 80% of people worldwide relying on them for some part of primary healthcare. Over the last decade, our laboratories have isolated and characterized natural products with analgesic properties from arid land plants or their associated fungi. Here, we aimed to characterize the molecular targets of a pentacyclic triterpenoid derivative of Betulinic acid (BA), derived from the desert lavender Hyptis emoryi.
Methods: We previously reported that betulinic acid attenuates paclitaxel-, HIV-, and nerve injury-associated peripheral sensory neuropathy via block of N- and T-type calcium channels (Bellampalli et al.,Pain 2019 160: 117-135). Using structure activity relationship (SAR) data, docking studies and virtual screening of BA analogs mined from the ZINC20 database, we designed second-generation BA analogs (BA-II analogs) with unique intellectual property and improved predicted PK properties. Here, we tested BA-II analogs for their in vitro mechanism of action on voltage-gated calcium channels in rat dorsal root ganglia (DRG) neurons using ratiometric calcium imaging and electrophysiology and in vivo in the spared nerve injury (SNI) model of neuropathic pain.
Results: Screening of the 30 BA-derivatives identified one BA-II analog (NPC 1-11) with significant inhibition of Ca^{2+} influx in DRGs. NPC 1-11 significantly downregulated total Ca^{2+} currents on DRGs without affecting voltage-dependence activation and inactivation curves. Inhibition by NPC-1-11 was limited to ($Ca_V2.2$) N- and T- type Ca^{2+} currents. Intrathecal delivery of NPC 1-11 (2µg/5µl) reversed mechanical allodynia induced by SNI.
Conclusions: Our *in vitro* and *in vivo* results demonstrate that inhibition of Ca^{2+} channels by the natural product derivative of betulinic acid alleviates neuropathic pain. Because of the wide-safety profile of pentacyclic triterpenoids, the mechanistic insights gleaned from these studies has the potential to lead to fast-tracking development of novel, non-addictive drugs for treatment of chronic neuropathic pain in humans.

Harnessing a unique $Na_V1.7$ regulatory domain for chronic pain

Kimberly Gomez
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Poster board 16

Despite identification of several small molecules directly targeting the voltage-gated sodium channel $Na_V1.7$, none has been clinically successful. We reported that preventing addition of a small ubiquitin-like modifier (SUMO) on the $Na_V1.7$-interacting cytosolic collapsin response mediator protein 2 (CRMP2) blocked $Na_V1.7$ functions and was antinociceptive in rodents. Here, we discovered a 15 amino acid CRMP2 regulatory sequence (CRS) unique to $Na_V1.7$ that is essential for this regulatory coupling. CRMP2 preferentially bound to the $Na_V1.7$ CRS over other isoforms. Substitution of the NaV1.7 CRS with the homologous domains from the other eight voltage-gated sodium channel isoforms decreased tetrodotoxin-sensitive $Na_V1.7$ currents in rodent sensory neurons. A cell-penetrant version of $Na_V1.7$-CRS reduced $Na_V1.7$ currents and trafficking, decreased presynaptic $Na_V1.7$ localization, reduced spinal neurotransmitter release, and reversed mechanical allodynia in a rat spared nerve injury model of neuropathic pain. Interfering with $Na_V1.7$-CRMP2 coupling did not produce motor impairment and spared thermal, inflammatory, and post-surgical nociception. As proof-of -concept for $Na_V1.7$-targeted gene therapy, we found that $Na_V1.7$-CRS packaged into an adeno-associated virus recapitulated the effects on $Na_V1.7$ function in both rodent and rhesus macaque sensory neurons and both reversed and prevented the development of mechanical allodynia in a neuropathic pain model in male and female rodents.

Poster Abstracts

Automated Patch Clamp Evaluation of Snake Neurotoxins and Recombinant Antibody Antivenoms

Weifeng Yu
Sophion Bioscience

Poster board 17

Snakebite was designated Neglected Tropical Disease (NTD) status by the WHO (2017), causing 100,000 yearly deaths and around 400,000 amputations. Each snake species has a unique venom, often consisting of dozens of different toxins.

The century-old, traditional technique to generate snake antivenoms involved purifying antibodies from horse blood serum following immunization with snake venom. However, there are several drawbacks: equine-human immunoreactivity and side effects; batch-to-batch variation; and specific to the snake venom used.

In the last decade, advances in antibody engineering have made antibody discovery and development more efficient and specific, including creating human recombinant antivenom antibodies to target and neutralize key toxin peptides. One of the most medically relevant groups of snake toxins are the α-neurotoxins, targeting the muscle α1-nicotinic acetylcholine receptor (α1-nAChR).For over two decades automated patch-clamp (APC) systems, have been used to advance our understanding of ion channel biophysics, pharmacology and their roles in physiology and disease.

Here, using QPatch II and Qube 384 APC, we functionally evaluate snake venom α-neurotoxins and anti-venom, toxin-neutralising IgG monoclonal antibodies (mAbs) on the muscle α1-nAChR.

The Role of TRPM3 Ion Channels in Opioid-Induced Analgesia and Pruritus

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Poster board 18

Morphine, a powerful opioid, is standard treatment for the management of severe pain. The desired analgesic effect of the neuraxial administration of morphine has the distressing side effect of pruritus. Opioid antagonists are the first line of treatment, which can counteract the analgesia from the initial opioid therapy. Antihistamine treatments are also ineffective against morphine-induced pruritus. Therefore, a more target-specific treatment for morphine-induced pruritus is needed. Pruritus from morphine is caused by the inhibition of inhibitory neurons, while the analgesia is caused by inhibition of excitatory neurons in the spinal cord. Morphine acts on opioid receptors which couple to heterotrimeric Gαi-proteins exerting acute effects on downstream ion channel targets to inhibit neuronal activity. However, the ion channel responsible for morphine-induced pruritus is unknown. This project aims to investigate if the transient receptor potential melastatin 3 (TRPM3) ion channels are involved in opioid-induced pruritus. TRPM3 is a non-selective, heat-sensitive cation channel expressed in neurons that is inhibited upon opioid receptor activation. This inhibition is mediated by direct binding of Gβγ to a 10-amino acid binding site in the channel, encoded by an alternatively spliced exon. We created a genetically mutated mouse line (TRPM3DEx17) in which TRPM3 channels lack this exon, thus the channels are not inhibited by opioid receptor activation. Upon intrathecal injection of morphine, the TRPM3DEx17 mice experienced significantly less scratch bouts compared to wild-type (WT) mice, while histaminergic itch was conserved in the mutant mice. Exogenously activating TRPM3 with intrathecal co-injection of pregnenolone sulfate (PS) along with morphine significantly decreased scratching. Intrathecal injection of the TRPM3 antagonist, primidone, caused spontaneous itch in WT mice. As TRPM3 shows higher level of co-expression with the μ-opioid receptors in the inhibitory neurons responsible for the pruritic pathway compared to the excitatory neurons for the analgesic pathway, the results are consistent with the claim that the activation of TRPM3 can decrease morphine-induced itch without significant side effects. This study outlines the first distinct phenotype found in the novel mouse model $TRPM3^{DEx17}$ and provides evidence for a potential new therapy to relieve morphine-induced pruritus by targeting TRPM3.

Poster Abstracts

Skeletal muscle delimited myopathy and verapamil toxicity in SUR2 mutant mouse models of AIMS

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Poster board 20

ABCC9-related intellectual disability and myopathy syndrome (AIMS) arises from loss-of-function (LoF) mutations in the ABCC9 gene, which encodes the SUR2 subunit of ATP-sensitive potassium (KATP) channels. KATP channels are found throughout the cardiovascular system and skeletal muscle, and couple cellular metabolism to excitability. AIMS individuals show fatigability, muscle spasms, and cardiac dysfunction. We found reduced exercise performance in mouse models of AIMS harbouring premature stop codons in ABCC9. Given the roles of KATP channels in all muscle, we sought to determine how myopathy arises using tissue-selective suppression of KATP, and found that LoF in skeletal muscle, specifically, underlies myopathy. In isolated muscle, SUR2 LoF results in abnormal generation of unstimulated forces, potentially explaining painful spasms in AIMS. We sought to determine whether excessive Ca^{2+} influx through $Ca_V1.1$ channels was responsible for myopathology but found that the Ca^{2+} channel blocker verapamil unexpectedly resulted in premature death of AIMS mice and that rendering $Ca_V1.1$ channels non-permeable by mutation failed to reverse pathology, results which caution against the use of calcium channel blockers in AIMS.

STIM1 intronic mutation abolishes store-operated calcium entry and T-bet expression cause immunodeficiency

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Store-operated calciumentry (SOCE) through Ca^{2+} release-activated Ca^{2+} (CRAC) channels is the main Ca^{2+} influx pathway in lymphocytes and is crucial for T cell function and adaptive immunity.The ER Ca^{2+} sensor STIM1 is required for the activation of ORAI1, the pore-forming subunit of CRAC channel, and subsequent SOCE. Loss-of-function mutations in STIM1and ORAI1cause severe immunodeficiency with recurrent infections and autoimmunity which termed as CRAC channelopathy syndrome. Several studies have shown that SOCE contribute to the differentiation of T helper (Th) 2, Th17 and T regulatory (Treg) cells, however, the role of SOCE in Th1 differentiation and its underlying mechanisms are not wellunderstood. Here, we reported a patient with an intronic mutation in STIM1that creates a new splice acceptor site, abnormal STIM1mRNA splicing and loss of STIM1 protein expression. The immunodeficiency of the patient is associated with abolished SOCE and reduced Th1 response, including the lineage-specific transcription factor TBX21and IFNG. Mechanistically, loss of STIM1 impairs SOCE and the activation of nuclear factor of activated T cells (NFAT), which binds to distal region of the TBX21 promoter to regulate its transcription. Our study reveals how STIM1 and SOCE deficiency impairs Th1 differentiation which cause immunodeficiency in patients.

Synergistic role of BACE1 and potassium channels in TB

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Poster board 22

Traumatic brain injury (TBI), a condition characterized by extensive oxidative stress, is often associated with formation of β-amyloid (Aβ) within hours after trauma.This suggests that Aβ constitutes an element of toxicity in TBI, but the underlying mechanisms are not completely understood. Preceding the production of pathogenic Aβ species, β-secretase 1 (BACE1) incorrectly cleaves amyloid precursor protein (APP). BACE1 is strongly susceptible to two hallmarks of TBI: oxidative stress and inflammation. Prior research indicates that oxidative conditions, developed in injured brains, induce covalent modifications (cross-linking) in the delayed rectifier and voltage-gated K^+ channel, KCNB1 (alias Kv2.1), a protein broadly expressed in the cortex.Our results show that inhibiting the endoproteolytic activity of BACE1 through Verubecestat, limits large spectrum cellular/molecular lesions and behavioral deficits that occur in response to all forms of brain injury in the Lateral Fluid Percussion mouse model of TBI. Most importantly, limiting the oxidation of KCNB1 channels decreases oxidative stress and inflammation and consequently moderates the transcriptional activity of BACE1. In summary, our data indicate that KCNB1 channels are capable of influencing TBI pathology by enhancing the production of Aβ via BACE1.

Poster Abstracts

Cavβ1 regulates T cell expansion and apoptosis independently of voltage-gated Ca^{2+} channel function

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Poster board 23

T cell receptor (TCR) stimulation triggers a rise in intracellular Ca^{2+} that is critical for many T cell functions and immune responses. The best-characterized Ca^{2+} influx pathway in T cells is store-operated Ca^{2+} entry (SOCE) through Ca^{2+} release-activated Ca^{2+} (CRAC) channels encoded by ORAI1. In addition, voltage-gated Ca^{2+} channels (VGCC or Ca_V) have been reported to regulate Ca^{2+} signaling and the function of T cells, but their mode of activation and role in electrically non-excitable T cells remains elusive. We here identified the auxiliary $Ca_V\beta1$ subunit, encoded by the gene Cacnb1, as a regulator of T cell function by using an shRNA screen for ion channels and transporters expressed in immune cells. Cacnb1 was required for clonal T cell expansion after lymphocytic choriomeningitis virus (LCMV) infection. Deletion of Cacnb1 in T cells increased apoptosis, whereas proliferation, cytokine production and Ca^{2+} signaling were unaffected. Using patch clamp electrophysiology and Ca^{2+} recordings to detect VGCC activity, we were unable to detect voltage-gated Ca^{2+} currents or Ca^{2+} influx in human and mouse T cells upon depolarization with or without prior TCR stimulation. Deletion of stromal interaction molecule 1 (STIM1), previously shown to inhibit the L-type VGCC $Ca_V1.2$ in leukemic T cells, failed to reveal voltage-gated Ca^{2+} influx in primary T cells. Transcripts of several α1 pore subunits of VGCCs can be detected in human ($Ca_V3.3$, $Ca_V3.2$) and mouse ($Ca_V2.1$) T cells, but exon usage analysis showed that the most 5′ exons of these genes are not transcribed, likely resulting in N-terminally truncated and non-functional proteins. Our findings demonstrate that although $Ca_V\beta1$ regulates T cell survival and expansion, these effects are independent of VGCC channel function.

Investigation of novel ion channels as potential next-generation therapeutic targets for MS

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Poster board 24

Multiple Sclerosis (MS) is a demyelinating disease of the central nervous system.Encephalitogenic T cells play a crucial role in the pathogenesis of MS. Emerging evidence shows that some ion channels either contribute to the process of autoimmunity or neurodegeneration in MS. However, in general, little is known about the function of ion channels and transporters (ICTs) in encephalitogenic T cells. We designed and performed a shRNA based screen using the EAE mouse model for MS and complemented the screen with gene expression profiling by RNA-seq, which is aimed to identify ICTs that control the function of encephalitogenic T cells. Our screen identified > 30 ICTs that are depleted in T cells isolated from the CNS. we then prioritized the (i) most depleted and (ii) most highly expressed ICTs from the depletion hits and performed validation experiments by deleting these genes in 2D2 T cells using CRISPR/Cas9 gene editing. As a proof of concept, knockout of 4 depletion hits identified by shRNA almost completely prevent the development of EAE. Protection from EAE was associated with a strong reduction of encephalitogenic T cells in the CNS. Of note, deletion of the hits did not affect T cell number in the spleen nor did it decrease the production of encephalitogenic Th1/Th17 cytokines, suggesting that deletion of the hits do not simply kill T cells or affect T cell function globally, but has more specific effects on T cells in the CNS. These findings suggest that depletion hits regulate the migration of CD4+ T cells to the CNS, their proliferation or survival in the CNS. Future studies will i) focus on elucidating the mechanisms of these novel ion channels in encephalitogenic T cell function, and ii) assess if these channels can be the potential drug targets for curing EAE/MS.

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