BEB Day 2024

(Not) Knowing What Comes Next:
Tools for the Research of Tomorrow

BOOK OF ABSTRACTS

15-16 MAY 2024
COIMBRA

cnc CENTER FOR NEUROSCIENCE AND CELL BIOLOGY
PDBEB DOCTORAL PROGRAM EXPERIMENTAL BIOLOGY AND BIOMEDICINE
cibb CENTRE FOR INNOVATIVE BIOMEDICINE AND BIOTECHNOLOGY
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Book of Abstracts

15-16 May 2024

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Table of Contents

Program Overview

BEB DAY 2024
PROGRAMME

DAY 1 | May 15th

8h30-9h | Check-In (Registration)

9h | Opening Session with João Ramalho-Santos
(Director of the Institute of Interdisciplinary Research, University of Coimbra)

9h30 | The Power of Innovation
Mariangela Natale (former scientist at CellmAbs & BEB alumni)

10h15 | Coffee Break & BEB Rooms *(with BEB alumni: Mariangela Natale, António Preto, Manuela Ferreira, Ricardo Amorim)*

11h | Uses of AI in publishing: What to do, and what not to do!
Anthony Newman (Elsevier)

11h30 | AI use in biomedical research: Methods, transparency, limitations, and ethics
John Ioannidis (Stanford University)

12h | Publishing with AI: discussion

12h15 | New insights and novel methods in real time: Examples from development of *in vivo* gene therapy for Parkinson's disease
Cecilia Lundberg (Lund University)

12h45 | Lunch Break

14h15 | Selected Talks

15h | Poster Session 1

16h15 | Coffee Break

16h45 | What is Quantum Computing and Why We Should Care
Luís Soares Barbosa (University of Minho)

17h15 | AI for Biomedical Discovery
Joel Arrais (DEI-UC)

17h45 | Cultural Moment: Beer for Thought *(sponsored by John Jones)* **+ Desconcertuna**

DAY 2 | May 16th

8h30-9h | Check-In (Registration)

9h | Selected BEB Talks

9h45 | Roadblocks for clinical translation: Importance of physiological O_2 levels for high throughput screening of therapeutics in live cell models
Giovanni Mann (King's College London)

10h15 | Coffee Break

10h45 | From Research-driven Innovation to Academic Entrepreneurship
George Papamichail (Foundation for Research and Technology - FORTH)

11h30 | Building Bridges: Integrating Science with Market Demand
Cristina Simões (HiSeedTech)

12h15 | Lunch Break

14h | Poster Session 2

15h | Outdoors Activity
Rafael Nogueira Rodrigues (Desporto UC - Pausa+Ativa)

16h | Coffee Break

16h30 | Round Table: Democracy and Science
Joana Branco (Biocant Park), Euclides Pires (Emeritus CNC), Joana Guedes (CNC-UC), Camila Dias (ABIC), Rui Tavares - Comment (Portuguese Parliament - Livre)

18h | Awards Session

18h30 | Closing Session with Paulo Oliveira *(PDBEB Coordinator)*, **Nuno Empadinhas and John Jones** *(BEBDay Organizing Committee)*

20h | Networking Dinner - Praxis

@ Pavilhão Centro de Portugal, Coimbra

Abstracts

*[1] **OC** – Oral Communication

*[2] **P** - Poster

OC1- ANTAGONISM OF ATP-P2X7 RECEPTOR PREVENTS NEUROINFLAMMATION AND OXIDATIVE STRESS, IMPROVING COGNITIVE AND EMOTIONAL OUTCOMES IN A STREPTOZOTOCIN-INDUCED MODEL OF SPORADIC DEMENTIA

Ariádne E. Belo-Silva[1,3]; Geanne M. Andrade[2] ; Samira G. Ferreira[3]; Rui D. Prediger[1]; Rodrigo A. Cunha[3]

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Introduction The ATP-P2X7 purinergic receptor (P2X7R) is a trigger for inflammation signaling and microglial activation, being regulated around senile plaques in Alzheimer's Disease (AD). Streptozotocin (STZ) administered intracerebroventricularly (ICV) is an experimental model for sporadic AD in rodents, inducing oxidative stress, microgliosis, and neuroinflammation, leading to emotional and cognitive deficits.

Aim To investigate the effects of blocking P2X7R on anxiety, depressive-like behaviors, memory impairments, oxidative stress, and neuroinflammation in an ICV-STZ-induced model of AD.

Methods Sixty adult male Swiss mice were divided into four groups: control, ICV-STZ (3mg/kg), BBG (50mg/kg), and ICV-STZ+BBG. Brilliant Blue G (BBG), a P2X7R antagonist, was administered i.p for 15 days. Behavioral tests for anxiety, depressive-like behaviors, and memory performance were conducted. Oxidative stress was evaluated by measuring nitrite and malondialdehyde in the prefrontal cortex and hippocampus. Blood glucose levels were monitored throughout the experiment. Immunoreactivity for Iba-1 and GFAP in the hippocampus was assessed, and 3D reconstructions of microglia and astrocytes were made using confocal microscopy.

Results BBG treatment reduced anxious and depressive-like behaviors and memory deficits caused by ICV-STZ. No significant differences were observed in total Iba-1 and GFAP immunoreactivity among groups. However, alterations in the number of astrocytes

and microglia in the ICV-STZ group were prevented by BBG. Furthermore, 3D reconstructions showed that ICV-STZ-induced changes in astrocyte and microglia morphology were also prevented by BBG.

Conclusion BBG treatment reduced alterations in the number and morphology of astrocytes and microglia in an ICV-STZ-induced AD model. These findings suggest that blocking P2X7R may have therapeutic potential in reducing behavioral deficits and neuroinflammation associated with AD.

OC2- A NANOFORMULATION FOR GENE EDITION IN RETINAL DEGENERATIVE DISEASES

Carolina Santos[1], Pedro Antas[2,3], Luísa de Lemos[3], Miguel Seabra[2,4], Susana Simões[5,6], Lino Ferreira[1,5]

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Choroideremia is an inherited retinal degenerative disease that causes progressive vision loss. It occurs as a result of a mutation in the CHM gene that encodes ubiquitously expressed Rab-escort protein-1 (REP-1), leading to retinal pigment epithelium (RPE) degeneration. There is no effective treatment for the disease. Gene therapy using adeno-associated virus is the approach with higher maturity for a potential treatment, but it has several limitations since it does not correct the endogenous mutated REP-1 and has the risk of immune system activation. We aim to develop a non-viral gene editing nanoformulation (CRISPR-NP) for delivery of Cas9 mRNA and the sgRNA designed for correction of REP-1. As proof of concept, we evaluated the gene editing efficiency of CRISPR-NP to create REP-1 mutations in a differentiated RPE human cell line (ARPE-19) and in RPE derived from human iPSCs without the CHM mutation (iPSC-RPE). Cells were incubated with CRISPR-NP for 4 hours and collected 3 days after. Internalization and toxicity were assessed immediately after the 4 hours incubation, by fluorescence microscopy or fluorescence-activated cell sorting. To establish the efficacy of REP-1 knockout, REP-1 levels were evaluated by western blot. In the differentiated ARPE-19 cells, we observed that knockout efficiency was dependent on cell density. In iPSC-RPE cells cultured in a monolayer, CRISPR-NP was uptaken by cells, delivered the Cas9 mRNA, the sgRNA, and performed the REP-1 knockout. Our results show the nanoformulation can be efficiently internalized, is safe and has a good gene editing capacity in vitro.

Funding: The authors appreciate the funding of COMPETE2020 and FCT program (EXPL/BIA-CEL/0989/2021 (acronym: Editing_Vision) and EXPL/MED-OUT/0599/2021 (acronym: NECHOR). C.Santos. acknowledges the FCT fellowship 2022.13609.BD.

OC3- Addressing cerebellum defects in a mouse model of schizophrenia harbouring a human mutation in the *CACNG2* gene encoding stargazin

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Cerebellar dysfunction has been implicated in several neuropsychiatric disorders. Both connectivity impairments and structural alterations in this brain region have been found in schizophrenia patients, suggesting a potential role for the cerebellum in the expression of schizophrenia-related phenotypes. However, despite these findings, the precise cerebellar defects that result in cognitive impairment and social behaviour abnormalities remain poorly understood.

This project aimed to study cerebellar defects in a mouse model harbouring a human mutation in the *CACNG2* gene, which was identified in association with schizophrenia. Stargazin, the *CACNG2*-encoded protein, is a synaptic protein highly expressed in the cerebellum, where it plays non-redundant functions in targeting AMPAR to the synapse. Knock-in mice harbouring a stargazin mutation associated with intellectual disability show abnormal cognitive and social behaviours, as well as impaired motor learning. Therefore, we aimed to assess social behaviour and sensorimotor gating in knock-in mice expressing a schizophrenia-associated variant of stargazin (STGSN-KI mice), as well as to evaluate cerebellar neuronal excitability.

Behavioural characterization revealed that STGSN-KI mice show depressive-like behaviour, deficits in prepulse inhibition of the acoustic startle response and in social behaviour. Overall, these results indicate that STGSN-KI mice recapitulate phenotypes observed in schizophrenia patients and other animal models of schizophrenia. Expression of mutant stargazin led to aberrant intrisic pattern of firing and higher excitability of Purkinje cells in the Crus I region of the cerebellum of STGSN-KI mice, compared to wild-type animals. Altogether, this work unveiled specific behavioural features altered in STGSN-KI mice, shedding light into the excitability deficits in Crus I Purkinje cells that might contribute to the behavioural abnormalities observed in this animal model.

OC4- Molecular mechanisms underlying the impairment of LTP in *Fmr1* KO mice

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Fragile-X-Syndrome, the most common inherited form of intellectual disability, is caused by transcriptional silencing of the *Fmr1* gene, that encodes for fragile-X-messenger ribonucleoprotein (FMRP). FMRP is an RNA-binding protein involved in regulating many synaptic proteins. In fact, *Fmr1*$^{y/-}$ mice present impaired synaptic plasticity, but the mechanisms underlying such deficits are largely unknown; the aim of this work is to bridge this gap. Acute hippocampal slices were prepared from WT and *Fmr1*$^{y/-}$ mice and long-term potentiation (LTP) was induced with five theta-bursts, a protocol known to induce the release of endogenous BDNF. We observed an impairment in LTP of CA1 synapses of *Fmr1*$^{y/-}$ mice. Blockade of BDNF-TrkB signalling further impaired LTP in slices from *Fmr1*$^{y/-}$ mice, while blocking NMDA receptors (NMDAR) was without effect. *Fmr1* downregulation may impair LTP by affecting BDNF-mediated control of synaptic NMDAR. This was investigated using primary cultures of hippocampal neurons transfected with a shRNA to knock down (KD) *Fmr1* expression, and analysing BDNF-induced upregulation of synaptic surface NMDAR by immunocytochemistry. *Fmr1* KD had no effect on synaptic GluN2A- and GluN2B-containing NMDAR under resting conditions, but abolished BDNF-induced upregulation of synaptic NMDAR. Furthermore, *Fmr1* KD impaired BDNF-induced dendritic accumulation of Pyk2, a kinase regulator of NMDAR synaptic stability. Finally, single particle tracking by quantum dots in neurons after *Fmr1* KD showed a decrease in mobility of GluN2A-containing NMDAR when compared to control, while GluN2B-containing NMDAR become so after BDNF treatment. Our data show an impairment in BDNF-induced synaptic regulation of NMDAR after *Fmr1* KD which may account for the deficits in LTP.

Funding: Supported by MSCA [ITN-#813986] and FCT.

OC5- Unmasking Hidden Systemic Effects of Neurodegenerative Diseases: A Two-Pronged Approach to Biomarker Discovery

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Identification of reliable blood biomarkers for neurodegenerative diseases (NDs) is crucial for translational and clinical research. However, conventional omics struggle with blood samples complexity, hindering desired outcomes. In this work the potential of High Molecular Weight (HMW) fractionation under non-denaturing conditions as a complementary approach to the conventional proteomics for identifying serum biomarkers in NDs was explored. A cohort of 58 serum samples of Alzheimer's disease (AD), Parkinson's disease (PD) patients and control (CT) individuals was used to compare the two proteomics strategies: i) direct analysis of whole serum and ii) non-denaturing fractionation using 300 kDa cut-off filters (HMW serum).

Although both approaches quantified a similar set of proteins, each approach captured a distinct subset of differentially altered proteins, suggesting that HMW fractionation identified additional types of alterations beyond conventional protein level changes. A discriminant model combining altered proteins from both datasets effectively distinguished between the three groups (AUC = 0.999 and median sensitivity and specificity of 97.4% and 91.7%, respectively). Importantly, this performance surpassed that of any model created using each method individually.

Altogether, this work demonstrated that HMW fractionation can be a valuable complementary method to direct serum analysis and could enhance biomarker discovery. The 10 proteins included in the model (5 from each strategy), comprise clear evidence for the contribution of apolipoproteins for the diagnosis of NDs, revealing potential changes within lipid metabolism and the organization of macromolecules and their complexes, thereby uncovering effects that remain hidden from a conventional serum proteome analysis.

OC6- Avian Host Antibody Production for SARS-CoV-2: Epitope-Specific Insights

Tiago Ochôa-Pires[1,2], Rafaela Seabra[1,3], Beatriz Vaz[1,2], Ana Beatriz Marques[1,3], Guilherme Gabriel[1], Rui Jorge Nobre[1,4,5], Ricardo S. Vieira-Pires[1,7*]

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Following the pandemic outbreak of the Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), researchers approached the issue by developing antibodies targeting the Spike glycoprotein of the SARS-CoV-2 virus, a critical component for host-cell entry and proliferation. One possible host for the development of this antibodies, are avian hosts, which offer unique advantages for antibody production, such as generating distinct antibody repertoires and enabling cost-effective, high-yield production of polyclonal IgY antibodies in eggs.

Grounded on these, we employed a proprietary scaffold to recombinantly produce five Spike peptides previously identified as immunodominant in avian systems (Lu et al., 2020). These peptides were used to immunize ISA-Brown chickens through three distinct vaccine formulations, a multimix of all five and two individual protocols.

Over a 90-day period post-immunization, daily egg collection enabled the evaluation of anti-Spike antibody titers via ELISA assays, leading to the selection of hyperimmune yolk pools. Subsequently, we purified total IgY polyclonal antibodies using a water dilution method and PEG precipitation. These final anti-Spike IgY polyclonal antibodies underwent analysis in terms of titer, target specificity, and sensitivity through ELISA and Western blotting (WB).

Examination of the five ELISA assay response profiles revealed that the epitope Ep3 significantly contributed to the response in the mixed formulation, while Ep2 was responsible for notable peaks in both individual and mixed formulations. A Neutralization Study was performed, indicating higher virus neutralization from IgY antibodies purified from the Spike Ep1 individual formulation.

These findings suggest that different formulations lead to diverse antibody responses, with Ep1, Ep2, and Ep3 showing the most promise. However, further research is needed to confirm the potential of these formulations to effectively combat SARS-CoV-2.

P01- Allele-specific silencing for Spinocerebellar Ataxia Type 3 via AAV9 polycistronic microRNA-based therapy

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Spinocerebellar ataxia type 3 (SCA3) is an autosomal dominantly inherited neurodegenerative disorder characterized by expansion of CAG trinucleotide repeats located in the *ATXN3* gene. This mutation induces a toxic gain-of-function in the encoded ATXN3 protein, leading to neurodegeneration, particularly in the cerebellum. Despite ongoing investigations, no treatment currently exists to alter disease progression in SCA3 patients. In this study, we explored the therapeutic potential of adeno-associated virus (AAV9) encoding polycistronic artificial microRNAs (miATXN3) targeting the mutant ATXN3 allele to induce gene silencing.

First, silencing capacity and selectivity for the mutant allele of different silencing constructs was evaluated in HEK293T cell lines transiently expressing human mutant or wild-type ATXN3. Our *in vitro* results identified a lead construct displaying significant and selective gene silencing abilities. Next, AAV9 encoding the lead silencing construct was delivered via intra-cisterna magna (ICM) injection, on post-natal day 1 in severely impaired transgenic SCA3 mice. Significant and robust improvements in motor behavior were observed at 5-, 8-, and 11-weeks post-injection. Importantly, histological analysis indicated a reduction in the number of ATXN3 aggregates and a trend toward preventing layer thickness shrinkage within the cerebellar lobules in AAV9 miATXN3-injected mice. These findings were supported by a dose-dependent reduction in mutant ATXN3 mRNA levels and decreased expression of neuroinflammatory markers typically elevated in the cerebellum of SCA3 mice. Notably, a significant increase of neuronal marker NeuN at the protein level was also observed. Finally, widespread detection of AAV genome copies and miATXN3 levels in disease-relevant brain regions was observed 13 weeks post-ICM injection.

In summary, our results support allele-specific AAV-based therapy in SCA3, representing a step toward closer clinical translation for SCA3 patients.

P02- Harnessing AI for Enhanced Drug Safety Evaluation

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Artificial Intelligence (AI) plays a significant role in the field of pharmaceutical innovation, significantly improving the precision and speed of drug toxicity prediction. The incorporation of AI with big data analytics, Machine Learning (ML), and Deep Learning (DL) has significantly enhanced the accuracy and efficiency of early toxicity detection. This advancement is crucial in refining drug assessment methods and ensuring AI's vital role at every stage of the drug discovery process. AI's ability to streamline compound safety assessment across the drug discovery pipeline not only minimizes resource and time investments, but also reduces the likelihood of late-stage drug development failures. The deployment of AI in this sector promises a substantial reduction in the timeline for delivering safe drugs to the market, potentially transforming patients' access to effective therapies. Emphasizing the importance of AI, its application across target identification, pre-clinical testing, clinical trials, and regulatory compliance highlights its contribution to the advancement of public health and medicine.

Keywords: Drug discovery; Toxicity prediction; Artificial intelligence; Machine Learning; Deep Learning.

P03- 2-HIT-SCZ: Unveiling the Gut-Immune-Brain axis in Schizophrenia

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Schizophrenia (SCZ) has been linked to gastrointestinal comorbidities suggesting that aberrant gut microbiota can impair brain functions through the Gut-Brain axis. It was observed that prenatal infection increases the risk of developing SCZ by 10- to 20-fold arguing that alterations in offspring immune responses may be involved in SCZ. The link between gut dysbiosis, inflammation and neural function remains largely unexplored. Our innovative proposal argues that modulation of the maternal immune system due to a bacterial infection during pregnancy allied to maternal gut dysbiosis can lead to the activation of innate immunity in the offspring and consequently neuroinflammation and synaptopathy. So far, we analysed fecal material from SCZ patients and age-match healthy controls by Next Generation Sequencing allowing the characterization of the gut microbiome. Our results suggest that SCZ patients have lower microbiome diversity. Moreover, post-mortem brain samples exhibit elevated pro-inflammatory markers and decreased anti-inflammatory cytokines, suggesting an activation of inflammatory pathways that are possibly involved in the onset and disorder progression. Interestingly, preliminary results from our “two-hit” SCZ-like animal model transplanted with human intestinal microbiota showed that, despite SCZ being more associated with males, females seem to be more affected. Behaviourwise, these animals seem to have hyperactivity and alterations in working memory and sociability. Increase in pro-inflammatory cytokines were detected in the plasma, ileum and prefrontal cortex indicating an overall inflammation. Our aim is to provide the first evidence linking the ‘leaky gut’ hypothesis to SCZ by developing a new SCZ-like mice model where maternal immune activation and dysbiotic gut microbiome from SCZ patients act in concert to alter immune responses that ultimately lead to synaptopathy characteristic of SCZ.

P04- Unraveling the brain areas behind prosociality

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Prosocial behaviors, defined as actions that benefit others – are phenomena observed in the lives of different social species [1]. These types of behaviors are fundamental aspects of animal and human interactions since they promote social bonding and cohesion. However, the mechanisms that drive these behaviors and their neural correlates are still unclear.

My PhD project aims to dissect the neural correlates of prosocial decision-making in rats. We have strong hypothesis regarding the role of the anterior cingulate cortex (ACC) and its putative connections to downstream areas, and we will perform gain and loss of function experiments to dissect its role in prosocial decisions. However, as a first step, I will evaluate c-fos signal in animals performing prosocial actions, as a proxy of neural activity, and compare it with control animals (home cage). For this, we will study brain activity of animals performing the prosocial choice task (PCT)), a task previously developed by my laboratory [2]. This behavior paradigm involves a two-choice behavior apparatus, where a focal rat can choose between one of the two sides of the maze, which yields food only to itself (selfish choice), or the opposite side, which yields food to itself and the recipient rat (prosocial choice). Previous results in [2] showed that rats have a high proportion of prosocial choices.

Preliminary results from my brain-wide c-fos analysis revealed positive cells in the pre-frontal cortex, insular cortex, lateral septum, paraventricular nucleus and other brain regions. In the future, these results could shed light on the brain areas that are involved in the regulation of prosociality and guide our circuit-specific gain and loss of function experiments.

1. Cronin, K.A., Prosocial behaviour in animals: the influence of social relationships, communication and rewards.Animal Behaviour, 2012. 84(5): p. 1085-1093.
2. Márquez, C., S.M. Rennie, D.F. Costa, and M.A. Moita, Prosocial choice in rats depends on food-seeking behavior displayed by recipients. Current Biology, 2015. 25(13): p. 1736-1745.

P05- Obstructive Sleep Apnea and Cellular Aging: Exploring Extracellular Vesicle Dynamics

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Introduction: Obstructive Sleep Apnea (OSA) is a prevalent sleep disorder, associated with chronic comorbidities, and may accelerate aging (Gaspar, et al, 2017). Extracellular Vesicles (EVs) play a crucial role in intercellular communication. We hypothesized that EVs contribute to accelerate aging/aging-related disorders in OSA patients. This study aims to investigate the impact of EVs-OSA on aging hallmarks.

Methods: EVs isolated from plasma of 12 men diagnosed with severe OSA (54±10 years) in the moment of diagnosis (T_{0M}), 24 months of treatment (T_{24M}) with continuous pressure positive mask, and from 14 controls of the same sex and age group (49±8 years) and 12 young controls (24±2 years) were characterized. This study was approved by the ethical committees of the FMUC and Coimbra Hospital. Moreover, human fibroblasts were incubated with EVs-OSA and the following hallmarks of aging were evaluated: senescence markers (p16, p53, p21, b-gal), genomic instability (ATM, ATR) and biological clock markers (Per1, Cry2, Dec1, Bmal1).

Results: Plasma from patients with OSA at T_{0M} and T_{24M} show an increased concentration of EVs in comparison with controls. Dysregulation in senescence gene expression and disruptions in biological clocks' profile in OSA-EVs seems to be mediated by SIRT1 as evidenced by their deregulation.

Conclusion: These results suggest OSA might aggravates/promotes some hallmarks of aging, some of which are not reverted by CPAP treatment.

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P06- Unveiling miR-486-5p role in Parkinson's Disease: mitochondrial dysfunction and inflammation *in vitro*

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Parkinson's disease (PD) is a neurodegenerative disorder that is clinically diagnosed upon the onset of motor symptoms. However, it has been described the existence of a prodromal phase, during which non-motor features emerge, such as gastrointestinal dysfunction, thus suggesting that some PD cases might start in the gut and spread to the brain many years before the first motor symptoms. In fact, gut inflammation and dysbiosis have been reported in PD patients, but it has not been elucidated how gut microbiota variations alter brain function. MicroRNAs (miRNAs) have been highlighted as important contributors. Several studies show that miRNAs influence bacterial growth and that gut microbiota metabolites can regulate miRNA expression in intestinal cells, which may alter intestinal homeostasis and allow the spread of gut contents to the brain through the bloodstream. Specifically, a recent study focused on the importance of the gut in body-first PD; it showed that miR-486-5p was overexpressed in colon biopsies of PD patients, which correlated with age and disease severity. The relevance of this study to gut-first PD led us to study miR-486-5p role in mitochondrial dysfunction and inflammation, two key pathological mechanisms involved in PD pathophysiology, in SH-SY5Y and Caco-2 cells. We determined that overexpression of miR-486-5p in SH-SY5Y cells downregulates cardiolipin synthase, leading to depletion of cardiolipin levels, which potentiated mitochondrial dysfunction. Mitochondrial dysfunction, through the production of ROS, induced the activation of inflammatory responses. Moreover, we found that miR-486-5p overexpression in Caco-2 cells also downregulates interleukin 23 receptor, resulting in increased levels of IL-1β and reduced levels of IL-17, consequently leading to decreased integrity of the intestinal epithelial barrier. Overall, our results suggest that this miRNA contributes to PD pathogenesis through mitochondrial dysfunction and inflammatory signalling.

P07- Unravelling the role of lipid metabolism of unconventional T cells

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Lifestyle and intestinal inflammation are key risk factors for colorectal cancer. Despite advancements in understanding the tumor microenvironment, the mechanisms by which immune cells perceive environmental cues and their implications for malignancy remain largely unknown.

Intraepithelial lymphocytes (IELs), specialized T cells residing closely with the intestinal epithelium, play a crucial role in immune defence and epithelial integrity maintenance. Notably, our research has revealed that CD8αα IELs are intricately regulated by retinoic acid, highlighting their responsiveness to environmental cues for both thymic development and intestinal maintenance.

Furthermore, recent findings have demonstrated that IELs play a role in systemic metabolism and exhibit heightened levels of enzymes involved in cholesterol biosynthesis compared to peripheral T cells.

Despite these findings, the lipidic metabolic profile of IELs and their impact on intestinal immunophysiology remain elusive. Consequently, there is an urgent necessity to investigate the role of cholesterol pathways in IELs and their broader implications for intestinal health.

This study delved into lipid pathways within CD8αα IELs by examining the gene expression of key players involved in various lipid pathways. Our analysis compared IEL populations with T cells from lymph nodes, affirming a notable upregulation of the cholesterol biosynthesis pathway in IELs at the mRNA level. Additionally, scrutiny of a proteome-based open dataset (Brenes et al., 2021, eLife 2021;10:e70055) unveiled potential candidates for CD8αα-specific markers, which can potentially be utilized to target CD8αα.

Through unravelling the impact of lipid metabolism on the development and functionality of unconventional T cells, particularly CD8αα IELs, the insights garnered from this project offer promise in devising novel strategies to enhance human well-being and pave the way for innovative therapeutic interventions in intestinal malignant diseases.

P08- Novel evidence of white matter contribution in Spinocerebellar Ataxia type 3 pathology

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Spinocerebellar ataxia type 3 (SCA3) is a devasting neurodegenerative disorder that belongs to the family of polyglutamine disorders. Despite the CAG repeat expansion beneath SCA3 disease having been discovered 30 years ago, there is still no cure or treatment able to delay its progression. One of the reasons for this lag may be attributed to the clinical heterogeneity among individuals, both phenotypic and neuropathological.

To overcome this gap, we decided to investigate the contribution to the pathology of specific brain regions that have been consistently reported to be the most degenerated in SCA3 patients, the cerebellar cortex, namely lobule IV-V, VIII and X, deep cerebellar nuclei (DCN) and the pons. For this purpose, we used lentiviral vectors to deliver human mutant ataxin-3, the SCA3-causing gene, to these specific regions in mice.

In the present study, we observed that the overexpression of mut*ATXN3* in different hindbrain regions led to the formation of ataxin-3 aggregates and alterations in motor phenotype. Neurons in the pons were more vulnerable to mut*ATXN3* overexpression than in the cerebellum. There was an increase in astrocytes and microglia recruitment that may be behind myelin damage and consequently, white matter loss in the cerebellum. Indeed, white matter loss was the most broadly observed pathological feature upon overexpression of mut*ATXN3* in different regions of the hindbrain.

In this work, we provide novel evidence that white matter changes are one of the hallmarks of SCA3 neuropathology. we believe that white matter changes have often been overlooked in SCA3 animal models and should be regarded as a biomarker for evaluating disease progression and novel therapies.

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P09- AntiOxBEN2, a mitochondria-targeted gallic acid antioxidant, improved hepatic lipid accumulation in male and female MASLD mouse model

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The global prevalence of Metabolic dysfunction-associated steatotic liver disease (MASLD) is approximately 24%. MASLD progression is commonly linked to hepatic fat accumulation, increased oxidative stress, and mitochondrial dysfunction. Addressing mitochondrial impairment through bioactive compounds has been a prevalent approach in disease intervention. Here, we present AntiOxBEN2, a mitochondriotropic molecule synthesized by conjugating the antioxidant gallic acid with the triphenylphosphonium cation, designed to specifically accumulate within the mitochondrial matrix. To study the impact of AntiOxBEN2 on the MASLD mouse model, male and female C57BL/6J mice were subjected to a high-fat (30%) and high-sucrose (30%) (HFHS) diet for 16 weeks, followed by injection of AntiOxBEN2 (0.5 or 2.5 mg/Kg, 3x/week) for 14 weeks, initiated 2 weeks after diet introduction.

AntiOxBEN2 did not induce significant changes in the body weight. Nevertheless, in females, AntiOxBEN2 (2.5 mg) exhibited a tendency towards reduced liver weight, diminished hepatic fat accumulation, and lowered plasma levels of liver damage markers (ALT, C-reactive protein, and leptin) compared to HFHS diet-fed animals. In males, AntiOxBEN2 (0.5 mg) reverted the significant increase in liver weight, plasma ALT, and insulin levels observed in HFHS diet-fed mice. Additionally, a trend towards increased activity of mitochondrial complexes was observed in animals treated with AntiOxBEN2.

Our findings suggest that AntiOxBEN$_2$ prevents hepatic steatosis in both male and female mice subjected to an HFHS diet, positioning it as a promising strategy for the prevention and treatment of MASLD.

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P10- Optimization of a cryopreservation protocol for precision medicine applications: a paired- analysis comparison of human urine- derived stem cells' viability and proliferation

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Introduction: Stem cell research, a dynamic and promising frontier in medicine, leverages pluripotency and self-renewal for clinical applications. Urine-derived stem cells (UDSC) possess mesenchymal-like features and differentiation potential, while offering cost-effective, and non-invasive collection. Their expandability and genomic stability render them valuable for disease modeling, biomarker discovery, drug screening, and therapeutics. Cryopreservation, an underexplored prospect, further enhances experimental assay design and UDSC biobanks foundation.

Objective: To evaluate the proliferation, viability, and potential gender-specific effects of UDSC cryopreservation, aiming to determine their suitability for post-thawing applications.

Methods: UDSC were isolated from 7 healthy volunteers (3 males, 4 females; aged 26-50), expanded to P5, and cryopreserved in 5% dimethyl sulfoxide supplemented culture media. Growth curves over 14 days and proliferation rates pre- and post-thawing were compared using resazurin and SRB assays. Paired- comparison of potential gender-specific effects on viability, was performed by flow citometry using the Apoptosis/Necrosis kit (Abcam). GraphPad Prism 9 was used for statistical analysis.

Results and Conclusion: Cryopreservation didn't impact UDSC viability and no significant differences in cell death were observed, including between genders. Comparable proliferation rates were noted pre- and post-thawing. Given this results, within this age interval and for healthy subjects, UDSC are suitable for experimental applications post-thawing, without the need for gender-based segregation.

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P11- The role of α-synuclein in neuronal innate immunity activation

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α-Synuclein (aSyn) is a protein found primarily in the brain, and its role in Parkinson's disease (PD) has been a subject of extensive research. aSyn aggregates in structures called Lewy bodies, which are believed to contribute to the death of nerve cells that produce dopamine. This loss of dopamine producing cells leads to the movement symptoms of PD, such as tremors, stiffness, and slowness of movement.

Recently, a seminal hypothesis was proposed for body-first PD etiology where gut inflammation triggers PD if systemic inflammation, acting as a facilitator, is present. Inflammatory changes that occur in the blood and brain of PD patients have been viewed as part of the cause of the progressive nature of the disease.

We hypothesize that under permissive conditions like gut dysbiosis or infection, enteric neurons induce aSyn expression that targets the mitochondria, as part of a neuronal innate immune response. A fragmented mitochondrial network in enteric neurons can further trigger innate immunity due to the exposure of danger associated molecular patterns.

To understand the role of aSyn expression in neuronal innate immune activation and disclose the contribution of mitochondria in this control, we treated SH-SY5Y cells and mice midbrain neurons with LPS (innate immune activation), MPP+ (mitochondria-mediated innate immune activation), or recombinant aSyn. We are measuring GATA-2 activation and levels, aSyn oligomers, IL-1β levels, caspase-1 activation, and mitochondrial function and morphology.

P12- Pathogenic mechanisms mediated by anti-$GABA_AR$-autoantibodies in autoimmune epilepsy

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Epilepsy is a chronic brain disorder characterized by recurrent unprovoked seizures due to abnormal hypersynchronous neuronal activity. In six out of ten cases, epilepsy's aetiology remains unknown, and a substantial proportion of patients develops pharmacoresistance to the available medication. Improving epilepsy diagnose and treatment are challenges of modern society.

The identification of circulating autoantibodies (aAbs) against neuronal proteins in epileptic patients suggests a new model for epileptogenesis where seizures can be immune-mediated. Particularly, human aAbs targeting different subunits of $GABA_AR$ are associated with acute refractory seizures and severe status epilepticus in autoimmune encephalitis. However, multiple aAbs (including anti-$GABA_AR$-aAbs) are not routinely tested in clinical practice, hindering patients' diagnosis and the understanding of aAbs pathogenicity on autoimmune epilepsy.

This work combines functional and molecular approaches to analyse the impact anti-$GABA_AR$-aAbs on rat hippocampal neurons. Briefly, hippocampal neurons were exposed to patient's serum containing anti-$GABA_AR$-aAbs and neuronal network activity analysed with microelectrode arrays (MEA), revealing an increase in the strength and rhythmicity of networks excitability and connectivity, resembling patients' clinical seizures. We also disclosed a mechanism of internalization of anti-$GABA_AR$-aAbs in hippocampal neurons preceding the observed functional alterations, pointing to a pathogenic effect of anti-$GABA_AR$-aAbs mediated by their internalization. Moreover anti-$GABA_AR$-aAbs serum significantly increased γ2-$GABA_AR$ internalization rate, as revealed by a fluorescence assay for receptor internalization.

Our work uncovered pathogenic mechanisms mediated by anti-$GABA_AR$-aAbs that might underly epileptogenesis in autoimmune epilepsy. Such findings lay the foundations for new strategies to cure autoimmune epilepsy, which could highly improve patient's quality of life.

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P13- The role of IL-23 in ileum inflammation: implication to gut-first PD

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease worldwide, with its incidence increasing significantly with age. Emerging research suggest that PD may originate in the enteric nervous system (ENS) before affecting the central nervous system (CNS). Within PD patients, there have been observed alterations in the microbiota composition, that lead to intestinal dysbiosis. This dysbiosis can stimulate a pro-inflammatory environment, triggering an excessive immune response. Importantly, a significant correlation exists between inflammatory bowel diseases (IBD) and Parkinson's disease, where individuals with IBD carry an elevated risk, reaching up to 22% of developing neuroinflammation and subsequently PD. IBDs are characterized by an immune system overactivation, particularly the interleukin 23 (IL23) immune axis, thereby fueling chronic inflammation. Consequently, it appears that intestinal inflammation may underlie the progressive nature of PD. Targeting the IL23 immune axis emerges as a promising therapeutic strategy for PD, as no current treatments effectively slow disease progression. To investigate this, our research team used mice transplanted with PD fecal microbiota to model the disease. These mice were treated with a specific monoclonal antibody (mAB) against the IL23 receptor (0,25 mg/kg) once a week for 6 weeks. Remarkably, this treatment resulted in decreased intestinal inflammation, restoration of the epithelial barrier, and decreased cerebral inflammation. These findings suggest that targeting the IL23 immune axis to alleviate intestinal inflammation holds promise in slowing the progression of PD.

P14- Unraveling the impact of mitochondrial-lysosomal axis dysregulation on the release of proinflammatory cargo in extracellular vesicles

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Impairment in the mitochondrial-lysosomal (mito-lyso) axis play key roles in neurodegenerative diseases pathogenesis. During this process, the release of small extracellular vesicles (sEVs), a nano-size vesicles, carrying mitochondria damage-associated molecular patterns can occur. The process might activate microglia contributing to neuroinflammation. The aim of our work was to evaluate if sEVs released from cells with mito-lyso axis impairment modulate the inflammatory response in microglia.

Mito-lyso dysfunction was induced in human fibroblasts (NHDF) by FCCP and FCCP+chloroquine (CQ), respectively. Subsequently, sEVs, DNA associated to sEVs and intact mitochondria were isolated 24h after stimulation. N9 microglia were utilized as recipient cells to assess the inflammatory response to these stimuli.

Our results showed that FCCP and/or CQ-treated NHDF displayed an increase in mitochondrial fission, ROS levels, and decreased mtDNA copy number. Moreover, the inhibition of mito-lyso axis increased the sEVs secretion containing more mtDNA copies with high levels of DNA oxidation, promoting an increase in the expression of pro-inflammatory cytokines in microglia. Moreover, mito-lyso axis inhibition increases the phagocytic index by microglia and the NF-kβ activation upon treatment with different stimuli.

In summary, our findings suggest that mito-lyso axis impairment impacts sEVs cargo and the ability to modulate microglial activation, thereby contributing to neuroinflammation.

P15- Electrospun Fibrin-Gelatin Scaffold for Oral Mucosa Regeneration

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Tissue engineering shows promise for regenerating bioartificial human oral mucosa to repair damages. Synthetic and natural biomaterials, including fibrin and collagen, are used for crafting scaffolds due to their biocompatibility. This study aimed to create a fibrinogen-based electrospun nanofibrous scaffold using gelatin as an "electrospinning-driving" polymer.

Fibrin, along with 17% gelatin (type B), 25% acetic acid, 15% ethyl acetate, and 50% 1,1,1,3,3,3-Hexafluoro-iso propanol (HFIP), was prepared and loaded into a syringe connected to a delivery device. This mixture was dispensed at a flow rate of 1 mL/h under a voltage of 22 kV through a needle onto an aluminum foil attached to a grounded mandrel positioned 10-15 cm from the needle tip. After electrospinning, the scaffold was crosslinked and morphologically characterized using SEM.

Electrospinning of natural polymers like fibrinogen requires volatile solvents like HFIP, which can be toxic. To reduce toxicity and maintain biological activity, a low HFIP concentration was used. Gelatin was employed as an "electrospinning-driving" polymer due to fibrinogen's low solubility in HFIP. Parameters such as flow rate and electric field affected the electrospinning process. However, SEM analysis showed a uniform nanofibrous structure in the scaffold.

This study presents a novel electrospinning approach for crafting nanofibrous fibrin-based scaffolds. However, further investigations, encompassing in vitro and in vivo biocompatibility analyses, are imperative to ascertain the potential applicability of this innovative scaffold in oral mucosa tissue engineering protocols.

P16- The protective effect of the mitochondria-targeted antioxidant AntiOxCIN4 against oxidative/nitrosative stress in amyotrophic lateral sclerosis *SOD1^{G93A}* mouse brain and skeletal muscle

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Amyotrophic lateral sclerosis (ALS) is an adult-onset, fatal neurodegenerative disease. Mitochondrial dysfunction and oxidative stress play a key role in ALS pathophysiology, and thus the use of novel mitochondria-targeted antioxidants (*e.g.,* AntiOxCIN4) may constitute promising therapies against ALS progression. We hypothesized that AntiOxCIN4 could mitigate brain and skeletal muscle oxidative/nitrosative stress in *SOD1^{G93A}* ALS mice.

Early adult *SOD1^{G93A}* mice were treated with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. The effect of AntiOxCIN4 was evaluated in animals' longevity, oxidative/nitrosative stress markers, and antioxidant enzymes' activities of brain and skeletal muscle homogenates, by using colorimetry-based methods.

AntiOxCIN4 extended the survival rate of female ALS mice (P=0.05) while slightly enhancing their brain activities of total superoxide dismutase and superoxide dismutase 2 by 51% and 92%. This was accompanied by an increase in glutathione peroxidase (by 69%, 23%) and reductase activities (P=0.008, P=0.01), together with a decrease in nitrites levels (P=0.002, P<0.001) in their brain and skeletal muscle.

In sum, AntiOxCIN4-mediated amelioration of brain and skeletal muscle oxidative/nitrosative stress could improve ALS mice longevity. Further studies are required to discover how this protection delays ALS progression.

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P17- The mitochondria-targeted antioxidant AntiOxCIN4 mitigates cardiac oxidative/nitrosative stress in the amyotrophic lateral sclerosis *SOD1^{G93A}* mouse

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease, whose patients often exhibit cardiovascular alterations that may culminate into heart failure and death. Since mitochondrial dysfunction and oxidative stress appear to play a key role in ALS pathophysiology, novel mitochondria-targeted antioxidants (*e.g.,* AntiOxCIN4) may represent promising therapeutic strategies against disease progression. We hypothesized that AntiOxCIN4 could mitigate cardiac oxidative/nitrosative stress in *SOD1^{G93A}* ALS mice.

Early adult *SOD1^{G93A}* mice were injected subcutaneously with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We assessed the effect of AntiOxCIN4 administration in animals' longevity, oxidative/nitrosative stress markers, and antioxidant enzymes' activities in heart homogenates, by using colorimetry-based methods.

Longevity of female ALS mice was extended (P=0.05), and the activities of total superoxide, superoxide dismutase-2, glutathione peroxidase, and reductase were increased (P=0.05, P=0.03, P=0.0002, P=0.008, respectively) upon AntiOxCIN4 treatment. Conversely, AntiOxCIN4 slightly reduced (by 39%) their cardiac nitrites levels.

In conclusion, AntiOxCIN4 may improve antioxidant defenses and counteract oxidative/nitrosative stress in ALS hearts. Further studies are pivotal to unravel if this protection delays cardiac damage upon ALS.

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P18- Novel nanoparticle formulation for combined gene therapy and chemotherapy application in hepatocellular carcinoma

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Liver cancer is the sixth most common cause of cancer and the third leading cause of cancer death worldwide, with hepatocellular carcinoma (HCC) accounting for about 90% of primary liver tumors. Presently, liver transplantation and surgical resection are the most effective therapeutic approaches for patients with early stage HCC. However, in the vast majority of patients with HCC the diagnosis is made in advanced stages of the disease. In these cases, the administration of chemotherapeutic drugs corresponds to the most common treatment, although it only slightly improve the survival of these patients. The combination of different chemotherapeutic drugs that inhibit crucial signaling pathways of hepatocarcinogenesis and the combination of tumor suppressor transgenes expression with different drugs that inhibit the referred signaling pathways may result in high antitumor effect. In this regard, the main objective of this work was to develop a new developed HNP consisted of a PLGA core, in which selumetinib was encapsulated, coated with a DNA plasmid containing the PTEN transgene and a pH-sensitive lipid bilayer containing the drug perifosine and the GalNAc ligand, to allow interaction with the asialoglycoprotein receptor. The PTEN transgene encodes the PTEN tumor suppressor protein, and perifosine is an AKT inhibitor whose activation is essential for cell survival. Our results showed that this HNP formulation has not only a high capacity to transport both drugs but also the genetic material, allowing the efficient expression of the PTEN transgene in target cells. In addition, this nanosystem has high stability, suitable physicochemical properties, and high specificity for HCC cells. This formulation resulted in a high antitumor effect, by combining chemotherapy with gene therapy, being observed not only an increase in programmed cell death but also a decrease in cell proliferation, both in 2D and 3D cell cultures. Overall, the hybrid nanosystem developed in this work, constitute promising platform for the generation of novel therapeutic approaches to HCC.

P19- Microelectrodes for monitoring lactate and oxygen dynamics in vivo in the rat brain with high spatial and temporal resolution

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Increases of lactate in the brain have been considered a consequence of inadequate oxygen delivery or disruption of oxidative metabolism. However, recent research recognizes lactate as a supplemental fuel, signalling molecule, and potential biomarker of brain activity. Oxygen is essential for sustaining the brain's metabolic processes and energy supply to support neural activity. Lactate and oxygen concentration dynamics can be altered in a wide range of perturbations that can be linked with situations of neural distress and disease. The ability to measure lactate and oxygen levels under conditions of large metabolic and hemodynamic responses has become gradually important in understanding and managing certain medical conditions such as TBI and epilepsy.

Microelectrodes, when coupled with fast electrochemical techniques, can provide an attractive analytical tool for monitoring the concentration dynamics of electroactive neurotransmitters and metabolic substrates such as lactate and oxygen levels in the brain extracellular space with high spatial and temporal resolution, sensitivity, selectivity, and minimal tissue damage.

In this work, microelectrode-based biosensors have been designed and developed using modified carbon fiber microelectrodes (CFM) and ceramic-based microelectrode arrays (MEA) as sensing platforms for the detection and monitoring of lactate and oxygen. Lactate oxidase was immobilized onto the electrode surface in the presence of bovine serum albumin and glutaraldehyde. To extend the microbiosensor lactate linear range, the microelectrodes were coated with an exclusion layer of polyurethane. To minimize the interference of undesired compounds the microbiosensors were coated with exclusion layers of Nafion® and/or m-phenylenediamine.

The micro(bio)sensors were successfully used to detect and monitor lactate and oxygen with high spatial and temporal resolution in the brain extracellular space in response to local lactate changes and upon stimulation.

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P20- Characterization of cerebellar pruning and microglia function in mice deficient for IL-4 signaling in microglia

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Interleukin (IL)-4 is a type II cytokine essential for the development of allergic responses. In the brain, IL-4 regulates learning and memory and its receptor (composed of 2 subunits, including the mandatory subunit IL4Rα) is expressed by both neurons and microglia. Microglia provide tissue surveillance and take part in neuronal circuit assembly and refinement through neuronal and synaptic pruning.

Our lab recently demonstrated that, in mice, IL-4 peripheral administration in the second postnatal week (which mimics early-life allergies and induces a premature increase in IL-4 levels) results in reduced neuronal pruning by microglia in the cerebellum, increased density of granule cells, circuit dysfunction and behaviors reminiscent of Attention-Deficit/Hyperactivity Disorder (ADHD). These alterations were not observed in mice lacking IL4Rα signaling in microglia, suggesting that these cells are primary targets of IL-4 during postnatal cerebellar maturation.

We now aim to study the physiological role of IL-4 signaling in microglia-dependent cerebellar pruning. To achieve this goal, we are characterizing conditional knockout mice (cKO) for IL4Rα in microglia (Cx3cr1-CreERK$^{I/wt}$::Il4rα$^{fl/fl}$ mice treated with tamoxifen). Preliminary results show a differential microglial developmental program in cKO mice characterized by increased percentage of microglia with phagocytic cups at postnatal day (P) 7 and microglial hyperamification at P13. These results are opposite to those observed in IL-4-administered mice and corroborate the hypothesis that IL-4 signaling in microglia is critical in shaping their function during the neuroimmune critical period of cerebellar maturation. Considering that allergies and ADHD share a strong comorbidity in humans, this work will help to further understand how IL-4 defines the maturation of the cerebellum and how early-life allergies can constitute a risk factor for the development of ADHD.

P21- Studying the metabolism of Urine-Derived Stem cells: Analysis of the impact of gender, cell passaging and cryopreservation in the mitochondrial and glycolytic function

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Stem cells are a key element in scientific research, serving as platforms for disease modeling, diagnosis, and therapeutic interventions. Nonetheless, traditional stem cell sources often involve limited or invasive sampling and the challenges of expensive culture. Urine-derived stem cells have emerged as a promising alternative, addressing these limitations. Despite their potential, key aspects remain unknown, particularly regarding gender-specific differences and the impact of cell passaging. Moreover, the absence of a standardized long-term cryopreservation protocol hinders coordinated batch studies. To address these gaps, this study evaluates the differences evoked by gender, passage, and cryopreservation in different mitochondrial and glycolytic function parameters, using Seahorse technology, in samples from healthy volunteers, aged 26-50. Our findings suggest no gender-based biologically relevant differences in stem cell functionality, allowing for the pairing of cells from both groups. A similar outcome was observed between cells at passages 4 and 5, indicating the stability of stem cell function across one passage. Furthermore, our cryopreservation protocol utilizing 5% DMSO in cell culture media demonstrated no discernible differences between frozen and thawed cells, validating its efficacy for long-term storage, considering the age interval of the healthy subjects participating in this study. These results open new ways to plan experiments with these cells, leveraging their unique advantages.

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P22- Metabolic syndrome's brain twist: Exploring the interplay of metabolism and mechanotransduction

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Metabolic syndrome (MetS) is a major public health concern, contributing to various non-communicable diseases development, including Alzheimer's disease (AD) and other neurodegenerative disorders (ND). Despite the mechanisms underlying MetS-driven ND pathophysiology are not fully understood, it is known that metabolic derangements impact tissue mechanics, and vice-versa, but how they mutually regulate, and whether they could play a role as upstream initiators of ND pathology and progression remains unclear. We aim to explore how MetS-related metabolic dysfunction impacts brain mechanosignaling pathways and its potential role in triggering or enhancing ND. Hence, we fed C57BL/6J mice with a standard diet (SD) or Western diet (WD) for 16 weeks to induce MetS, and we used a mouse neuronal cell line (HT22) cultured on hydrogels mimicking brain tissue stiffness (physiological (~6.5/7.5kPa) or AD (2.5/2.0kPa)). We observed that brains from WD-fed mice had no alterations in mitochondrial markers (TOM20, VDAC, ATP5), while increased levels of glucose metabolism (GLUT1, HK) and fatty acid-oxidation (CPT1) markers, and the activity of mitochondrial respiratory chain complexes, when compared with brains from SD-fed mice. Measurement of brain stiffness through AFM revealed that WD-fed mice presented a soft brain (450Pa vs. 240Pa). In vitro, we confirm that substrate stiffness influences cellular mechanotransduction signals (p-cofilin/cofilin, YAP subcellular localization). Interestingly, mitochondrial or fatty acid-oxidation markers (TOM20, CPT1) were not altered, while lower basal oxygen consumption rate and decreased levels of metabolism-associated proteins (HADHA, HK) and ATP were observed in cells cultured on soft substrate. Our results suggest that primary alterations in brain metabolism precede subsequent changes in brain tissue mechanics in WD-induced MetS mice, potentially leading to overall decay in cell metabolism later in the disease.

P23- Integrating cognitive training with physical exercise to enhance brain arousal and improve cognitive function in older people

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Cognitive training is a promising non-pharmacological tool to improve cognition in older people. Physical exercise enhances brain arousal by increasing noradrenaline and acetylcholine, two neuromodulators important for synaptic plasticity and learning. In this project, we aimed at taking advantage of the arousing effect of physical exercise in the brain to boost the effect of cognitive training. For this, we designed and implemented a cognitive training program integrated with aerobic exercise that we hypothesize will have long-lasting effects on cognition. We designed a twice-a-week, 12-week multimodal exercise program that aggregates cognitive training with aerobic exercise and is applied in group classes of 4 participants each. The cognitive training includes tasks that engage cognitive skills like decision-making, processing speed, inhibitory control, attention, and working memory, which are performed standing while moving and intercalated with aerobic exercises. The aerobic exercise includes rhythmic bodily movements with the intensity at 60-70% of HR reserve. Each session takes 50 min (5-min warm-up, 40 minutes aerobic or aerobic+cognitive training exercise, and 5-min cooldown), and includes 4 different cognitive training tasks of 5-min each. During the cognitive training tasks, visual stimuli are presented on an LCD screen and participants are instructed to give the most accurate and fastest possible responses using pedals placed on the floor while marching in place to keep arousal levels increased. When the average group accuracy of the task is above 80%, the difficulty level of the cognitive program is increased the following week. Preliminary data analyses revealed that older participants (55-77 years old) were able to use the system correctly and improved rapidly their weekly performance in the trained tasks, as observed in increases in accuracy and decreases in reaction time and reaction time variability.

P24- Measuring Ovarian Tissue Oxygen Consumption Rate – A Novel Approach

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Young cancer patients that undergo oncological treatments can have their fertility severely compromised. Cryopreservation of ovarian tissue (OT), followed by its autologous transplantation, has emerged as a recent fertility preservation technique that has allowed cancer survivors to recover their endocrine function and reproductive independence. To maximize recovery outcomes, good tissue health is necessary, and metabolic and energetic function is required to guarantee the survival of the graft. In the present study, we designed and validated a new strategy to measure oxygen consumption rates (OCR) in whole OT grafts. Fresh bovine OT was collected, and OCR was measured using the XF24 Extracellular Flux Analyzer (Seahorse). Whole OT fragments successfully internalized and responded to all mitochondrial targeted compounds. Various parameters, including basal, ATP-linked, maximal respiration, and OT spare capacity, were assessed. This method allowed us to evaluate, for the first time, OT mitochondrial health without compromising tissue integrity and viability during homogenization protocols.

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P25- Targeting cell senescence in the blood-brain barrier with senotherapeutic Nanoparticles

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Aging is the leading cause of cognitive dysfunction and cerebrovascular diseases. The function and structure of the blood-brain barrier (BBB) is impaired during physiological aging, promoting cognitive loss and increased risk of cerebrovascular diseases due to its disruption, followed by blood-to-brain extravasation of neurotoxic molecules. A potential trigger of BBB aging is the accumulation of senescent cells. Senotherapeutics have been proposed to revert the senescent burden by inducing cell apoptosis or reducing senescent cell secretory signals. Although the use of these compounds for the treatment of chronic diseases is under intense scrutiny in ongoing clinical trials, their translation to the brain is limited due to low BBB permeability and potential side effects. Here, we investigated the use of biodegradable nanoparticles (NPs) to release senotherapeutics in senescent cells from the BBB, increasing their safety and therapeutic effect.

A library of 42 NPs containing Navitoclax was synthesized. NPs are cleaved at the glycosidic linkage when in the presence of β-galactosidase, an enzyme overexpressed in the lysosomes of senescent cells. Two hit NPs were evaluated in brain microvascular endothelial cells and compared to soluble Navitoclax. Moreover, therapeutic effect of the formulations in terms of anti-senescence program and BBB permeability was accessed in aged rats. Both NPs were efficiently internalized by cells after 4h of exposure and were able to selectively kill senescent endothelial cells after 48h, more efficiently than soluble Navitoclax and at a lower dose. In vivo administration of one selected hit - NP17 – was able to rescue vasculature integrity, reduce the endothelial senescent burden and

improve spatial memory at the hippocampal level, therefore ameliorating brain function dependent of aging. Overall, we have developed a promising pharmacological strategy to eliminate senescent brain endothelial cells at the BBB.

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P26- Dietary nitrate improves nitric oxide-mediated neurovascular coupling and spatial memory in a rodent model of type 2 diabetes

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Background:

The temporal and spatial coupling between neuronal activity and cerebral blood flow (CBF) - neurovascular coupling (NVC) – in the brain is crucial for cognition. In the hippocampus, a brain region involved in memory processing, NVC is critically dependent on nitric oxide (•NO) via the NMDAr-nNOS-sGC pathway. Coherently, limited •NO bioavailability is associated with NVC impairment, negatively impacting cognitive function associated with several conditions, including type 2 Diabetes mellitus (T2DM).

Aim:

In a rodent model of T2DM (Goto-Kakizaki rats), we aimed to 1) assess the functionality of NVC in connection with neuronal-derived •NO bioavailability and cognitive function and 2) explore the potential of dietary nitrate, a metabolic precursor of •NO via the nitrate-nitrite-•NO pathway, to nourish the •NO-dependent NVC and thus to prevent neural and cognitive dysfunction.

Methods:

Male Goto-Kakizaki (GK) rats and Wistar rats (controls) were evaluated at 4 and 8 months of age in terms of glycemic profile, spatial learning, and memory performance (Barnes maze, Novel Object Recognition, and Y Maze), the functionality of NVC (in vivo simultaneous measurements of •NO and CBF dynamics) and plasma nitrate/nitrite load (gas-phase chemiluminescence). Nitrate supplementation was provided in water ad libitum for 12 weeks.

Results/Conclusions:

Diabetic animals exhibited compromised •NO bioavailability (regarding real-time concentration dynamics) to glutamatergic activation and dysfunctional NVC, correlated with impaired spatial learning and memory performance from an early age (4 months).

Dietary nitrate intervention abrogated the neurovascular uncoupling and improved spatial memory performance in GK rats. Data supports the potential of dietary nitrate to counteract the cognitive decline in T2DM via functional NVC.

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P27- Role of GluN2B-containing NMDARs on network activity and synapse maturation and its potential interaction with EphA4

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Receptors at synapses such as NMDA and AMPA receptors and their subunits take part in synaptic plasticity along with shaping neuronal circuits. Their disruption is linked to neurodegenerative diseases like Alzheimer's disease (AD). EphA4 plays a vital role in regulating dendritic spines, affecting network activity, and the endocytosis of AMPARs via the synaptic proteosome. Past research showed decreased EphA4 levels in mice cortical neurons with NMDARs lacking the GluN2B subunit (KO).

We propose that EphA4 forms a signaling complex with NMDARs through GluN2B, significantly contributing to the regulation of spine structure and synaptic activity. Our study tackled three main objectives: characterize spine structure and network activity in KO mice cultures; analyze EphA4 expression and activity within these cultures; and explore the interaction between EphA4 and NMDARs.

Multi-electrode array analysis revealed that KO cortical cultures had increased firing rates, bursts, and erratic bursting patterns compared to wild-type (WT), indicating altered network activity. Spine morphology assays showed that KO neurons had more immature dendritic spines, revealing the preponderant role of GluN2B in spine maturation. EphA4's expression was reduced in KO neurons, reinforcing its involvement in spine maturation via the GluN2B subunit. EphA4 impacts spine maturation by pruning unwanted immature spines. Our immunoprecipitation assays also disclose a potential EphA4-GluN2B-NMDARs interaction, possibly impacting spine development, and network activity. In AD, there is an aberrant activation of EphA4 and GluN2B-NMDARs, which opens the possibility that this complex may contribute to AD synaptopathy.

Overall, these results contribute to a more comprehensive understanding of how this novel EphA4/GluN2B-NMDARs interaction influences network activity and synapse maturation and proposing that this interaction may become a therapeutic target for AD in the future.

P28- Unravelling the role of retinoic acid-regulated unconventional intraepithelial lymphocytes in intestinal immune defence

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Infectious diseases and diarrhoea are the leading causes of death of children under-5. Thus, a better understanding of crosstalk between intestinal immunophysiology and nutrients is needed. Unconventional Intraepithelial Lymphocytes (IEL), characterized by the TCRαβCD4-CD8β-CD8αα phenotype (CD8αα IEL), originate from thymic precursors (IELp), and inhabit the intestinal epithelium (IE). Despite recent advances, the transcriptional program governing the development of CD8αα IEL and its function remains unknown.

Preliminary data shows expression of retinoic acid (RA) receptors by IELp, and that respond to RA. Furthermore, thymocyte-targeted disruption of RA-signalling compromises both IELp and CD8αα IEL population, rendering mice more susceptible to enteric infections (protozoans and bacteria) and, in vitro co-culture demonstrated deficient cytotoxic responses in RA-signalling disrupted CD8αα IEL. Thus, showing a critical role of RA-signalling in CD8αα IELs and intestinal immune defence. Additionally, our results show that by transferring RA signalling-competent cells into mice with disrupted RA-signalling or lymphopenic mice, it results in a protective effect on the IE.

Importantly, our findings highlight the influence of RA signals on CD8αα IEL and their role in early-life enteric microbial protection and unveils a molecular link between nutritional cues and IEL responses, shedding light on their crucial role in host defence during infancy.

P29- Maternal obesity alters the metabolic and redox aging trajectories of the male and female offspring

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Obesity incidence is rising in women of reproductive age increasing maternal obesity (MO) prevalence and predisposing the offspring (F1) to heart disease. We have previously identified metabolic decline as an early aging-related mechanism in the baboon heart. We aimed to understand how MO-*in utero* environment modulates the F1's cardiac aging trajectory.

A MO-Sprague-Dawley rat model was obtained with maternal high-fat/high-sugar diet consumption starting 6 weeks(w) before pregnancy. F1 were kept under a control diet. Heart tissue was collected at 6, 16, and 32w (C=34; MO=32) for male and female offspring. Luminescence and spectrophotometric assays were used to evaluate metabolic and redox states. A two-way ANOVA test was performed and $p<0.05$ was set as statistical significance.

Cardiac age-related increase in total ATP levels ($p<0.01$) is exacerbated in male and female MO-F1 ($p<0.05$). ATP phosphorylation rate and capacity are decreased due to MO only in male F1 hearts ($p=0.03$; $p=0.05$). Nevertheless, mitochondrial ATP and NAD(P)H levels are only increased in female F1 ($p<0.01$; Interaction(I):$p=0.05$). Slower rates of ATP production along with an overall accumulation of ATP suggest slower cardiac metabolism in MO-F1. Total antioxidant capacity increases at 16w in control animals reverting to similar levels at 32w, concomitant with the rise in protein carbonylation and lipid peroxidation. This age-related behavior is attenuated or inexistent in MO-F1 in both sexes: lipid peroxidation (I:$p<0.03$), protein carbonylation ($p<0.10$), and total antioxidant activity (I:$p<0.02$). Free thiol levels are impaired only in male MO-F1 (I:$p<0.01$) while catalase activity is affected by MO in male (I:$p=0.01$) and female (I:$p=0.03$) F1).

Maternal Obesity decreases the cardiac aging-related metabolic rate in the offspring. The redox state of offspring from MO shows an altered aging trajectory. Altogether these events may predispose MO offspring to heart disease.

P30- Long-Lasting Neurobehavioral Effects of Early-Life Stress: The Role of MeCP2

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Methyl-CpG binding protein 2 (MeCP2) is an epigenetic factor that binds to methylated DNA, thereby modulating the expression of genes. Brain-derived neurotrophic factor (*Bdnf*) and corticotropin-releasing hormone gene (*Crh*) are two such genes; being implicated in synaptic plasticity and in the stress response, respectively, they play a critical role in anxiety disorders. Mutations in the *MECP2* gene are the main cause of Rett syndrome (RTT), a rare neurodevelopmental disorder affecting mainly girls, characterized by motor and cognitive impairments, breathing abnormalities and stereotypies. Anxiety is a main component of RTT, yet largely under-investigated.

Anxiety disorders are characterized by excessive and uncontrollable apprehension, and constitute the most common mental disorders, affecting 4.8% of the world population. Early-life stress (ELS) is a known risk factor for developing anxiety disorders later in life. It comprises adverse events occurring during periods of high brain plasticity in the early development, which leave epigenetic marks in the DNA that ultimately may influence gene expression by *MeCP2*. Previously, we found that in female mice Mecp2 deficiency per se recapitulates the anxiety-like behavior observed in mice subjected to maternal separation (MS), a protocol that mimics ELS.

Here, we aim to characterize a two-hit stress model on the effects of ELS and late life stress on anxiety. We found that female, but not male, mice submitted to MS that experience a later stressor display blunted anxiety and stress response, as assessed by the elevated plus maze and corticosterone levels. We also investigated whether changes in maternal care induced by MS explained the anxiety changes by analyzing maternal behavior. To further characterize our model, we evaluated the contribution of neurotrophin and MeCP2 functions in regions of the anxiety neurocircuitry. We hope to clarify how MeCP2 can constitute a risk factor in the development of anxiety disorders.

P31- Inhibitory synaptic alterations in neuroimmune insults mediated by IL-4

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Attention-deficit/hyperactivity disorder (ADHD) is the most common neurodevelopmental condition (1) and its incidence is influenced by both genetic and environmental factors (1). ADHD is associated with dopamine dysfunction (2,3) and people with early-life allergies present an increased risk of developing ADHD (4).

We previously demonstrated that increasing the levels of IL-4 in the postnatal period, during a neuroimmune critical window for cerebellar development, leads to ADHD-like behaviours in mice. IL-4 prevents pruning of granule cells, which increases the frequency of excitatory postsynaptic currents in Purkinje Cells (PCs) (5). PCs are the sole output of the cerebellar cortex and inhibit neurons in the deep cerebellar nuclei (DCN) (6), which connect the cerebellum to other brain regions. In addition to the thalamus, the DCN outputs to the ventral tegmental area (VTA) via monosynaptic glutamatergic connections (7). The VTA is a dopaminergic nucleus that influences the dopaminergic tonus of the prefrontal cortex (PFC) (8,9). The PFC is crucial for sustained attention, decision-making, and executive control and is critically implicated in ADHD (10).

This project aims to deepen our understanding of the cerebellar circuit in IL-4-injected mice and anatomically validate the pathway cerebellum-VTA-PFC, whose dysfunction might be the basis of ADHD-related behaviours in this model. We are using mice stereotaxically injected with an AAV-Syn-GFP virus in the DCN and an AAV-Syn-RFP virus in the PFC. We aim to evaluate the putative colocalization of green and red fluorescence in tyrosine hydroxylase-positive neurons in the VTA. Importantly, we are also performing whole-cell patch clamp in inhibitory neurons of the molecular layer of the cerebellum. We hope this work will further unveil the mechanisms through which early-life allergies induce ADHD in mice.

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P32- Characterization of clock genes expression in Fatal Familial Insomnia

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Familial Fatal Insomnia (FFI) is a rare and fatal prion neurodegenerative disease caused by a mutation in the prion protein gene. FFI is clinically characterized by persistent and progressive insomnia, accompanied by neurological and psychiatric symptoms, as well as disruptions in circadian rhythm. Circadian rhythm disturbances are common in neurodegenerative disorders, even in early stages, being considered potential disease biomarkers. Considering the importance of clock genes in regulating circadian rhythms and their relevance to various neurologic disorders, we hypothesize that FFI patients may exhibit disrupted clock gene expression, which could aid in understanding disease onset and disease progression.

The aim of this study is to evaluate circadian rhythm clock genes expression in FFI patients-derived fibroblasts compared to healthy controls. This study was approved by the ethical committees of the Faculty of Medicine, University of Coimbra and Coimbra's Local Health Unit.

In this study, skin fibroblasts from FFI patients (n=3) and controls (n=4) were synchronized using horse serum shock for 2 hours. Circadian rhythmicity was assessed through the expression of clock genes (*Clock, Bmal1, Per1, Cry1, Dec1*) in these cells by qRT-PCR every 4 hours, for 24 hours.

Preliminary results suggest that expression of clock genes in fibroblasts from patients with FFI and healthy controls were not significant different. However, *Cry 1*, Clock and Bmal1 gene expression levels between 8 and 20 h after cell synchronization. These preliminary results suggest the need further studies to better understanding the cellular mechanisms of FFI.

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P33- Identification of A_1, A_{2B}, and A_3 receptors in the mice telencephalon at mid-late embryogenesis

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Caffeine is the most consumed psychoactive drug, and its consumption during pregnancy is associated with several adverse effects (1) However, its impact in brain development is poorly defined. Caffeine has a well-established antagonistic action on adenosine receptors (P1R) (2). During brain development, $A_{2A}R$ was shown to be involved in both radial (3) and tangential neuronal migration (4), and synapse stabilization (5). While caffeine mimicked the $A_{2A}R$ blockage delay in tangential migration (4), our unpublished results have shown in mice that caffeine consumption at E13.5-E17.5 accelerated radial migration, in contrast to the observed with $A_{2A}R$ antagonism (3), indicating the involvement of other P1R(s) in radial migration.

We detected by Western blot analysis immunoreactivity for A_1, A_{2B} and A_3 receptors in mice telencephalon at both E14.5 and E16.5. While $A_{2A}R$ was observed predominantly in the intermediate zone (IZ) (3), A1R displayed an increased density in ventricular regions, and $A_{2B}R$ and A_3R were detected predominantly in the IZ and cortical plate (CP). This suggests the existence of an integrated action of adenosine in the radial migration through activation of different P1Rs, from neurogenesis, eventually through A_1R, to the IZ-CP transition through $A_{2A}R$ (3), and integration in the CP through $A_{2B}R$ and/or A_3R.

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P34- A matter of time: effect of the antidepressant paroxetine on the cellular circadian clock

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Depressed patients experience a wide range of circadian rhythms, neuroinflammation and sleep-cycle disruptions. Core circadian clock gene polymorphisms have also been associated with depression. This suggests that drugs capable of affecting the circadian clock could be of interest for the treatment of depression. Indeed, antidepressants may convey their therapeutic effects via the modulation of circadian rhythms. Paroxetine, a selective serotonin reuptake inhibitor, has been previously proposed to significantly shorten the period of rat fibroblasts. However, no clear evidence is available regarding the ability of antidepressants to affect circadian rhythms. On contrary, here we found a lengthening effect of paroxetine *in vitro*.

For this, we generated multiple *Per2-luc* and *Bmal1-luc* circadian reporter cell lines. Cells were seeded in 35 mm dishes, synchronized with dexamethasone. DMSO or paroxetine (between 1-10 µM) were added directly after synchronization, or at 24 h or 36 h post-synchronization to assess the phase shifting properties of the drug. Bioluminescence was recorded in a Lumicycle (Actimetrics).

In human HME1;*Per2-luc* cells, paroxetine dose dependently increased period length. Additionally, when paroxetine was added to HME1;*Per2-luc* cells at 24 h or 36 h post-synchronization, the phase of the clock was delayed or advanced, respectively ($p<0.05$). This period phenotype and phase-shifts were replicated in other human (U2OS;*Bmal1-luc*) and mouse fibroblast (PER2::LUC) cell lines. Moreover, HME1;*Per2-luc* cells concomitantly treated with paroxetine and TPCA1, a NFkB inhibitor, did not show additive period lengthening. In conclusion, this suggests that paroxetine significantly lengthens the period of the circadian clock in human and mouse cells in vitro through a NFkB dependent mechanism. Further studies are needed to test the implications for patient dosing but might point towards incorporating timing as a variable to adjust and improve antidepressant therapy.

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P35- TARPs in the regulation of M-channel function and neuronal excitability

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Neuronal excitability dysfunction is a common feature of neurodevelopmental disorders, such as epilepsy. Understanding the mechanisms that underlie these processes is crucial for designing safe therapeutical strategies. M-type potassium channels are a breakthrough target for seizure suppression, however, the available drugs that activate M-channels display adverse side effects and have been withdrawn from clinical use. Alternatively, targeting interactors of the channels to indirectly modulate their activity could overcome this problem.

We identified a new interactor of the Kv7.2 subunit of M-channels, Stargazin, a member of the TARP family. We previously demonstrated that Stargazin-Kv7.2 interaction functionally impacts M-currents.In this work, we developed methods that allow for the study of this interaction. Additionally, we engineered molecular tools to examine how other TARP family members could regulate Kv7.2 function. By using splitFAST, a fluorescence complementation assay, we further validated the interaction, demonstrating its regulation upon cellular depolarization and PKC activity. The designed molecular tools also revealed that other TARPs can interact and differentially regulate Kv7.2.

Overall, this study produced significant evidence of the Stargazin-Kv7.2 interaction. Additionally, it identified a whole family of proteins as bona fide auxiliary subunits of Kv7.2 channels. The newly implemented splitFAST assay enabled the real-time study of the interaction, addressing its dynamics and providing spatial-temporal detail of the Stargazin-Kv7.2 interaction. This work lays the foundation for searching for modulators targeting newly identified Kv7.2 regulatory mechanisms.

P36- Unravelling the role of retinoic acid-controlled *Hes1* in iNKT cells

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Invariant Natural Killer T (iNKT) cells play a crucial role in immune responses, including cancer immunosurveillance. However, how environmental signals affect iNKT development and its effector function remains largely unknown. Our research indicates that retinoic acid (RA), derived from vitamin A metabolism, is crucial for iNKT cell development.

Preliminary data show that iNKT cells express RA receptors and thymocyte RA signaling abrogation results in iNKT cell development disruption and increased colon tumor growth and malignancy. Moreover, RNA seq data reveals a compromised transcriptome in that RA-signaling-disrupted iNKT cells, notably showing significant downregulation of Hairy and enhancer split-1 (HES1), a critical regulator of T cell lineage commitment and proliferation.

Despite these findings, the precise mechanisms by which RA controls iNKT cells and its downstream targets remain unclear. Our ongoing research, using a CD4CreHes1Flox mice model, aims to elucidate the role of Hes1 in iNKT cells, shedding light on the intricate interplay between RA signaling and iNKT cell development and function.

In summary, our research underscores the importance of RA signals in iNKT cells, while also highlighting the need for further exploration into its specific intracellular regulatory mechanisms. Through our research, we aim to deepen our understanding of how RA signals influence iNKT cells and identify their downstream molecular targets. By unravelling the cascade of molecular events triggered by RA cues, we aspirate to elucidate novel pathways for enhancing cancer immunosurveillance.

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