BELGIAN SOCIETY OF

PHYSIOLOGY AND PHARMACOLOGY

NATIONAL COMMITTEE OF PHYSIOLOGY AND PHARMACOLOGY

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Autumn Meeting

Friday, November 24th 2023

PROGRAMME

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Venue

Palace of the Academies
Royal Academy of Medicine of Belgium
"Rubens room"
Rue Ducale / Hertogstraat 1
1000 Brussels

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Local host

Prof. Dr. Alain Labro
Department of Basic and Applied Medical Sciences
Ghent University
Belgium

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with support of the

Royal Flemish Academy of Belgium for Science and the Arts

BELGIAN SOCIETY OF PHYSIOLOGY AND PHARMACOLOGY

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Autumn Meeting
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Venue

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Royal Academy of Medicine of Belgium
"Rubens room"
Rue Ducale / Hertogstraat 1
1000 Brussels

09.15-10.00 Welcome with coffee and tea

Keynote lecture

10.00-11.00 **Acute stress can be fatal in cardiac cells**
Prof. Guiscard Seebohm (University of Munster, Germany)

Oral communications (morning session)

11.00-11.15 **Assessing the roles of miR-199a in cardiac and vascular adaptations**
Marchand D., Joris V., Dessy C. (UCLouvain)

11.15-11.30 **Role of the adipocyte beta3-adrenergic receptor on myocardial remodeling**
Cascarano L, Esfahani H, Balligand JL , Michel LY (UCLouvain)

11.30-11.45 **Potential of "peroxiporin" blockers to improve vascular oxidant stress in humans**
Hasnae Boughaleb, Virginie Montiel (UCLouvain)

11.45-12.00 **Starvation resistant cavefish reveal conserved mechanisms of starvation-induced hepatic lipotoxicity**
Macarena Pozo-Morales, Ansa E Cobham, Cielo Centola, Mary Cathleen McKinney, Peiduo Liu, Camille Perazzolo, Anne Lefort, Frédérick Libert, Hua Bai, Nicolas Rohner, Sumeet Pal Singh (ULB & Stowers Institute for Medical Research, Kansas City, MO & Iowa State University, Ames, IA)

12.00 – 12.30 Lunch

12.30 – 13.00 Guided Poster Session

Posters:

- **Succinate Receptor as an Emerging Target in Ischemic Stress**
  A. Szedleski, J. Huart, J. Hanson, F. Jouret (U Liège)

- **Effect of Episodic Hypoxaemia on skeletal muscle: which association with Ad pathway modifications?**
  Lise Paprzycki, Yamina Gourari, Alexandre Legrand, Florence Debacq-Chainiaux, Alexandra Tassin (UMons & UNamur)

- **Organotypic slice cultures from the P1 mouse dorsal raphe: Methodological development and first results**
  ROBAYE Laura, ENGEL Dominique, LIEGEOIS Jean-François, KERFF Frédéric, QUERTEMONT Etienne, SEUTIN Vincent (ULiège)

Oral communications (afternoon session)

13.00-13.15 **A photopharmacology approach to reduce spontaneous seizures in a mouse epilepsy model**
Sofie Bournons, Surajit Sahu, Roman Sarott, Miroslav Kosar, Bilal Kicin, Patrick Pfaff, Matthias Westphal, Michael Schafroth, Erick M. Carreira, Dimitri De Bundel, Ilse Smolders (VUB & Eidgenössische Technische Hochschule Zürich)

13.15-13.30 **Role of TRPC1 channel in synaptic plasticity**
Farah Issa, Xavier Yerna, Thibaud Parpaite, Olivier Schakman, Nicolas Tajeddine, Roberta Gualdani, Philippe Gailly (UCLouvain)

13.30-13.45 **Fluoroquinolones and the Risk of Aortic Aneurysm or Aortic Dissection: Evidence From a Nationwide Nested Case-Control Study Paralleled With Matched Experimental Models**
Callan D. Wesley, Jarl Emanuel Strange, Anders Holt, Gunnar H. Gislason, Cédric H.G. Neutel, Dustin N. Krüger, Celine Civati, Mart Theunis, Tania Naessens, Lynn Roth, Guido R.Y. De Meyer, Wim Martinet, Peter Vibe Rasmussen, Pieter-Jan Guns (UAntwerp & Copenhagen University Hospital)

Closing lecture

13.45-14.30 **Drug-induced vascular toxicity in safety pharmacology and cardio-oncology**
Pieter-Jan Guns (UAntwerp)

14.30 Coffee – Tea and Networking

15.00 General Assembly for the Members

ORAL PRESENTATION 1

Assessing the roles of miR-199a in cardiac and vascular adaptations

Marchand D., Joris V., Dessy C.

UCLouvain - IREC institute - FATH group

INTRODUCTION| Numerous clinical studies have shown that patients suffering from cardiovascular disorders such as cardiac hypertrophy have a highly specific miR expression profile. Several of these have been characterized and potential roles suggested in the development of hypertrophy. Our previous work has documented an up-regulation of both miR-199a-3p and -5p in heart and vessels of TAC and angiotensin II treated mice; as well as a downregulation following chronic moderate exercise. Currently, we are investigating the actors implicated is this dynamic regulation. Among the signaling pathways regulating cardiac hypertrophy, we focus on microRNAs and AMPK.

METHODS| Heart from C57BL/6J AMPKα1 KO or WT mice were processed for miR profiling using Maxwell miR extraction and RT-qPCR. Neonatal rat cardiomyocytes were treated with pro-hypertrophic stimuli or transfected with siRNA targeting QKI or Gld2, partners of Argonaute (Ago2). After 5 days in culture, cells were harvested for miR profiling as before. An immunoprecipitation was also performed on those cells.

RESULTS| Our results show that, in both mice and cultured cardiomyocytes, miR-199a-5p's expression is regulated by AMPKα1. By measuring the expression of pre-miR, we highlighted that this regulation is carried via stabilization. In parallel, PKA is also implicated. Finally, our results show that a downregulation of Gld2 and QKI-7 induce a downregulation of miR-199a.

CONCLUSION| Taken together, our results suggested a role of AMPK in miR-199a-5p stabilization. Our current working hypothesis is that AMPK or PKA activates by phosphorylation Gld2. This enzyme adds a single adenine to miRNAs, the latter appears to stabilize.

ORAL PRESENTATION 2

Role of the adipocyte beta3-adrenergic receptor on myocardial remodeling

Cascarano L, Esfahani H, Balligand JL , Michel LY

FATH-IREC-UCLouvain

Aside from its role in energy storage, the adipose tissue (AT) modulates tissue remodeling in distant organs, including the heart, through endocrine mechanisms that vary according to the metabolic status and type of adipocyte. We hypothesize that modifications of AT during obesity impacts cardiac remodeling via AT endocrine communication. We further postulate that increasing the pool of beige adipocytes - that share brown-like characteristics while dispersed in white AT - through stimulation of adipocyte beta-3 adrenergic receptor (β3AR), could prevent AT remodeling and remotely protect against cardiac remodeling upon metabolic imbalance.

C57Bl/6J mice were subjected to a high-fat +sucrose diet (HF-S) or a control diet during 5 months. Echocardiographic follow-up and histological analysis confirmed that HF-S diet produced cardiac hypertrophy and interstitial fibrosis as well as initial signs of left ventricular diastolic dysfunction. Notably, these phenotypic changes were less marked in the C57Bl/6N strain, possibly owing to NNT deletion in the -6J strain. Moreover, obese mice showed impairment of glucose disposition (OGTT) after 1 month of HF-S diet. Transcriptional study of primary adipocytes from HF-S mice revealed a shift towards white phenotype compared to a more beige-like phenotype in control. Treatment with the β3AR agonist, CL-316243 increased the expression of thermogenic brown/beige markers in AT, and prevented weight gain and metabolic dysregulation under HF-S.

Altogether, HF-S-fed C57Bl/6J mice recapitulate key features of obesity-related metabolic and cardiac phenotypes. The model is being used to test the AT cross-talk with the heart through local and systemic effects of β3AR on AT.

ORAL PRESENTATION 3

Potential of "peroxiporin" blockers to improve vascular oxidant stress in humans

Hasnae Boughaleb, Virginie Montiel

FATH IREC UCLouvain

Bacopaside II from the Ayurvedic plant, Bacopa monnieri (BM) is a specific blocker of the water channel, Aquaporin 1 (AQP1). We showed that AQP1 also mediates the transport of H2O2 across the membrane of cardiac myocytes, endothelial cells and erythrocytes (1), thereby implicating AQP1 as a "peroxiporin" in the regulation of vascular oxidative stress.

Aim: Evaluation of the safety and potential of Bacopa-400®, an oral BM extract to block transmembrane passage of H2O2. Methods: 20 healthy volunteers; Group A: 400 mg/d; Group B: 800 mg/d; 6 weeks of treatment; Evaluation (V0-V4) of: i.safety: clinical routine biomarkers; ii.vascular oxidative stress: subjects'erythrocytes were loaded ex vivo with the fluorescent ROS tracer, dichlorodihydrofluorescein diacetate (DCFDA), and fluorescence was monitored after exposure to H2O2 by FACS.

Results: Oral BM extract: i.did not alter blood count or muscular enzymes; ii.slightly increased LDL-cholesterol (LDL_V0=104.2±38.8 vs LDL_V4=122.1±47.4 mg/dL; p=0.01) in group A; and total-cholesterol (Chol_V0=188.0±29.9 vs Chol_V4=210.0±47.7 mg/dL; p=0.03) and LDL-cholesterol (LDL_V0=111.3±29.2 vs LDL_V4=135.4±44.1 mg/dL; p=0.01) in group B; iii.decreased the area under the curve (AUC) of the DCFDA signal at V4 in both groups (Group A: AUC_V0=14711±2239 vs AUC_V4=12270 ± 1613; Group B: AUC_V0=14711±2239 vs AUC_V4=10422±1520;), albeit non-significantly.

Conclusion: Chronic oral intake of BM is clinically well tolerated. Despite a slight increase of the lipid profile, it tended to dose-dependently decrease the transmembrane passage of extracellular H2O2 in erythrocytes, a proxy of vascular oxidant stress. Higher doses may be needed to fully block AQP1 in vivo. (1) Montiel et al. Sci Transl Med 2020

ORAL PRESENTATION 4

Starvation resistant cavefish reveal conserved mechanisms of starvation-induced hepatic lipotoxicity

Macarena Pozo-Morales1†, Ansa E Cobham2†, Cielo Centola2, Mary Cathleen McKinney2, Peiduo Liu3, Camille Perazzolo1, Anne Lefort1, Frédérick Libert1, Hua Bai3, Nicolas Rohner2,4*, Sumeet Pal Singh1*

1 IRIBHM, Université Libre de Bruxelles (ULB), Route de Lennik 808, 1070 Brussels, Belgium. 2 Stowers Institute for Medical Research, Kansas City, MO 64110, USA. 3 Department of Genetics, Development, and Cell Biology, Iowa State University, Ames, IA 50011, USA. 4 Department of Cell Biology & Physiology

INTRODUCTION | Starvation causes the accumulation of lipid droplets in the liver, a somewhat counterintuitive phenomenon that is nevertheless conserved from flies to humans. Much like fatty liver resulting from overfeeding, hepatic lipid accumulation (steatosis) during undernourishment can lead to lipotoxicity and atrophy of the liver. To uncover potential strategies for protecting the liver, we turned to adaptation strategies observed in nature.

METHODS | To gain mechanistic insights, we used a genetically tractable and naturally occurring model of starvation resistance - the Astyanax mexicanus model system. Cave populations of this species have adapted to survive extreme starvation, while the surface populations of the same species display relatively normal vertebrate physiology. We took advantage of this unique system to study the response of the liver to starvation.

RESULTS | We found that while surface populations of Astyanax mexicanus undergo this evolutionarily conserved response to starvation, the starvation-resistant cavefish larvae of the same species do not display an accumulation of lipid droplets upon starvation. Moreover, cavefish are resistant to liver atrophy during starvation, providing a unique system to explore strategies for liver protection. Using comparative transcriptomics, we identified the fatty acid transporter slc27a2a/fatp2 to be correlated with the development of fatty liver. Pharmacological inhibition of slc27a2a in zebrafish rescues steatosis and atrophy of the liver upon starvation. Further, down-regulation of FATP2 in drosophila larvae inhibits the development of starvation-induced steatosis.

CONCLUSION | This demonstrates that the identified pathway is evolutionary conserved for over 400-million-years, highlighting its potential as a druggable target.

ORAL PRESENTATION 5

A photopharmacology approach to reduce spontaneous seizures in a mouse epilepsy model

Sofie Bournons[1], Surajit Sahu[1], Roman Sarott[2], Miroslav Kosar[2], Bilal Kicin[2], Patrick Pfaff[2], Matthias Westphal[2], Michael Schafroth[2], Erick M. Carreira[2], Dimitri De Bundel[1], Ilse Smolders[1]

1:Vrije Universiteit Brussel, Department of Pharmaceutical Chemistry, Drug Analysis and Drug Information, Center for Neurosciences (C4N), Brussels, Belgium, 2:Laboratorium für Organische Chemie, Eidgenössische Technische Hochschule Zürich, Vladimir-Prelog-Weg 3, 8093 Zürich, Switzerland

Epilepsy is one of the most common neurological disorders, affecting up to 50 million people globally. Over the last decade, cannabinoids received considerable interest for the treatment of refractory epilepsies. Δ9-tetrahydrocannabinol (Δ9-THC) has a significant seizure suppressing potential but its therapeutic use is limited due to its extensive side effect profile. To evade this problem, a photoswitchable Δ9-tetrahydrocannabinol (azo-THC-3) derivative has been developed. Two isomers (cis-ON, trans-OFF) of this compound exists, each one characterized with its own pharmacological properties. Using UV-A light (365 nm), azo-THC-3 could be photo-activated to the cis-ON configuration at the site of seizure onset, thereby reducing off-target side effects. We here assess its anticonvulsant potential in a clinically relevant mouse model of drug-resistant epilepsy.

C57Bl/6J mice were unilaterally injected with kainic acid, and an optofluid cannula coupled to a depth electrode was implanted into the affected dorsal hippocampus. Azo-THC-3 (n=7), or vehicle (n=6) were infused directly into the hippocampus after a baseline period, and we analyzed seizure occurrence for two hours post-infusion following irradiation at 365 nm (cis-ON) and without irradiation (n=7, trans-OFF).

Hippocampal infusion of azo-THC-3 significantly reduced number ($p=0.0257$) and duration ($p=0.0344$) of seizures compared to a vehicle control group following irradiation at 365 nm. Infusion of azo-THC-3 without irradiation did not result in significant changes compared to vehicle controls. Intrahippocampal infusion of azo-THC-3 light-dependently decreases the seizure burden in a mouse model of drug-resistant temporal lobe epilepsy. In future experiments we aim to photoactivate azo-THC-3 following systemic administration of the photoswitchable drug.

ORAL PRESENTATION 6

Role of TRPC1 channel in synaptic plasticity

Farah Issa, Xavier Yerna, Thibaud Parpaite, Olivier Schakman, Nicolas Tajeddine, Roberta Gualdani, Philippe Gailly

Université Catholique de Louvain, Institut de Neuroscience

INTRODUCTION |Group I metabotropic glutamate receptors (mGluR) are involved in various forms of synaptic plasticity that underlie declarative memory by inducing long-term depression (mGluR-LTD). mGLUR-LTD results from an endocytosis of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) underlying synaptic depression. The lab has previously shown that mGluR5 specifically activates TRPC1 channel, a Transient Receptor Potential channel that is highly expressed in CA1-3 regions of the hippocampus.

METHODS |To investigate the role of mGluR-TRPC1 in synaptic plasticity, and to decipher its downstream pathway, we induced an acute deletion of Trpc1 gene, using the Cre-tamoxifen conditional system, in a murine model of Fragile X Syndrome (FX). FX is a genetical disorder characterized by an enhanced activation of mGluR5, an accelerated memory extinction, and an exaggerated mGluR-LTD that persists even in the presence of anisomycin, a protein synthesis inhibitor.

RESULTS |Acute deletion of Trpc1 almost abolished the mGluR-LTD observed in FX mice (FX Cre), and results in a maintained surface AMPARs that did not undergo endocytosis. Furthermore, memory extinction was reduced in both Morris Water Maze and Passive Avoidance tests after inhibiting Trpc1. Finally, in hippocampal brain slices that lack Trpc1, activation of mGluR5 by its agonist, DHPG, failed to increase Arc, an activity regulated cytoskeleton associated protein and failed to phosphorylate ERK, the extracellular signal-regulated kinases, both essentials for mGluR-LTD.

CONCLUSION |Here we show that TRPC1 is involved in maintaining a normal mGluR-LTD, and is required for memory extinction, an important process in synaptic plasticity. Deciphering its pathway is in progress.

ORAL PRESENTATION 7

Fluoroquinolones and the Risk of Aortic Aneurysm or Aortic Dissection: Evidence From a Nationwide Nested Case-Control Study Paralleled With Matched Experimental Models

Callan D. Wesley, Jarl Emanuel Strange, Anders Holt, Gunnar H. Gislason, Cédric H.G. Neutel, Dustin N. Krüger, Celine Civati, Mart Theunis, Tania Naessens, Lynn Roth, Guido R.Y. De Meyer, Wim Martinet, Peter Vibe Rasmussen, Pieter-Jan Guns

University of Antwerp and Copenhagen University Hospital

INTRODUCTION | Fluoroquinolones (FQ) have been linked to aortic aneurysms and dissections (AA/AD), resulting in an official warning. However, recent large-scale epidemiological studies have reported lack of FQ-AA/AD association. This study aimed to scrutinize FQ-AA/AD risk by implementing a combined epidemiological and experimental approach.

METHODS | Danish nationwide registers (2003-2021) were used for a nested case-control analysis. FQ-AA/AD risk was evaluated in a main and high-risk FDA cohort. Further, mortality and aortic interventions linked to FQ were investigated in patients with aortic disease. Additionally, ciprofloxacin (100 mg/kg/day, 2x2 weeks) was administered to wild-type, hypertensive or Marfan mice. Aortic diameters and pulse wave velocity (PWV) were measured longitudinally to investigate aortic remodeling.

RESULTS | The main cohort comprised 5.10 million individuals with 58,919 cases and 1,767,510 sampled controls. Compared with amoxicillin exposure. FQ exposure was not associated with increased AA/AD risk (30-day hazard ratios (HR) 1.00 [95% confidence intervals (CI): 0.74-1.34]; 90-day HR1.07[CI 0.94-1.22]; 1-year HR0.95[0.90-1.01]). In a high-risk cohort, there was no FQ-AA/AD association (30-day HR 0.83 [0.61-1.12]; 90-day HR0.99[0.86-1.15]; 1-year HR0.97[0.90-1.05]). In patients with aortic disease, FQ were not associated with increased aortic interventions or mortality (30-day HR0.98[0.79-1.22]; 90-day HR1.06[0.95-1.19]). Additionally, ciprofloxacin did not affect aortic diameters or PWV in wild type, hypertensive, and Marfan mice, while differences between models proved the sensitivity of the methodology.

CONCLUSION | The data clearly do not support the current precautions and warnings pertaining to risks of aortopathies and FQ should not be discouraged when clinically indicated.

POSTER PRESENTATION 1

Succinate Receptor as an Emerging Target in Ischemic Stress

A. Szedleski1,2, J. Huart1,2, J. Hanson2,3,*, F. Jouret1,2,*

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Renal ischemia/reperfusion (I/R) is the leading cause of acute kidney injury, and necessarily occurs during kidney transplantation or cardiovascular surgery. Renal ischemic preconditioning (RIP) includes the mechanical or pharmacological manoeuvres aiming to reduce the ischemic insult. However, the current RIP methods fail to completely mitigate I/R injuries that still lead to well-established poor consequences for kidney function. During ischemia, succinate concentration increases in intracellular and extracellular compartment. The intracellular effects of succinate during ischemia are well-studied, in contrast with its extracellular impact, notably mediated by its interaction with the Succinate Receptor (SUCNR1), highly expressed by macrophages. SUCNR1 has recently emerged as an ischemic stress sensor, and we hypothesize that it may represent an innovative drug target in pharmacological RIP. Additionally, preliminary observations from our laboratory showed a significant decrease in kidney injuries following I/R in Sucnr1 knocked-out mice compared to wild-type.

Our research project plans to answer these outstanding questions : 1. What is the in vivo role of SUCNR1 during renal I/R, with a specific focus on the contribution of the SUCNR1 expressed by macrophages ? 2. What is the in vivo impact of the SUCNR1 pharmacological modulation on the RIP, and what are the mechanisms of action for various SUCNR1 ligands ? Our experimental strategy consist in renal I/R model, applied both to (i) macrophages and proximal tubular cells, and (ii) transgenic mice lines (conditional Sucnr1-KO and Sucnr1 tagged with StayGold, a photostable and bright GFP). The impact of their exposition with SUCNR1 ligands will also be studied.

POSTER PRESENTATION 2

Effect of Episodic Hypoxaemia on skeletal muscle: which association with Ad pathway modifications?

Lise Paprzycki[1], Yamina Gourari[1], Alexandre Legrand[1], Florence Debacq-Chainiaux[2], Alexandra Tassin[1]

[1] Laboratory of Respiratory Physiology, Pathophysiology and Rehabilitation, Research Institute for Health Sciences and Technology, University of Mons, Mons, Belgium. [2] URBC-Narilis, University of Namur, Namur, Belgium.

Episodic hypoxaemia, a major pathological component of progressive respiratory insufficiencies, is associated with systemic comorbidities including altered skeletal muscle function and regeneration. Adiponectin (ApN), an adipo/myokine favouring muscle oxidative metabolism, exerts anti-inflammatory, anti-ageing, pro-myogenic and antioxidant effects. Since converging evidence suggest its alteration upon hypoxia, ApN pathway constitutes an attractive therapeutic target to counteract the effects of episodic hypoxaemia at the muscle level.

To decipher the specific effect of episodic hypoxaemia on skeletal muscle, we used a reductionist mouse model of Sustained Intermittent Hypoxaemia (SIH; FiO_2: 10%, 8h/day). Muscle structural changes, expression of myogenic markers and ApN pathway were investigated in fast and slow-type muscle fibers at early and late timepoints. At 35 days, the Cross-Sectional Area of the soleus muscle is increased, with a dominant effect on slow-type fibers. This hypertrophy is not observed in the fast tibialis anterior (TA) muscle. However, in this muscle, we observed a decreased expression of Myogenin (Myog) or Myod (Myod) at 7 and 35 days, respectively. No change was observed in plasmatic ApN levels. However, a decreased expression of AdipoR2 receptor (Adipor2) was observed at 7 and 35 days in the TA muscle. AdipoR2 protein level was also shown affected in the soleus muscle at early timepoints.

In conclusion, SIH mice exhibit an early decrease in the expression of myogenic markers and a muscle hypertrophy taking place over time, mainly in slow-type myofibers. Those effects are associated with perturbations of the AdipoR2 receptor at the mRNA or protein levels.

POSTER PRESENTATION 3

Organotypic slice cultures from the P1 mouse dorsal raphe: Methodological development and first results

ROBAYE Laura, ENGEL Dominique, LIEGEOIS Jean-François, KERFF Frédéric, QUERTEMONT Etienne, SEUTIN Vincent

Université de Liège

Previous data have suggested that blockade of SK channels in the dorsal raphe leads to antidepressant effects and that it induces cognition enhancing effects via the hippocampus. In order to expand on these ideas, it would be important to assess the behavioural consequences of a reduction in the expression of SK2 and SK3 subunits in these areas. To achieve this, we decided to selectively inhibited SK2 and/or SK3 expression in the two areas in mouse brain using local injection of CRISPRi constructs.

In order to determine the most effective sgRNAs, we used the organotypic brain slice culture technique using acute slices from P1 mice dorsal raphe and hippocampus. Preliminary whole-cell recordings demonstrate the viability of neurones after 2 weeks in slice cultures. In the dorsal raphe, all recorded neurons had overshooting action potentials and displayed input resistance of 155 ± 22 MΩ (Mean ± SEM, n=9). When applying 1 µM apamin, the classical SK channels blocker, we observed an increase in firing during depolarizing pulses, indicating the presence of functional SK channels in these neurons.

Organotypic brain slice cultures from P1 mice are the method of choice for determining the most efficient sgRNAs to use for our further study of SK channels.