
Parque Florestal de Vieira do Minho
PARKING
ENTRANCE
SESSIONS
MEALS
Centro Cultural Casa de Lamas
Auditório Municipal de Vieira do Minho
WELL-BEING ACTIVITIES
ENTRANCE
N304
N304
PARKING
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Largo Prof. Brás da Mota
Avenida Barjona de Freitas

ICVS Retreat 2025

June 26-27th | Vieira do Minho

June 26th (Thursday)

08:20 Departure: Braga to Vieira do Minho

09:15 - 10:00 Welcome Message from the ICVS director (Patrícia Maciel)

10:00 - 10:40 Working groups - Session I

- Communication and Dissemination (Joana Silva and Nuno Alves)
- Onboarding/outboarding (Patrícia Maciel)

10:40 - 11:10 Coffee break

11:10 - 12:40 Working groups – Session II

- Lab Management (Rute Moura)
- Lab Safety (Alexandra Fraga and Rute Moura)
- Services (Luísa Pinto)
- B.ACIS (António Salgado and Roberto Barbosa)
- Green Lab (Oiá plast)

12:40 - 14:00 Lunch break

14:00 - 15:00 Time to discuss on:

- *How can admin staff and researchers work better together?*
- *What is the best strategy to get new and advanced equipment?*
- *How can ICVS and the School of Medicine collaborate more effectively?*
- *How can we improve collaboration between ICVS teams?*

15:00 - 16:00 Groups discussion outcomes

16:00 Well-being activity and Coffee break

18:00 Departure: Vieira do Minho to Braga

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ICVS Retreat 2025

June 26-27th | Vieira do Minho

June 27th (Friday)

08:00 Departure: Braga to Vieira do Minho

09:00 - 09:45 Plenary session I

Chairs: Fátima Baltazar and Sara Granja

"*Hypoxic Glycolysis Controls Cancers, Pathogens, tissue repair, and immunity*" - **Jacques Pouysségur**

9:45 - 10:45 Flash presentation – Session I

Chairs: Luísa Pinto and Isabel Veiga

- "*Prevalence of asthma in Portuguese adults - the EPI-ASTHMA study, a nationwide population-based survey*" – Dinis Brito
- "*DHA priming reprograms the metabolism of human adipose mesenchymal stromal cells and improves the therapeutic performance of its secretome in translational studies of spinal cord injury*" – Jonas Campos
- "*A cholinergic brainstem input to the nucleus accumbens bidirectionally mediate cocaine reinforcing effects*" – Bárbara Coimbra
- "*The path from the choroid plexus to the (sub)ventricular zone and back: implications for multiple sclerosis*" – Ana Mendanha Falcão
- "*The egli-1 gene as a possible link between autophagy and food-related behaviors: insights from the C. elegans nervous system*" – Jorge H Fernandes
- "*Characterization of microglial response to the activity of MCH neurons*" – João Frei

10:45 - 11:15 Coffee break

11:15 - 12:00 Posters Session I – Odd numbers

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June 26-27th | Vieira do Minho

12:00 - 13:00 Flash presentation – Session II

Chairs: Bruno Costa and João Oliveira

- "*Modulating Tau: Exploring novel ASOS for Down syndrome*" – Carlos Campos-Marques
- "*Shugoshin-1 (SGO1) is a novel glioblastoma biomarker predictive of patient prognosis*" – Joana M Ferreira
- "*Sex differences in astrocyte activation contributes to pain in experimental osteoarthritis*" – Diana Fonseca-Rodrigues
- "*Volume Reconstruction Algorithm for Tumor Assessment from Freehand Ultrasound Recordings in Mice*" – Raquel Lima
- "*From Memory to Misfiring: Lipid and Synaptic Drivers of Alzheimer's and Seizures*" – Tatiana P Morais
- "*Regulation of macrophage immunometabolic responses to Aspergillus fumigatus by PTX3*" – Rita Silva-Gomes

13:00 - 14:00 Lunch break

14:00 - 14:45 Posters Session II - Even numbers

14:45 - 15:30 Plenary session II

Chairs: Ana João Rodrigues and Tiago Gil Oliveira

"***Targeting Soluble Epoxide Hydrolase for Alzheimer's Treatment: Innovations in Drug Discovery*" - Mercè Pallàs**

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15:30 - 16:30 Flash presentation – Session III

Chairs: Hugo Almeida and Egídio Torrado

- "*Layer-specific imbalances in cortical PV and SST interneurons in the Dp(16)1Yey mouse model of Down syndrome*" – Sara Guerreiro
- "*Phospholipase D2 ablation leads to deficits in social memory*" – Marta Mendanha
- "*Genetic engineering of the CD81 large extracellular loop for targeted delivery of mesenchymal stem cell derived vesicles for spinal cord injury repair*" – Diogo Santos
- "*The IFNγ-JAK/STAT axis regulates macrophage transformation and granuloma formation in sarcoidosis*" – Diana Santos-Ribeiro
- "*A Robust Optical Flow-Based Tracker for Anatomical Landmark Detection in Laparoscopic Videos*" – Bruno Silva
- "*Astrowars: the return of the astrocytic metabotropic glutamate receptor 5 (in cortico-limbic function and behavior)*" – João Viana

16:30 Coffee break

17:00 Closing Session: Prizes & Final Remarks

18:00 Departure: Vieira do Minho to Braga

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ICVS Retreat 2025

Book of Abstracts

July 26-27th | Vieira do Minho

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Universidade do Minho
Escola de Medicina

ICVS Retreat 2025

Plenary Sessions

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Hypoxic Glycolysis Controls Cancers, Pathogens, tissue repair, and immunity

Pouysségur J[1,2] #, Marchiq I[1], Ždralević M[1], Vucetic M[2]

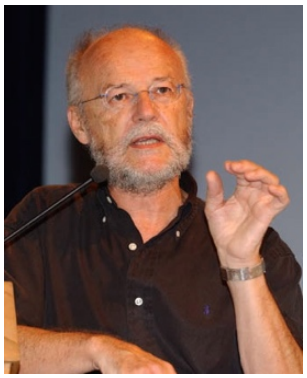
1 University Côte d'Azur, IRCAN, Lacassagne Cancer Centre, CNRS, Nice, France
2 Department of Medical Biology, Centre Scientifique de Monaco (CSM), 98000 Monaco

How glycolysis, a primitive hypoxic imprinted metabolic pathway present at the emergence of life is instrumental for the rapid growth of cancers, regenerating tissues, immune cells but also bacteria and viruses during infections? This pathway, activated via Myc and HIF-1 respectively in response to growth factors and hypoxia, is a Master bioenergetic pathway which satisfies energetic demands required for rapid Genome replication.

We will present the key role of lactic acid, the end-product of fermentative glycolysis able to move across cell membranes in both directions via monocarboxylate transporting proteins (MCT1 & MCT4) contributing to cell-pH homeostasis but also to the complex immune response via acidosis of the tumour microenvironment. Importantly lactate is recycled in multiple organs as a major metabolic precursor of gluconeogenesis and energy source protecting cells and animals from harsh nutritional or oxygen restrictions.

We will revisit the Warburg effect via CRISPR-Cas9 disruption of glucose-6-phosphate isomerase (GPI-KO) or lactate dehydrogenases (LDHA/B-DKO) in two aggressive tumours (human melanoma B16-F10, colorectal adenocarcinoma LS174T). Full suppression of lactic acid production reduces but does not suppress tumour growth due to reactivation of OXPHOS. In contrast, disruption of the lactic acid transporters MCT1/4 suppressed glycolysis, mTORC1, and tumour growth as a result of intracellular acidosis.

Finally, we will discuss the current clinical developments of an MCT1 specific drug AZ3965, and the recent progress for a specific in vivo MCT4 inhibitor, two drugs of very high potential for future clinical applications against cancers, bacterial and viral pathogens.



Biography: Pouysségur graduated from an Engineering School in Biochemistry of the University of Lyon, where he obtained his PhD in 1972. He spent two years as a post-doctoral scientist at the National Cancer Institute of NIH (USA) and established his own research group in 1978 at the CNRS Biochemistry Centre of the University of Nice. After directing the CNRS Institute of Signalling, Developmental Biology and Cancer, affiliated to the Cancer Centre A. Lacassagne up to 2008, J Pouysségur, joined the Cancer & Aging (IRCAN) in Nice, and later the Biomedical Department of the Scientific Centre of Monaco (CSM).

Jacques Pouysségur has previous experience in bacterial and somatic cell genetics, metabolism, Na-H exchanger, pH regulation, MAP kinase signalling in the context of growth control in mammalian cells. In the last 25 years his group developed a strong interest in hypoxia signalling, oxygen, nutrient sensing and Bioenergetics. He is member of AACR, EACR, EMBO, the French and European Academy of Sciences.

Targeting Soluble Epoxide Hydrolase for Alzheimer's Treatment: Innovations in Drug Discovery

Mercè Pallàs

Department of Pharmacology and Therapeutic Chemistry, Institut de Neurociències-Universitat de Barcelona, Avda. Joan XXIII, 27, 08028 Barcelona, Spain; Spanish Biomedical Research Center in Neurodegenerative Diseases (CIBERNED)-Instituto de Salud Carlos III, Madrid, Spain.

Alzheimer's disease (AD) is the most prevalent form of dementia globally, with cognitive decline as the main symptom. AD is characterized by the presence of extracellular accumulation of the amyloid-β (Aβ) plaques and the abnormal Tau phosphorylation (p-Tau), forming neurofibrillary tangles (NFTs), in the brain. Those AD hallmarks initiate a range of brain molecular alterations mediated by the activation of neuroinflammation, apoptosis and neuronal death, among others Altogether, these alterations cause a progressive neurodegeneration process. Despite the extensive research in this field, many clinical trials have been centered exclusively on the Aβ hypothesis, and at present, the molecular mechanisms triggering neurodegeneration are largely unclear. Thus, there is an urgent need to understand the etiopathogenesis of AD to develop novel disease-modifying therapies.

Growing evidence shows that uncontrolled neuroinflammation is well-identified in many pathological conditions, which would impair the typical structures and functionality of neurons. Therefore, the suppression of neuro inflammation has been proposed as an important strategy in neurodegenerative disease.

Epoxyeicosatrienoic acids (EETs) and epoxy-fatty acids (EpFAs) are derivatives of arachidonic acid endowed with potent anti-inflammatory properties. Interestingly, increased levels of EETs have been associated with microglial attenuation in AD mice models. Thus, EETs are potential neuroprotective agents, which are known to modulate several neurodegeneration-associated molecular pathways such as inflammatory, apoptotic, angiogenic, oxidative stress, among others. There is an ubiquitous enzyme in vertebrates (EC 3.3.2.10, EPHX2) called soluble epoxide hydrolase (sEH), which promotes the principal route of EETs degradation into their corresponding less-active diol metabolites, the dihydroeicosatrienoic acids (DHETs). Our group demonstrated that sEH expression is increased in AD patients' brain and AD mice models, altering the anti-inflammatory effects of EETs and boosting the DHETs. To this way, we and others have recently proposed the sEH as a new target for a novel approach to AD treatment.

To move forward in this therapeutic path, we design and synthesize a first in class adamantane derivatives (UB-EV-52) that showed promising neuroprotective effect on animal models of AD and prompted us to initiate a program of drug development that overcome pharmacokinetics and efficacy gaps in order to selected an optimized lead that should entry in a discovery and development process to forward this new strategy to regulatory and preclinical trials. After the validation of this enzyme as a putative target for AD, a completely new scaffold with improved pharmacological characteristics in front to sEH, UB-SCG-51. This compound effectivity in reverse neurodegeneration, ad was considered as a lead for the chosen target. Next, we designed several derivatives in order

to improve physicochemical, and pharmacokinetic properties to forward this led to a lead optimization. We tested several UB-SCG-51 prodrugs (tertbutyl, methyl and ethyl esters) and salts. Results in IC50 for sEH enzyme (rat, mice and human) prompted us to study in deep arginate. Therefore, based on these results we designated UB-SCG-74 as an optimized lead, and test its efficacy, safety, and potency, to demonstrated a good bioavailability and a suitable safety pharmacology profile. In addition, we studied the duration of the inhibitory effects on sEH and determine whether the treatment allows to limit the progression of AD. In sum, we develop a candidate compound endowed with strong sEH inhibition, favorable pharmacokinetic properties and an excellent safety profile, that was in the pool position for advancing it towards regulatory preclinical evaluation and eventual clinical development.

UB-SCG-74demostrated enhanced oral absorption compared with UB-SCG-51 resulting in increased oral biodisponibility. UB-SCG-74 also exhibited higher potency, with a dose of 1.5 mg/Kg providing complete prevention of working and spatial memory (figure 4 and 5) compared with 5 g/kg for UB-SCG-51 [17]. Additionally, UB-SCG-74 showed no toxicity, with a MTD exceeding 2000 mg/kg.

In terms of safety, UB-SCG-74 did not affect K+ currents through hERG, nor did it exhibit carcinogenesis or mutagenesis potential, supporting its classification as non-toxic, at least in rodent models. Notably, UB-SCG-74 maintained cognitive improvements up to four weeks after the cessation of oral administration, indicating potential disease progression-modifying activity in addition to symptomatic relief.

In summary, UB-SCG-74, the arginate derivative of UB-SCG-51, represents promising therapeutic candidate for the AD treatment and other neurodegenerative conditions with a proinflammatory processes.

Biography: Mercè Pallàs is a Full Professor of Pharmacology in the Department of Pharmacology, Toxicology and Therapeutic Chemistry at the Faculty of Pharmacy and Food Sciences, University of Barcelona (UB). She is also a founding member of the University of Barcelona's Institute of Neurosciences (NeuroUB). She has over 25 years of experience in preclinical pharmacology, focusing on cognitive decline associated with aging and neurodegenerative diseases, particularly Alzheimer's disease (AD). After completing her PhD at UB in 1991, she spent a postdoctoral year (1992–1993) at the Istituto di Ricerche Farmacologiche Mario Negri (Italy), working in Professor Daniela Corda's group on G protein-coupled receptors (GPCRs). She returned to Barcelona in 1993 as an Associate Professor and was promoted to Full Professor in 2011. Her research has included both non-pharmacological (diet, exercise, caloric restriction) and pharmacological strategies (melatonin and resveratrol) for brain aging. Currently, she is focused on elucidating the precise mechanisms of action and molecular pathways involved in the activity of new drug candidates developed by the ChemMedPharm group. These include small molecules targeting neuroinflammation (soluble epoxide hydrolase inhibitors), mitochondrial stress, oxidative stress (imidazoline 2 receptors), and pathological senescence, as well as exploring the intersection with epigenetic mechanisms (e.g., G9a inhibitors).

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Flash Presentations

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Prevalence of asthma in Portuguese adults - the EPI-ASTHMA study, a nationwide population-based survey

Dinis Brito[1,2], Cristina Jácome[3,4], Cláudia Bulhões[1,5], Maria João Barbosa[1,6], Nuno Pina[7], Ana Alves da Silva[8], Catarina João[8], Diana Gomes[8], Filipa Lopes[9], Janete Santos[8], Liliana Amorim[10], Marina Rodrigues[5], Marisa Pardal[11], Pedro M Teixeira[1,10], Tiago Jacinto[3,4], Ana Margarida Cruz[12,13], Ana Margarida Pereira[4,8,14], Ana Marques[11] , Bernardo Sousa-Pinto[3,4] , Cláudia Vicente[15], Elisete Ferreira[10], Luís Alves[13,16] , Maria Inês Fernandes[10] , Rafael Vieira[3,4], Rita Amaral[3,4,17,18], Rita Sousa[11], Rui Costa[10], Teresa Castanho[10], Filipa Bernardo[11], Jaime Correia-de-Sousa[1] , João A Fonseca[3,4,9,14] , EPI-ASTHMA Group

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Introduction: In 2010, 6.8% of the Portuguese adults had asthma. Contemporary studies employing more accurate methods are needed. We aimed to assess asthma prevalence in Portugal and to identify associated-factors.

Methods: A population-based nationwide study was conducted from May 2021 to March 2024. A multistage random sampling approach was applied to select adults from primary care. Stage 1 involved a telephone screening interview to collect socio-demographic and clinical data. Patients with an Adult Asthma Score (A2 Score) ≥1 were eligible for Stage 2, and 5% of those with an A2 Score = 0 were also invited to participate in Stage 2, which consisted of a diagnostic visit with a physical examination and diagnostic tests. We computed weighted asthma prevalence estimates and multivariable logistic regression models were used.

Results: A total of 7,556 participants completed Stage 1 and 1,857 Stage 2. The prevalence of asthma was 7.1% (95%CI = 6.3–8.0%), with slight differences by sex, age, and region. Education, family history of asthma, inhaler prescription, nasal/ocular symptoms, food allergies, and previous allergy skin tests were associated with an increased risk of asthma (R2 = 33%). Asthma diagnosis could also be predicted by the A2 score, either on its own (R2 = 43%) or in combination with family history and previous allergy skin tests (R2 = 45%).

Discussion:Asthma affects 7.1% of Portuguese adults. Family history of asthma, nasal/ocular symptoms, and comorbid food allergy are associated with increased risk of asthma.

DHA priming reprograms the metabolism of human adipose mesenchymal stromal cells and improves the therapeutic performance of its secretome in translational studies of spinal cord injury

Jonas Campos[1,2], Belém Sampaio-Marques[1,2], João Afonso[1,2], Marta F. Lima[1,2], Alice Miranda[1,2], Jorge R. Cibrão[1,2], Sara Rito-Fernandes[1,2], Filipa F. Antunes[1,2], Susana Monteiro[1,2], Melissa Carvalho[1,2], Andréia Monteiro[1,2], Maria M. Moura[1,2], Diogo Santos[1,2], Alexandra Teixeira[3], Sophia L. Baptista[1,2], Sofia C. Serra[1,2], Tiffany Pinho[1,2], Jorge R Cibrão[1,2], Nuno A. Silva[1,2], Adina T. Michael-Titus[4], António J. Salgado[1,2]

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[4]Blizard Institute - Faculty of medicine and Dentistry, Queen Mary University of London.

Mesenchymal stem cells have been increasingly used as a potential treatment strategy for spinal cord injuries (SCI). Their capacity to modulate SCI pathophysiology is derived from the array of bioactive molecules and extracellular vesicles secreted to the extracellular environment. Recently, cellular priming approaches have been proposed to further improve the therapeutic functions of MSCs and facilitate clinical translation. Herein, the effects of a newly developed priming approach using the omega-3 fatty-acid docosahexaenoic acid (DHA) on several aspects of adipose stem-cell biology and on the neuroregulatory profile of the secrcetome of hASCs were studied in the context of SCI.
By using dose-escalation studies coupled with metabolic viability and temporally resolved evaluation of cellular morphology, the optimal DHA priming protocol was determined to be 40 µM for 72h. Transcriptomic experiments and metabolic profiling revealed over-represented pathways that unify metabolic sensing and higher biosynthetic capacity with DHA priming. These transcriptional alterations were correlated with increased glycolytic output and mitochondrial activity which culminated in an increased concentration of proteins and extracellular vesicles in the secretome. Unbiased LC/MS proteomics revealed a link between the transcriptional landscape and the proteome of the hASCs secretome, with up-regulated proteins being functionally associated with anti-oxidant and neurotrophic responses.
Functionally, the DHA-primed secretome protected spinal cord cells and modulated astrogliosis and microglial cell reactivity by reducing their proliferation after a hyperosmotic stress injury in vitro. In a clinically relevant model of thoracic compression SCI, DHA-primed secretome reduces astrocyte and microglial reactivity in the grey matter and degenerating corticospinal tracts of the spinal cord. These effects correlated with a differential performance on the amelioration of SCI-induced behavioral deficits in sensory and spasticity domains. Overall, this work proposes a novel priming strategy that promotes significant improvements in the therapeutic potential of ASC secretome when applied for spinal cord injury.

A cholinergic brainstem input to the nucleus accumbens bidirectionally mediate cocaine reinforcing effects

L.A.A. Aguiar [1,2,6], L. Royon [3,4,5,6], R. Bastos-Gonçalves [1,2], T.T.A. Carvalho [1,2], E. Teixeira [1,2], L. Pinto [1,2], S.P. Fernandez [3,4,5], J. Barik [3,4,5], **B. Coimbra** [1,2], A. J. Rodrigues [1,2]

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[3]Institut de Pharmacologie Moléculaire & Cellulaire, CNRS, UMR7275, Valbonne, France
[4]Université Côte d'Azur, Nice, 06560, France
[5]Inserm U1323Divisão de Biomateriais,

The laterodorsal tegmentum (LDT) is a key mesopontine structure that projects to multiple brain regions involved in reward processing, including the nucleus accumbens (NAc). While LDT inputs to the ventral tegmental area (VTA) have been extensively studied, the role of direct LDT-NAc projections in addiction-related behaviors remains largely unexplored. Here, we demonstrate that repeated cocaine exposure induces persistent neuroplastic changes in LDT-NAc circuits, particularly within cholinergic neurons, and alters behavioral responses to cocaine reinforcement. Using a combination of cell type-specific viral tracing, in vivo electrophysiological recordings, optogenetics, and patch-clamp electrophysiology, we provide evidence that LDT cholinergic neurons preferentially innervate NAc medium spiny neurons (MSNs) and cholinergic interneurons (CINs). Large-scale in vivo recordings reveal that cocaine pre-exposure persistently modifies neuronal activity in both LDT and NAc, with LDT-NAc-projecting cholinergic neurons displaying heightened excitability, while non-projecting cholinergic cells exhibit reduced activity.

Optogenetic activation of LDT-NAc cholinergic projections enhances cocaine conditioning, whereas their inhibition significantly reduces cocaine's reinforcing effects. Using a generalized linear model, we reveal that cocaine pre-exposure alters the functional connectivity between LDT and NAc, reducing the influence of LDT neuronal activity on NAc responses to acute cocaine challenge. *Ex vivo* patch-clamp recordings show that LDT-NAc-projecting cholinergic neurons exhibit increased intrinsic excitability following cocaine exposure, a plasticity effect not observed in other LDT cholinergic cells. These findings highlight an asymmetric impact of cocaine exposure on distinct LDT cholinergic populations and suggest that LDT-NAc cholinergic signaling plays a critical role in mediating the reinforcing properties of cocaine.

Overall, our study establishes the LDT-NAc cholinergic pathway as a key circuit mediating cocaine's reinforcing effects and introduces novel insights into addiction-related neuroplasticity. These findings provide a mechanistic framework for understanding how cocaine alters mesopontine-striatal interactions and suggest that targeting LDT-NAc cholinergic signaling could offer new therapeutic strategies for substance use disorders.

The path from the choroid plexus to the (sub)ventricular zone and back: implications for multiple sclerosis

Monica Fernandes[1,2,3], Lili Li[1], Eduarda Correia[2,3,4], Alice Vieira[2,3], Jonas Campos[2,3], Neemat Mahmud[1], Mandy Meijer[1], Eneritz Agirre[1], Joao Canto[2,3], Joao Cerqueira[2,3], Cláudia Nobrega[2,3], Fernanda Marques[2,3], Gonçalo Castelo-Branco[1,5], Joao Carlos Sousa[2,3] and **Ana Mendanha Falcão[1,2,3]**

1Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, 171 77 Stockholm, Sweden.
2Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal. 3ICVS/3B's -PT Government Associate Laboratory, Braga/Guimarães, Braga, Portugal.
4Clinical Academic Center-Braga (2CA), Braga, Portugal
5Ming Wai Lau Centre for Reparative Medicine, Stockholm node, Karolinska Institutet, 171 77 Stockholm, Sweden

Can choroid plexus-borne molecules modulate the (re)generation of oligodendrocytes? In multiple sclerosis the lost oligodendrocytes are hardly replaced at later stages of disease leading to an irreversible symptomatology and ultimately to the patients' death. Despite efforts, it remains extremely difficult to come up with ways to induce the replacement of the lost cells. The choroid plexus is a tissue of the brain whose most described function is cerebrospinal fluid (CSF) production. Because epithelial cells from the choroid plexus produce most of the CSF they have privileged access to the brain, particularly to the neural stem cell niche lying at the ventricular- subventricular zone (V-SVZ). In this study we are investigating the choroid plexus as a shaper of brain milieu in multiple sclerosis and as a source of molecules that influence oligodendrocyte (re)generation. For that, we apply single-cell multi-omics and in vitro co-culture models to search for the competence of choroid plexus cells to phagocyte myelin debris, and to characterize the cellular composition and transcriptome profiles of the choroid plexus and V-SVZ cells upon a massive loss of oligodendrocytes, in both mouse models and in human choroid plexus samples collected from MS patients and non-MS controls. Our results reveal that choroid plexus cells: i) uptake myelin debris, ii) modulate and communicate with V-SVZ cells in response to inflammation, iii) change transcriptome in response to oligodendrocyte loss and rewire their communication and iv) populations are more prevalent or underrepresented in MS tissue.
Altogether, our data suggests that the choroid plexus is a promising, yet underexplored, target for modulation in multiple sclerosis.

The *egli-1* gene as a possible link between autophagy and food-related behaviors: insights from the *C. elegans* nervous system

Jorge H. Fernandes [1,2], Jorge Diogo Da Silva [1,2], Marta D. Costa [1,2], Stéphanie Oliveira [1,2], Andreia Teixeira-Castro [1,2], and Patrícia Maciel [1,2]

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2 ICVS/3Bs - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Introduction: The ability to sense and respond to food is crucial for animal survival, with imbalances potentially leading to various pathological conditions. Understanding the neuronal and cellular mechanisms underlying food-related behaviors is vital for insights metabolic disorders. The nematode *Caenorhabditis elegans* offers a powerful model system for this purpose due to its well-characterized neural circuitry and conserved behavioral responses. We previously identified the gene *egli-1* as a key regulator of food-related behavior in *C. elegans*. Interestingly, its mammalian orthologue, *TMEM41B*, has been implicated in autophagy. Our research aims to elucidate the role of EGLI-1 in autophagy in the nematode and underpin the precise subcellular changes underlying behavioral adaptation, providing mechanistic insights into how neuronal autophagy regulates food-related behaviors.
Methods: We investigated the role of *egli-1* in autophagy and behavior using genetic, molecular, and behavioral approaches. Autophagosome formation was assessed in vivo using an LGG-1::GFP reporter strain. To assess behavioral consequences, we performed neuronal RNAi-mediated silencing of *egli-1* and other autophagy-related genes and quantified food-related behavior under varying nutritional conditions.
Results: Loss-of-function mutations in *egli-1* led to a reduction in autophagosome formation under starvation, indicating a disruption in autophagy. Neuron-specific silencing of *egli-1* resulted in distinct behavioral impairments, including defective food chemotaxis and altered egg-laying patterns. These phenotypes were not due to general sensory deficits but to failures in adaptive behavioral responses. Remarkably, inducing autophagy in neurons through genetic manipulation was sufficient to rescue normal behavioral responses in *egli-1* mutants.
Conclusions: Our findings demonstrate that neuronal autophagy, mediated by *egli-1*, is essential for adaptive behavioral responses to food availability in *C. elegans*. We hypothesize that autophagy, through its link with vesicle trafficking and membrane remodelling, supports neuronal plasticity by enabling rapid modulation of synaptic transmission during changes in nutritional status. This work highlights a novel mechanism linking intracellular catabolism to behavioral regulation and offers potential insights into conserved pathways relevant to human metabolic and neurobehavioral disorders.

Characterization of microglial response to the activity of MCH neurons

João Frei [1,2], Ana Falcão [1,2], Sara Calafate [1,2]

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Introduction: Microglia, immune cells of the central nervous system, interact with neuronal elements and are sensitive to brain homeostasis changes, altering their morphology and molecular signatures in response to insults. Under physiological conditions, they are highly ramified with dynamic processes that refine synaptic activity and plasticity, influencing memory. Recently, sleep has been shown to modulate microglia surveillance. Melanin-concentrating hormone (MCH), a sleep-related neuropeptide produced by MCH neurons in the lateral hypothalamic area (LHA), is active during rapid-eye movement sleep and regulates sleep architecture and hippocampal-dependent memory. Notably, in early stages of Alzheimer's diseases, the MCH-system is vulnerable, affecting LHA projections to the CA1 region.
Objective: Microglial physiology may change during sleep-wake cycle, but the molecular organizers of this dynamic remain unclear. This study aims to determine whether MCH peptide orchestrates microglia responses during sleep via manipulation of MCH neuron activity.
Methods: We used Pmch-cre transgenic mice to modulate MCH neuron activity with adeno associated viruses expressing Designer Receptors Exclusively Activated by Designer Drugs, which are engineered receptors activated by the administration of the agonist Clozapine-N-oxide. We established three experimental groups: Pmch:Cherry (control group), Pmch:Gq (MCH neurons activated) and Pmch:Gi (MCH neurons inhibited). Microglial morphodynamics were assessed using Iba1 immunofluorescence for morphological reconstruction. Single-cell RNA sequencing was used to analyze microglial molecular profiles and gene expression heterogeneity.
Results: We found differences between Pmch:Cherry and Pmch:Gq in Iba1-covered area in CA1 region, but not in microglial numbers. Pmch:Gi showed increased microglial branches and total length compared to control. Branch intersection differences were also seen between Pmch:Cherry and both Pmch:Gi and Pmch:Gq. Molecular profile data are still under analysis.
Conclusion: The results suggest that the activity of MCH neurons influences microglial complexity without affecting their number. Furthermore, inhibition of these neurons appears to enhance the morphological complexity of microglia.

MODULATING TAU: EXPLORING NOVEL ASOS FOR DOWN SYNDROME

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Down Syndrome (DS) is the most common form of intellectual disability worldwide and it is characterized by a partial or complete trisomy of chromosome 21. Interestingly, individuals with DS develop many of the neuropathological markers found in Alzheimer's disease (AD), which is suggested to be driven by the presence of an extra copy of APP gene. Considering recent evidence highlighting the essential role of Tau protein in AD pathology, along with observed similarities between AD and DS brain pathologies, we previously developed and screened *in vitro* novel antisense oligonucleotides (ASOs) designed to reduce total Tau or 4R-Tau levels. From this screening, we selected the most effective ASOs to evaluate their efficacy on DS brain pathology using both *in vitro* models (primary neurons and human-derived induced pluripotent stem cells) and *in vivo* DS mouse model (Ts65Dn, expressing approximately two-thirds of human orthologs).

We first confirmed the efficacy of these ASOs reducing Tau mRNA and protein levels via qRT-PCR and Western blot analyses, respectively, both *in vitro* and *in vivo*. More importantly, we were able to revert cognitive deficits observed in DS mice in the Morris Water Maze test, ameliorating disease phenotype with ASOs. Subsequent proteomic analysis identified multiple pathways altered in DS mice, related with translation, synaptic signaling and AD, which were restored following ASO administration. Moreover, Golgi-based neuronal reconstruction revealed some reversion of the neuronal atrophy observed in DS mouse brain upon treatment with ASOs. Ongoing are the studies in hiPSCs from patients of DS, where we are optimizing neuronal differentiation settings, focusing on interneurons, which are heavily affected in DS brain.

In summary, this study provides the first comprehensive *in vivo* confirmation of the efficacy and therapeutic potential of novel ASOs targeting Tau in DS, underscoring their promise as a novel RNA-based therapeutic approach for DS.

Shugoshin-1 (SGO1) is a novel glioblastoma biomarker predictive of patient prognosis

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Introduction:Glioblastoma (GBM) is a highly heterogeneous and aggressive brain tumor, characterized by poor prognosis and limited treatment efficacy. The identification of novel molecular biomarkers is essential to improve our understanding of GBM biology and contribute to developing targeted therapies. Shugoshin-1 (SGO1), a key regulator of chromosome segregation, has been associated with increased aggressiveness in several cancers. Multi-omics data from our group identified *SGO1* as a candidate molecule of interest in GBM, yet its functional and clinical impact remain unexplored.

Methods

A sparse network was used to assess relationships in GBM multi-omics data. Endogenous *SGO1* mRNA expression was analyzed in 11 GBM cell models (5 cell lines and 6 patient-derived cultures). GBML42, a patient-derived culture with high *SGO1* expression, was selected for lentiviral shRNA-mediated gene silencing. The impact of *SGO1* expression was evaluated *in vitro*, through functional and molecular assays, and in an *in vivo* orthotopic mouse model. Prognostic value was evaluated in an in-house patient cohort and in publicly-available datasets, using univariable and multivariable models.

Results

In GBM sparse networks, *SGO1* presented numerous and strong gene relationships. *SGO1* was found to be highly expressed across all tested 11 GBM cell models. *In vitro*, *SGO1* silencing in GBML42 cells significantly decreased cell viability, colony formation capacity, wound healing, while increasing chemosensitivity. Molecularly, *SGO1* silencing associated with decreased expression and reduced activation key GBM oncogenic pathways. An *in vivo* experiment is ongoing to validate these findings in a more relevant model. Clinically, *SGO1* was identified as an independent prognostic biomarker for decreased overall survival in GBM patients.

Conclusions

This work associates *SGO1* with increased GBM aggressiveness and worse patient prognosis, and provides insights into mechanisms through which it may be operating. Ultimately, these findings identify *SGO1* as a candidate prognostic biomarker and a novel potential target for future precision medicine strategies.

Sex differences in astrocyte activation contributes to pain in experimental osteoarthritis

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Introduction. Osteoarthritis (OA) is one of the leading causes of chronic pain worldwide. Functional studies showed a critical role of descending facilitation, involving the rostral ventromedial medulla (RVM), in the establishment of pain chronicity. Additionally, glial cells such as microglia and astrocytes, have been recognized for their contribution to chronic pain through the facilitation of neuronal excitability. Our goal was to conduct an extensive analysis of the morpho-structural attributes of neuronal and glial cells in the RVM and to identify potential sexual dimorphisms, to increase our understanding of these contributing factors to osteoarthritis pain.

Methods. The kaolin/carrageenan model of experimental osteoarthritis (eOA) was induced in both male and female rats. Four weeks after induction, we evaluated changes in neuronal/glial cell density within the RVM through a stereological analysis. Additionally, glial activation was evaluated through the expression of GFAP and Iba1, astrocytic and microglial markers. Astrocyte proliferation and structural changes were further evaluated through immunofluorescence and a Sholl analysis, respectively.

Results. While no major differences were found between RVM structural and cellular characteristics of SHAM and K/C males, eOA led to an increase in the glia-to-neuron ratio in females. No differences in Iba1 immunoreactivity were found between experimental groups. However, females exhibited an increase in GFAP-positive cells and immunostained area after K/C induction, indicating an increase in astrogliosis, associated with an increase in mitotic activity and structural complexity.

Conclusions. Our findings indicate that RVM astrocytes respond differently to experimental osteoarthritis pain in males and females. By addressing the effects of biological sex and showing sex-dependent glial impact in the processing and modulation of pain, these data further highlight the need to include female subjects in preclinical research.

Volume Reconstruction Algorithm for Tumor Assessment from Freehand Ultrasound Recordings in Mice

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Tumor volume quantification in animal studies, namely in mice, is a critical component in oncobiology research, as accurate and reliable measurements are essential for assessing disease progression and/or treatment efficacy.

Currently, there are three main methods to measure tumor volume in mice: (1) calipers, which provide only rough estimates and are limited to subcutaneous tumors; (2) 2D ultrasound (US), which uses orthogonal images for approximate volume estimation; and (3) 3D US imaging followed by tumor segmentation, which gives accurate results but is time-consuming and requires anesthesia.

To overcome these limitations, this work proposes an alternative method based on deep learning (DL) to estimate spatial transformations between US frames, enabling 3D volume reconstruction.

The initial phase involved developing a motion tracking setup to provide ground truth data for training and validating the DL model. To do this, a six-degree-of-freedom sensor was attached to the US probe, and spatial and temporal calibration was carried out using a phantom. The calibration setup, installed at the ICVS animal facility, includes the Aurora electromagnetic tracking system, capable of capturing the probe's pose in real-time.

Once the system is fully assembled and calibrated, the next phase will involve acquiring freehand US sequences from mice. A DL approach will then estimate the spatial transformations between frames by analyzing image features and patterns. The model will be trained using loss functions that minimize the error between predicted and actual transformations, allowing it to assess whether a prediction deviates significantly from the ground truth and enabling accurate 3D volume reconstruction. Finally, model performance will be evaluated by directly comparing the transformations or by comparing the reconstructed volumes derived from predicted transformations with those from the motion tracking system.

Ultimately, this approach aims to reduce anesthesia use and animal stress, potentially improving accuracy and reliability in preclinical tumor volume assessment.

From Memory to Misfiring: Lipid and Synaptic Drivers of Alzheimer's and Seizures

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Alzheimer's disease (AD) is the most common dementia, affecting 56 million people worldwide. It involves progressive cognitive decline and deposition of beta-amyloid (Aβ) plaques and tau tangles in the brain. Amyloid precursor protein (APP), which is cleaved into Aβ peptides, plays a central role; dysregulated APP processing and impaired Aβ clearance contribute to neurotoxicity and inflammation. Despite its immense burden, few therapies exist for slow AD progression.

An emerging, underexplored aspect of AD is its association with epileptic seizures. People with AD have an 87-fold increased risk of epilepsy, though many seizures are subclinical and undetected. The relationship is bidirectional: seizures exacerbate cognitive decline, and AD pathology increases seizure susceptibility. Animal models, such as J20 and APP knock-in mice, display early-onset seizures and cognitive impairment, but the molecular connection between seizures and AD remains unclear.

Lipids, essential for brain function, may serve as a missing link. They regulate synaptic activity and excitability—key factors in seizure susceptibility. Altered lipid pathways are found in AD and epilepsy, suggesting their involvement in the AD-epilepsy interface. PLD2, a lipid-modifying enzyme producing phosphatidic acid, is elevated in AD models. Deletion of PLD2 protects against memory loss and synaptic dysfunction. Our unpublished data shows PLD2 knockout mice exhibit seizure resistance in AD models, altered lipid profiles, and synaptic protection similar to tau knockout mice.

At the synapse, PLD2 influences lipid composition and NMDA receptor (NMDAR) function. Fyn, a kinase dysregulated in both AD and epilepsy, phosphorylates the NR2B subunit of NMDARs, increasing excitability. Tau recruits Fyn to synapses, enhancing NR2B phosphorylation. Aβ oligomers promote NR2B clustering at extrasynaptic sites, triggering excitotoxicity.

Since membrane lipids modulate Fyn-NR2B signaling, PLD2 may regulate this pathway. This study uses electrophysiology, behavior, histology, and lipidomics to identify lipid signatures linking seizures and AD, aiming to uncover new lipid-based therapeutic strategies.

Regulation of macrophage immunometabolic responses to *Aspergillus fumigatus* by PTX3

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Introduction: Fungal infections represent a global health problem, affecting over 1 billion individuals worldwide and resulting in an estimated 3 million deaths annually. Immunocompromised patients are at higher risk of developing life-threatening fungal infections, such as invasive pulmonary aspergillosis (IPA), primarily caused by the opportunistic fungus *Aspergillus fumigatus*. The long pentraxin PTX3, a fluid-phase pattern recognition molecule produced by macrophages and neutrophils, plays a central role in antifungal immunity by recognizing and opsonizing fungi. Importantly, previous studies have highlighted the significance of metabolism in regulating macrophage function in response to *A. fumigatus*. This study aimed to elucidate the mechanistic links between the intracellular functional activity of PTX3 and immune cell metabolism in the pathogenesis of IPA.

Methods: By resorting to a full knockout mouse model for Ptx3 (*Ptx3*$^{-/-}$), we have generated wild-type (Wt) and *Ptx3*$^{-/-}$ bone marrow-derived macrophages. Macrophages were infected with *A. fumigatus* and their functional activity and metabolic state were compared at different time-points.

Results: *Ptx3*$^{-/-}$ macrophages show a defective capacity to eliminate internalized conidia and to control its germination. In response to *A. fumigatus*, *Ptx3*$^{-/-}$ macrophages exhibit a significant increase in pentose phosphate pathway (PPP) metabolites, while Wt cells accumulate glycolytic intermediates. Importantly, increased expression of sedoheptulose kinase (Shpk), a critical regulator of the PPP, is observed in *Ptx3*$^{-/-}$ macrophages. Shpk, known to co-localize with glucose- 6-phosphate dehydrogenase (G6pd), has been previously shown to modulate macrophage activation. Although we do not observe the modulation of G6pd or Shpk proteins in macrophages upon infection, we hypothesize a regulation of Shpk and/or G6pd activity by Ptx3.

Conclusions: Our findings indicate that the impaired capacity of *Ptx3*$^{-/-}$ macrophages to control fungal elimination is associated with an inadequate reprogramming of cellular metabolism towards glycolysis. Further studies need to be conducted to better understand how Ptx3 modulates the intracellular responses to *A. fumigatus.*

Layer-specific imbalances in cortical PV and SST interneurons in the Dp(16)1Yey mouse model of Down syndrome

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Introduction. Down Syndrome (DS), caused by triplication of the human chromosome 21, is the most common neurodevelopmental disorder worldwide, associated with altered brain structure and function, physical abnormalities, and intellectual disability. A cortical excitatory/inhibitory (E/I) circuitry imbalance was described in DS patients' brains, but how specific cortical inhibitory circuit mediate DS pathophysiology remains unclear. Thus, we aimed at characterizing the DS mouse model Dp(16)1Yey, throughout development, and comprehend how cortical interneurons are affected in DS.
Methods. For that, we used a battery of behavioral tests to asses cognition, anxiety, obsessive compulsive and innate behaviors as well as motor function, at 1 and 5 months of age, in male and female WT and Dp(16)1Yey mice. Next, to characterize the different subtypes of cortical interneurons, we performed immunostainings in the prefrontal cortex (PFC) of 5 months old mice, and the slices were aligned with the Allen Brain Atlas.
Results. We observed that, starting at 1 month of age, Dp(16)1Yey mice showed anxiety- and obsessive compulsive-like behaviors, impaired long-term, spatial and social memories and absence of sociability deficits. This was accompanied by coordination and balance difficulties and hindlimb hypotonia, that worsened with age. At the neuronal level, at 5 months of age, we observed reduced number of neurons in some layers of the anterior cingulate and prelimbic areas of the PFC along with an increased number of total GABAergic interneurons in Dp(16)1Yey mice. Also, the number of different interneuron subtypes (parvalbumin-, somatostatin- and neuropeptide Y-positive neurons) was altered in this DS model, pointing towards an imbalance in the E/I circuitry.
Conclusions. Overall, our results confirm that the Dp(16)1Yey model mimics the clinical manifestations observed in humans, being a good model to study the different cellular and molecular mechanisms of the disease, pointing to interneuron subtype imbalances as an important component of the disease.

Phospholipase D2 ablation leads to deficits in social memory

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The hippocampus is a structure located in the brain's temporal lobe that plays a key role in learning and memory. The hippocampal formation consists of the dentate gyrus, the cornu ammonis(CA)regions 1-3, and the subiculum in rodents. Electrophysiological, gene, and functional studies focusing on the longitudinal axis (ventral-dorsal in rodents) showed that the dorsal and ventral portions have different connectivities.

Phospholipase D (PLD) is a phosphodiesterase that hydrolyses phosphatidylcholine into phosphatidic acid (PA). A PA gradient along the longitudinal axis of the rodent hippocampus suggests that PLD might play a role in regulating the DH-VH axis.

Work from our team showed that PLD1 is a major contributor to total PA production in the mouse forebrain. PLD1 ablation leads to altered dendritic arborization and behavioral deficits. Pld1 KO mice had deficits in Long Term Depression and alterations in synaptic proteins in the DH. Unpublished data from our group showed that PLD2 ablation has no impact on the performance of cognitive and memory, which contrasts with the impact of PLD1 ablation. *Pld2* KO mice showed no differences in sociability or preference for social novelty. However, *Pld2* KO mice lost the ability to discriminate between a littermate and an unfamiliar mouse. Our findings suggest that PLD2 ablation impairs littermate recognition and decreases social exploration in a CA2-dependent task.

PLD2 is highly expressed in CA2, and CA2 impacts social memory in a circuit that depends on VH CA1. Our results suggest a potential role for PLD2 in ''dorsal CA2''- ''ventral CA1'' circuit regulation with an impact on social memory recall. Therefore, we designed a social behavioural task, to further evaluate the impact of PLD2 ablation on social memory and this circuitry, where following exposure to familiar or novel mice, we performed counterstaining against c-Fos, a marker for neuronal activity.

Genetic engineering of the CD81 large extracellular loop for targeted delivery of mesenchymal stem cell derived vesicles for spinal cord injury repair

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Spinal cord injury (SCI) is a devastating condition. Its consequences are related with the acute and chronic inflammatory response, and the establishment of an environment that limits regenerative processes, resulting in lifelong impairments. We have previously shown that systemic administrations of adipose-derived mesenchymal stem cell (ASC) secretome can improve motor function in SCI rodent models, however, most of the extracellular vesicles (EVs) present in the secretome do not reach the spinal cord. Here, we aim to genetic engineer CD81 (highly expressed in EVs), for targeting of ASC derived EVs towards the SCI site. To achieve this, we used a previously established yeast library displaying the large extracellular loop (LEL) of CD81, mutated in specific solvent exposed amino acids. By consecutive rounds of MACS and FACS sorting we selected versions of the CD81 LEL that have binding ability to relevant proteins found specifically in the SCI site, both acutely (myelin associated glycoprotein, MAG) and chronically (neurocan) which were recombinantly expressed using HEK-293 cells. We further confirmed that the selected LELs, when expressed in full length CD81, can be expressed and maintain their binding ability in HEK cells (mammalian system) and that EVs from these cells also bind to MAG/neurocan. We are currently performing lentiviral transduction of immortalized ASCs with the binder CD81s. We will then assess the homing ability of EVs from the transduced ASCs towards the SCI site as well as their therapeutic potential in comparison with wild type (WT) CD81 transduced cells, in a rodent model of SCI. By directing the EVs to the specific growth inhibiting environment of SCI, we hope to maximize the potential of systemic administrations of ASC secretome in the context of SCI.

The IFNγ-JAK/STAT axis regulates macrophage transformation and granuloma formation in sarcoidosis

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Introduction: The pathological hallmark of sarcoidosis is the formation of granulomas incited by the accumulation of activated macrophages and T-cells, and the local production of proinflammatory cytokines. Despite their clinical significance, the molecular sequences of events that promote macrophage aggregation and transformation into epithelioid cells that initiate and maintain granulomas remain elusive.
Many cytokines implicated in sarcoidosis, such as IFN-y and others, signal via the JAK/STAT (Janus kinase/signal transducers and activators of transcription) pathway. Persistent activation of JAK/STAT has been demonstrated in tissues and blood of patients with sarcoidosis. Although the importance of JAK/STAT in sarcoidosis has been suggested, no study to date has determined the mechanistic bases linking the constitutive activation of JAK/STAT pathway and disease pathogenesis.
Methods: By taking advantage of mononuclear cells from patients with sarcoidosis and using advanced in vitro models of macrophage transformation and granuloma formation, we assessed the contribution of JAK/STAT signaling to macrophage reprogramming and the acquisition of granuloma-promoting features.
Results: PBMCs subjected to an in vitro model of granuloma formation showed increased IFN-y production and STAT1 activation, resulting in larger granuloma-like structures. Treatment with ruxolitinib, a JAK1/2 inhibitor, abrogated STAT1 activation, leading to decreased levels of IFN-y and cellular aggregates with reduced size. Moreover, in a giant cell formation model, ruxolitinib treatment and consequent JAK/STAT signaling inhibition restrained macrophage transformation and multinucleation.
Conclusions: Our results shed light on the important role of JAK-STAT activation in driving macrophage transformation and aggregation in sarcoidosis and highlight the therapeutic potential of ruxolitinib in targeting JAK-STAT-driven granulomatous inflammation.

A Robust Optical Flow-Based Tracker for Anatomical Landmark Detection in Laparoscopic Videos

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Introduction: Minimally invasive surgery (MIS) improves patient recovery but increases the technical demands on surgeons. Tracking anatomical landmarks during laparoscopic procedures can provide real-time feedback and assist in navigation, offering benefits for training and clinical outcomes. However, existing tracking solutions struggle with occlusions, rapid camera movement, and tissue deformation.
Methods: We propose a two-stage optical flow-based tracker to estimate the motion of anatomical landmarks in laparoscopic videos. The first stage uses a coarse full-frame estimation to handle large displacements, while the second stage refines predictions using a localized region of interest. To enhance robustness under instrument occlusions, we introduce a training strategy called Instrument Motion Inpainting (IMI), allowing the model to infer tissue motion behind surgical instruments using only visual context.
Results: The tracker was evaluated on the SurgT dataset using both standard and event-based metrics. Compared to existing methods, our approach demonstrated improved accuracy and robustness, especially in challenging scenarios such as occlusions (when instruments block the view) and fast camera movements. The Instrument Motion Inpainting (IMI) strategy significantly reduced errors following occlusions and improved recovery consistency. Additionally, the two-stage tracking method reduced the recovery failure rate to 0% while maintaining high accuracy under normal conditions, confirming the benefit of combining global context with local refinement.
Conclusion: By combining coarse and refined optical flow estimation with instrument motion inpainting to handle occlusions, our method enhances the robustness of landmark tracking in laparoscopic surgery. This technology could support future surgical navigation systems, improve training tools, and ultimately contribute to safer and more efficient procedures.

ASTROWARS: THE RETURN OF THE ASTROCYTIC METABOTROPIC GLUTAMATE RECEPTOR 5 (IN CORTICO-LIMBIC FUNCTION AND BEHAVIOR)

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Introduction: Evidence has demonstrated a key role of astrocytes in the brain, namely in the modulation of neuronal networks. Indeed, astrocytes gained recognition as the third active element of a synapse. While interacting closely with neurons, astrocytes modulate neuronal activity by sensing, processing, integrating, and responding to synaptic transmission. Astrocytes were shown to sense glutamate, the primary excitatory neurotransmitter, which occurs by activation of metabotropic glutamate receptor 5 (mGluR5). Despite the importance of mGluR5 for circuit function, the studies on astrocytic mGluR5 were performed mostly ex vivo in young rodents, missing the physiological properties and functional consequences during adulthood, which are crucial for fully understanding glutamatergic signaling in brain circuits. To address these, we performed gain VS. loss of astrocytic mGluR5 function in mice to allow a detailed characterization of the different levels of activity: behavior-circuit-cellular-molecular.
Methods: We used a Cre-lox system to drive the deletion of mGluR5 in astrocytes from the whole brain in adult mice. Additionally, we used a viral approach to delete or overexpress mGluR5 specifically in hippocampal astrocytes. To confirm the specific manipulation and loss/gain of mGluR5 function in astrocytes, a combination of molecular biology and calcium imaging techniques was used. This confirmation was followed by a behavioral and electrophysiological evaluation of these models.
Results: Our results revealed that deletion of mGluR5 in the whole brain induced anxious- and depressive-like phenotypes, impairments in recognition and spatial memory, and enhancements in behavior flexibility, behaviors also altered when mGluR5 manipulation was performed specifically in hippocampal astrocytes. Moreover, we confirmed that astrocytic mGluR5 is involved in the induction of long-term potentiation and modulating astrocytic structure in the hippocampus.
Conclusion: We show the involvement of astrocytic mGluR5 signaling in mood and cognitive processing, suggesting a possible therapeutic potential in diseases characterized by a hippocampus-dependent cognitive decline, such as Alzheimer's disease.

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1

Astrocytic Foxo1 regulates hippocampal spinogenesis and synaptic plasticity and enhances fear memory

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Introduction: Astrocytes respond to neuronal activity through calcium signals with complex spatiotemporal properties: global (soma/processes) and focal (microdomains). These are expected to underline the astrocyte involvement in synaptic transmission, metabolism, and brain homeostasis.

Aims: This study aimed to investigate the role of global calcium elevations via the IP3 receptor type 2 (IP3R2) in astrocytes and their impact on behavior, synaptic structure, and gene expression.

Methods: We used the IP3R2 KO mouse model, which lacks global calcium elevations in astrocytes, to perform transcriptomic analysis of hippocampal tissue and identify differentially expressed genes. We also examined pyramidal neuron morphology and hippocampus-dependent behaviors. After identifying the astrocyte-specific transcription factor Foxo1 as a potential contributor to these changes, we overexpressed Foxo1 in hippocampal astrocytes of C57BL/6J mice and assessed the resulting structural, synaptic, and behavioral outcomes. To confirm its role, we finally then silenced Foxo1 in hippocampal astrocytes of IP3R2 KO mice.

Results: Transcriptomic analysis of hippocampal tissue of IP3R2 KO mice revealed that the lack of astrocytic global calcium causes the differential expression of hundreds of genes. Among these, 76 are regulated by the astrocyte-specific Foxo1 transcription factor, which is upregulated in hippocampal astrocytes of these mice and regulates genes involved in spinogenesis and synaptic coverage. Morphological analysis revealed a shift towards immature dendritic spines, potentially explaining reduced long-term depression and enhanced fear memory. To confirm causality, we overexpressed Foxo1 in hippocampal astrocytes in C57BL/6J mice, reproducing the structural, synaptic, and behavioral effects observed in mice lacking global calcium elevations in astrocytes. Lastly, silencing Foxo1 in IP3R2 KO astrocytes reversed the fear memory enhancement, confirming its causal role.

Conclusions: Our findings demonstrate that astrocytic calcium signaling, through Foxo1, influences hippocampal circuit structure, synaptic function, and fear memory. These findings underscore the significance of astrocytes in shaping behavior and memory processes.

2

Effect of iPSCs-derived Mesenchymal Stem Cells and their Secretome on Spinal Cord Injury: Focus on Neurovascular and Cellular Responses

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Spinal cord injury (SCI) is a devastating condition that disrupts the communication between the brain and the body. Its complex interconnected mechanisms demand a combinatorial therapeutic approach for a higher chance of success, with a strong window of opportunity in the acute phase. Mesenchymal stem cells (MSCs) and their secretome have independently demonstrated beneficial effects for SCI, due to their immunomodulatory, angiogenic and neuroregenerative/protective activity. Furthermore, MSCs derived from induced pluripotent stem cells (iMSCs) present a similar secretory profile with a rejuvenated phenotype, while being obtained from a less invasive and more clinically translatable source. However, functional validation studies of iMSCs and their interaction with neural cells remains limited. For that reason, this project aims to understand how iMSCs and their secretome modulate neural cells under injury conditions. Using an *in vitro* hyperosmotic stress model of SCI, the effect of cell secretome on spinal cord cells viability, morphology and phenotype was evaluated. Additionally, the combination of iMSCs and their secretome was studied in an acute SCI mouse model. Given the critical role of vascular function in tissue regeneration and homeostasis, a focused analysis on the neurovascular unit was performed. Immunofluorescence staining was employed to evaluate glial and immune responses, barrier phenotype, vessel maturation, cell proliferation and neurovascular integrity. Although iMSCs treatment seem to produce an overall positive effect, further studies are required to achieve full functional recovery and to elucidate the mechanisms behind the treatment positive effects. This work provides a comprehensive characterization of iMSCs effects on different injury paradigms, while highlighting cytoarchitectural alterations following SCI.

3

Optimization of the Delivery Routes of Mesenchymal Stem Cells Secretome in a Rodent Animal Model of Parkinson's Disease

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Introduction: Parkinson's disease is the second most common neurodegenerative disorder, affecting around 1% of the population. Motor symptoms include tremors, rigidity and bradykinesia. As of now, the available therapies, like Levodopa and Deep Brain Stimulation only offer amelioration of the symptoms, with none addressing the neurodegenerative process. Novel research on regenerative medicine has opened the door for new therapies, having neuroprotection in mind. Mesenchymal stem cells (MSCs), a type of non-hematopoietic stem cell secrete molecules, their "secretome", with neuroprotective and immunomodulatory properties. Previous work in ICVS's laboratory has shown that they might be the key to fight neurodegeneration in Parkinson's disease. Nonetheless, having clinical application in mind, it is essential to standardize procedures, mainly involving the administration of this novel therapy. In this work, we will be looking at the available methods of administration of secretome, such as intravenous, intracerebral and a novel method, intranasal administration and comparing its effects in a 6-hydroxidopamine (6-OHDA) mice model of Parkinson's disease.
Methods: 50 10-week old mice were divided into 5 groups, one control (non-lesioned and non-treated), and 4 unilaterally lesioned with 6-OHDA, of which two were treated with intranasal administration, one of secretome, and a control, of saline, and two were treated with intracerebral administration under the same conditions. During the following 10 weeks the animals passed through 4 behavioral essays, spaced 2 weeks from each other, composed of the beam balance, cylinder, motor swimming and pole tests. Afterwards the mice were occised and their brains collected for histology. Comparison with intravenous administration was based off literature.
Results: We expect to see non-treated lesioned mice behave poorly in behavioral tests. They should also present a noticeable less number of dopaminergic neurons in the lesioned side. Intracerebrally treated mice should present better results in the behavioral tests, achieving almost the same performance as the non-lesioned mice and a rescue of dopaminergic neuron population should be seen in the lesioned side. Intranasal treated mice should present similar results, or even better than intracerebrally treated mice.
Conclusion: With this work, we expect to contribute to a standardization of the procedures of administration of secretome, as a potential therapy for Parkinson's disease, having the clinical translational aspect in mind. Intranasal administration should be an easier, safer, and less expensive form of administration of this therapy if it presents as being comparable to other forms of administration in laboratory settings.

4

The role of astrocytic metabotropic glutamate receptor 5 in the medial prefrontal cortex

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Astrocytes have been identified as crucial players in the central nervous system, either by modulating synapses and entire neuronal networks or by maintaining the homeostasis of the brain. Astrocytes were first shown to sense glutamate, the most abundant excitatory neurotransmitter in the brain, which occurs mostly by activation of metabotropic glutamate receptor 5 (mGluR5). The activation of astrocytic mGluR5 triggers somatic Ca2+ elevations, which lead to the release of gliotransmitters, modulating synaptic activity in cortico-limbic regions that are critical for cognitive processing. However, most of the evidence was based on studies that only explore the biological role of mGluR5 in young rodents, often using in vitro or ex vivo approaches. Therefore, the impact of astrocytic mGluR5 on behavior in adult mice needs to be further investigated. Preliminary data from our laboratory show that mGluR5 activation is important for cognitive processing in behavioral tasks dependent on the medial prefrontal cortex and hippocampus.
Here, we took advantage of a mouse model with the mGluR5 gene flanked by loxP sites, and through an intracranial stereotaxic injection of an adeno-associated virus, we targeted specifically astrocytes in the medial prefrontal cortex. The generated mouse model with regional and temporal controlled deletion of astrocytic mGluR5 was then subjected to a detailed behavior characterization, covering mood, cognition, and sociability.

5

The role of phospholipase D1 in myelin turnover

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Myelin sheaths, formed by oligodendrocytes, play a crucial role in axonal isolation, protection and signal transmission, and their dysfunction has already been linked to several neurodegenerative diseases, including Alzheimer's disease (AD) and Multiple Sclerosis (MS). Magnetic Ressonance Imaging (MRI) metrics, including myelin thickness, white matter volume, diffusion tensor imaging (DTI), and the Grey/White Matter contrast (GWC) are useful to give insight into myelin's integrity and monitor possible changes associated both with age and neurological conditions.

Phospholipase D1 (PLD1) is a lipid metabolizing enzyme responsible for the conversion of phosphatidylcholine (PC) into phosphatidic acid (PA) and free choline, involved in membrane trafficking, receptor signaling and cytoskeletal dynamics. Interestingly, PLD1 has been described to have a significant impact in brain GWC and a prominent role in myelination, suggesting that this enzyme can be both a therapeutic target to enhance myelin regeneration and an important marker for diagnosis through imaging techniques. Previous MRI studies of our group found significant differences between mice models with or without a knock-out of PLD1 or and AD-like model, in corpus callosum, a region highly myelinated. This study assesses histological differences among glial cells that can correlate with these results, giving us insight into how PLD1 ablation affects myelin turnover, and the glial network involved in the maintenance and regeneration of white matter. Our preliminary data shows no significant differences both in astrocyte and microglia cell numbers and area covered in corpus callosum and hippocampal dorsal CA1, and in myelin covered area.

6

Structural and Functional Analysis of Brain Maps in Alzheimer's Disease

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This study investigates the application of the Neuromaps toolbox for comparative analysis of brain maps related to Alzheimer's disease (AD) and primary age-related tauopathy (PART) with particular emphasis on Lewy body (LB) co-pathology. Working in collaboration with researchers examining LB influence on cognitive outcomes and brain atrophy, this research aims to identify distinct patterns in subjects already classified as AD or PART with or without LB pathology. This study leverages pre-classified subjects to examine how these distinct pathological entities and their co-pathologies affect brain structure.

The methodology involves implementing a systematic workflow through the Neuromaps toolbox, utilizing neuroimaging data post-mortem correlated with MRI scans (T1's specifically) obtained during life. The dataset contains subjects classified with either AD or PART, with or without LB, retrieved from the NACC Institute (n=319 with LB and n=514 without LB). The research employs pre-processed maps from Freesurfer which passed through a group analysis, using general linear modeling, and then converted to standardized coordinate systems, transformations between different coordinate spaces, and applied statistical analyses that preserve spatial autocorrelation of brain maps through various null models. The implementation uses a modular Python-based pipeline for flexible processing and comparison.

The initial implementation successfully demonstrated proper functioning of the Neuromaps workflow. Performance metrics revealed significant processing time differences between volumetric, surface-based and parcellated analyses. The pipeline effectively processes brain maps and provides correlation values and statistical significance between pathology types. Although the current final results aren't the most scientific because of some irregularities in the MRI scans, the final approach will eliminate this kind of problems and hopefully show significant results (e.g. detecting pathology-specific patterns).

The developed methodology provides a systematic approach for analyzing spatial relationships between AD and PART with and without LB co-pathology. This framework enables reproducible analyses of brain maps while maintaining spatial integrity of the data. The secondary objective of implementing a web interface will further democratize access to these complex analyses for researchers and medical professionals.

7

Unraveling lipid-dependent mechanisms in heme detoxification

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Malaria remains a major global health challenge, with *Plasmodium falciparum* accounting for most of the 263 million cases and 597,000 deaths reported in 2023. During the blood stage of infection, *P. falciparum* relies on detoxifying free heme—a toxic by-product of hemoglobin digestion—by converting it into an inert crystalline form called hemozoin. This detoxification process is essential for parasite survival and is the target of many antimalarial drugs.

Recent studies have highlighted the involvement of lipid droplets within the digestive vacuole in hemozoin formation. However, the molecular mechanisms remain unclear. We hypothesize that perilipins—proteins known to coat lipid droplets and regulate lipid metabolism—may play a crucial role in this pathway. Notably, perilipins have not yet been characterized in *P. falciparum*.

Using bioinformatic analyses, we identified eight *P. falciparum* proteins with potential perilipin- like signatures. Our aim is to validate the presence and function of these proteins in the heme detoxification process. To achieve this, we are engineering conditional knockdown parasite lines targeting these candidates. We constructed plasmids using genome-editing approaches, integrating the genes into a selection-linked integration (SLI) system coupled with the glmS ribozyme for post-transcriptional regulation.

We have successfully cloned the candidate genes into the initial vector and are proceeding with the final SLI plasmid assembly. The next step will involve transfecting these constructs into *P. falciparum* Dd2 and 3D7 strains via electroporation.

This study aims to uncover a novel regulatory mechanism in hemozoin formation. Confirming the role of perilipins in *P. falciparum* could open new avenues for antimalarial drug development.

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8

Regulation of anti-fungal immunity by the c-MET signaling pathway

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Introduction: Invasive pulmonary aspergillosis (IPA), primarily caused by *Aspergillus fumigatus*, impacts an estimated 2 million individuals worldwide annually with mortality rates up to 85%. Recent shifts in the epidemiology of IPA have highlighted innate immune dysfunction as a key contributor to disease progression. Consequently, understanding the mechanisms involved in the antifungal immune response to *A. fumigatus* could aid the development of innovative anti-fungal therapies to improve patient outcomes. c-MET, an important tyrosine kinase receptor, involved in recognition of bacteria and parasites, has recently been identified as an epithelial receptor for invasins present in *Candida albicans* hyphae. However, c-MET's function in the immune response to *A. fumigatus* infection remains unexplored.

Methods: In this work, we interrogated the effector functions of macrophages to *A. fumigatus* infection *in vitro*. We also explored the role of c-MET in the antifungal immune response to *A. fumigatus* using an immunocompetent mouse model of IPA, with c-Met conditionally deleted in murine hematopoietic and endothelial cells. Subsequently, immunoblotting was harnessed to investigate potential fungal ligands responsible for c-MET activation by *A. fumigatus*.

Results: *In vitro* analysis confirmed that deletion of c-Met in macrophages impaired phagocytic and conidiacidal capacities, along with production of reactive oxygen species (ROS). Moreover, our results also revealed that c-Met is essential for pro-inflammatory cytokine production, namely IL-6 and IL-1β, as well as for the metabolic reprogramming of macrophages during *A. fumigatus* infection. Additionally, c-MET deletion *in vivo* resulted in increased fungal burden and lung inflammation, impaired neutrophil phagocytic capacity, and reduced conidiacidal activity and ROS production by interstitial macrophages.

Conclusions: This study highlights the importance of c-MET in the immune response to *A. fumigatus* and begins to uncover additional molecular mechanisms underpinning this host-pathogen interaction to support future investigation of the receptor as a novel target for antifungal therapeutics.

9

Sialidase-1 as a Key Modulator of Immune Response and Virulence in *Sporothrix brasiliensis*

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Sporotrichosis, a subcutaneous mycosis caused by *Sporothrix*, affects humans and animals. Initially linked to *Sporothrix schenckii*, later research identified a pathogenic clade of cryptic species. Though globally distributed, its relevance has increased due to recent zoonotic outbreaks. *Sporothrix brasiliensis,* the most virulent species, its notably linked to feline epidemics, spreading from Brazil worldwide.

A recent dual RNA-sequencing analysis of *S. brasiliensis* and *S. schenckii* following *in vitro* human macrophage engulfment unveiled sialidase-1 (SbSia1), also called neuraminidase, as a key virulence factor, with its expression increasing more than 700 times in intracellular *S. brasiliensis* after phagocytosis. SbSia1 removes sialic acids and modifies cell surface glycoconjugates in both pathogens and mammalian cells. Its high expression correlates with significant transcriptional reorganization in infected macrophages, enhancing cellular transcription and proliferation while impairing pyroptotic responses. Unlike *S. schenckii*, which activates protective apoptotic signalling, *S. brasiliensis* alters host cell responses, leading to heightened inflammation and cytotoxicity. Our findings suggest that SbSia1 enables *S. brasiliensis* to evade host macrophages defenses by modifying glycoprotein sialylation, contributing to its persistence and pathogenesis. This imbalance likely exacerbates inflammation and complicates sporotrichosis management. Given SbSia1's critical role in host-pathogen interactions, targeting this enzyme may represent a promising therapeutic strategy. As proof-of-concept, treating *S. brasiliensis* with the neuraminidase inhibitor oseltamivir significantly reduced fungal burden in bone marrow-derived macrophages (BMDMs) infected *in vitro*.

Evaluating SbSia1's role in *S. brasiliensis* pathogenicity is key to understanding its persistence, dissemination, and feline sporotrichosis. Using isogenic *S. brasiliensis* and *S. schenckii* strains with knocked-out or overexpressed SbSia1, we will assess sialic acid levels in cell-walls and membranes and analyse inflammatory responses in infected BMDMs. Future studies should determine whether SbSia1-driven immune modulation aids fungal adaptation and explore its potential as a therapeutic target. Unravelling these mechanisms will support better treatment strategies and disease management.

10

Methotrexate-Induced Immune Modulation: Impact on T Cell Function in *Leishmania* Pathogenesis

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Human visceral leishmaniasis (VL), caused by the *Leishmania* parasites, is a neglected infectious disease endemic in the Mediterranean basin and poses a significant risk, particularly to immunosuppressed individuals. *Leishmania infantum* disrupts T cell metabolism, promoting immune evasion and chronic liver infections. A reduced Th1 response, associated with metabolic disruptions and deregulated nutrient-sensing pathways, weakens the host's ability to clear the parasite and its response to treatment. Additionally, certain immunosuppressant therapies exacerbate VL severity. However, the impact of these immunometabolic alterations on chronic infection dynamics remains poorly understood. In particular, the metabolic basis of antigen-specific T cell dysfunction during chronicity is not well characterized. Identifying key metabolic regulators may provide immunotherapeutic targets to restore T cell function and improve drug efficacy. Using a well-established murine model of *L. infantum* infection, we assessed the immune cell phenotypes and parasite burden in immunocompetent and immunosuppressed mice treated with miltefosine, a first-line antileishmanial drug for VL, through flow cytometry and qPCR analysis. Building on the immunological characterization under chronic and immunosuppressed conditions, we will next investigate the metabolic landscape of T cells and its relationship with anti-leishmanial immune response. This will use an integrated approach with RNA sequencing and metabolic profiling of sorted splenic effector $CD4^+$ and $CD8^+$ T cells. By elucidating the interplay between T cell metabolism and immune function during infection, this work will provide insights into the pathogenesis of chronic VL and drive innovative immunometabolic therapies for VL.

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Sentinels for synaptic function: exploring RNA-binding proteins in Down Syndrome Synaptic degeneration

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Down syndrome (DS), caused by an extra copy of human chromosome 21, is associated with progressive neurodegeneration and cognitive decline, which are often exacerbated by aging. Increasing evidence links DS with early-onset Alzheimer's disease (AD) pathology and disruptions in proteostasis, RNA metabolism, and cellular stress responses, alongside a range of clinical complications.

This study uses the Ts65Dn mouse model of DS to investigate molecular mechanisms underlying age-related neuronal dysfunction associated with AD pathology; aiming to elucidate the relationship between RNA-binding proteins (RBPs), stress granules (SGs), synaptic dysfunction, and neurodegeneration in DS. For that, we collected hippocampal tissue from Ts65Dn and wild-type (WT) littermates at 8 and 18 months of age and conducted proteomic analyses, followed by RIPA-insoluble protocol to study SG marker levels and expression levels of the different RBPs involved in this process. These studies were complemented with neurostructural analysis using Golgi-cox and behaviour.

Ts65Dn mice showed significant age-dependent alterations in the hippocampal proteome, including dysregulation of proteins involved in translation, synaptic function, and plasticity. This was accompanied by reduced dendritic spine plasticity and reduced neuronal complexity with age in the DG of Ts65Dn mice, in contrast to an age-related increase in spine number in WT mice that compensates for the loss of dendritic complexity, suggesting disrupted synaptic plasticity in DS. Regarding RNA metabolism and SG formation, we observed alteration in the protein levels of several RBPs in the insoluble and soluble fraction, with a particular focus on TIA1, eIF4e and G3BP increase in DS, that is sustained during aging. Additionally, PABP staining shows the presence of SG- like aggregates in DS brain at both ages.

Our results show for the first time that DS hippocampus presents a dysregulation in RBPs levels and their accumulation in SG-like aggregates similar to the ones observed in Tauopathy models, accompanied by neuronal atrophy and spine alterations; changes that are sustained and increased along aging.

These molecular alterations may underlie neurodegeneration and related cognitive deficits in DS and offer insights into novel mechanisms of disease.

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Astrocytic Exocytosis: Molecular Mechanisms Underlying Cognitive Function and Neuronal Synchronization

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Introduction: Astrocytes are now recognized as dynamic partners in brain function, actively participating in neuronal communication and metabolism. Exocytosis from astrocytes plays a crucial role in releasing various gliotransmitters, including glutamate, ATP, and D-serine, which modulate synaptic strength, synchronize neuronal activity, and regulate synaptic plasticity. Recent research has highlighted the importance of astrocytic exocytosis in higher cognitive functions, including learning, memory formation, and sleep regulation. The dnSNARE mouse model, which allows for selective inhibition of astrocytic vesicular release, has been instrumental in revealing these functions. Our laboratory showed that blocking SNARE-dependent gliotransmitter release from astrocytes using the dnSNARE mouse model caused desynchronization of theta oscillations between the hippocampus and prefrontal cortex and led to impaired performance on working memory tasks, highlighting the importance of astrocyte signaling in cognitive functions

Methods: To elucidate the molecular underpinnings of this cognitive impairment, we conducted microarray analysis on hippocampal tissue from adult dnSNARE mice. Our transcriptomic analysis aimed to identify differentially expressed genes, enriched biological pathways, potential upstream regulators, and disease associations.

Results:The analysis identified 481 differentially expressed genes, including key proteins involved in synaptic plasticity, neuroprotection, extracellular homeostasis, and the inflammatory response. Predicted upstream regulators of these transcriptional changes are associated with learning and memory processes. The gene expression profile shows strong enrichment for neurological disorders such as Alzheimer's disease, epilepsy, and frontotemporal dementia, which present similar cognitive impairment as in dnSNARE mice.

Conclusions:These findings elucidate the broader neurobiological consequences of impaired astrocytic exocytosis, emphasizing its fundamental role in maintaining neural network function and contributing to neuropathological processes.

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IL-37 genetic variant influences susceptibility to invasive pulmonary aspergillosis by modulating macrophage metabolic responses to fungal infection

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Introduction: Invasive pulmonary aspergillosis (IPA) is a frequently fatal opportunistic infection among immunocompromised patients, particularly those receiving allogeneic hematopoietic stem cell transplantation (HSCT). Genetic predisposition is considered a major factor influencing susceptibility to IPA. Interleukin-37 (IL-37) is a recently identified immunosuppressive cytokine of the IL-1 family that plays a central role in suppressing innate and adaptive immune responses and acts as a modulator of cellular metabolic homeostasis during inflammation. The present study identified a single nucleotide polymorphism (SNP), rs3811047, within the *IL37* locus as a risk factor for the development of IPA and aims to further dissect the underlying mechanisms behind this association.

Materials & Methods:The cumulative incidence of IPA was evaluated in patients undergoing HSCT stratified by *IL37* genotypes at the rs3811047 SNP. The SNP's functional role in antifungal immune responses was explored using *A. fumigatus*-infected human monocyte-derived macrophages (hMDMs) from genotype-defined healthy donors.

Results: In a disease-relevant context, this SNP was found to influence the alveolar cytokine milieu within the bronchoalveolar lavage (BAL) of IPA patients. Moreover, our results indicate that risk genotype hMDMs exhibit increased intracellular expression of IL-37, accompanied by a profound defect in their fungicidal activity and pro-inflammatory cytokine secretion, notably IL-1β, which correlated with NLRP3-inflammasome suppression. Treatment of monocytes with rhIL-37 reduced the fungicidal capacity of macrophages after differentiation, particularly in those with the risk genotype, underscoring a regulatory role for IL-37 in the epigenetic modulation of macrophage function. This phenotype was associated with alterations in the metabolomic profiling of risk carrier macrophages, which revealed a shift toward increased glucose metabolism alongside inhibition of purine metabolism, the glutamine/glutamate axis, and the tricarboxylic acid (TCA) cycle.

Conclusions:In conclusion, our findings suggest that the dual function of IL-37 in limiting inflammation and modulating metabolism may have important clinical implications for antifungal immunity and susceptibility to IPA.

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Modeling Spinal Cord Injury in a Dish with Hyperosmotic Stress: The Effects of Mesenchymal Stromal Cell Secretome Treatment

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Innovations in spinal cord injury (SCI) models are crucial for developing effective therapies. This study introduces a novel *in vitro* SCI model using cultures of primary mixed spinal cord cells from rat pups, featuring key spinal cord cell types. This model offers distinct advantages in terms of feasibility, reproducibility, and cost-effectiveness, requiring only basic cell culture equipment. Following hyperosmotic stress via sorbitol treatment, the model recapitulated SCI pathophysiological hallmarks, with a 65% reduction in cell viability and gradual cell death over 48 hours, making it ideal for evaluating neuroprotective agents. Notably, the human adipose tissue stem cells (hASCs) secretome provided significant protection: it preserved metabolic viability, reduced β-APP expression in surviving neurons and modulated the shift of astrocytic morphotype. A transcriptomic profile of the effect of the hASCs secretome treatment showed significant functional enrichments related to cell proliferation and cycle progression pathways. In addition to supporting the use of the hASCs secretome as a therapy for SCI, this study is the first to use sorbitol as a hyperosmolar stressor to recapitulate key aspects of SCI pathophysiology. Thereby, this model can be used as promising platform for evaluating therapeutic agents targeting neuroprotection and neuroregeneration, offering outputs related to cell death, neuronal stress and protection as well as induction of glial reactivity.

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LIPID PRIMING OF ASCS SECRETOME FOR SPINAL CORD INJURY: AN INVESTIGATION OF ITS MODULATORY ROLES IN MICROGLIAL PRUNING AND PHAGOCYTOSIS

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Microglia are involved in the modulation of synaptic plasticity through pruning events, selectively removing weak synapses and strengthening appropriate ones. While crucial for recovery, dysregulated microglial activity—either excessive pruning or reduced debris clearance—can contribute to maladaptive plasticity. Adipose tissue-derived mesenchymal stem-cells (ASCs) are highly valued in regenerative medicine due to their secretome (SEC), and to enhance its neuroregulatory potential for spinal cord injury (SCI) repair, priming strategies have been developed. Previous studies from our lab have shown that targeting the FFAR4 receptor in the primary cilium of ASCs with the omega-3 fatty acid docosahexaenoic acid (DHA) produces a protein-rich secretome (ω-SEC) that has protective effects in SCI models. Animals treated with ω-SEC showed fewer activated microglial cells—similar to healthy controls—and experienced less spasticity compared to those treated with the unprimed secretome. These results suggest that the primed secretome may modulate microglial behavior, potentially affecting processes like synaptic pruning and phagocytosis, known to influence neural plasticity after SCI. Building on that, our goal is to investigate the development of maladaptive plasticity following SCI associated with microglial function, exploring how DHA-primed secretome could be a potential treatment to mitigate pathological outcomes. Results of *in-vitro* testing in mixed SCI cultures demonstrated that ω-SEC restored microglial interaction with myelin debris, while injured controls showed reduced microglial presence and debris clearance, which suggests restored microglial structural- functional coupling after treatment. I*n-vivo* experiments revealed that, in histological sections of the spinal cord, the size of microglial processes at the peri-somatic space of motor neurons is positively correlated with the size of GABAergic inputs arriving; specifically, the ω-SEC-treated group exhibited significantly enhanced GABAergic input area along with a trend toward larger microglial contact areas comparing to NBA-treated group, potentially counteracting spasticity by enhancing inhibitory tone. These preliminary results underscore the need for further investigation into the multifaceted role of microglia in SCI.

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Unraveling the role of EVs and PBMCs crosstalk as inflammation mediators in Lysosomal Storage Diseases

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Lysosomal storage diseases (LSDs) are a group of rare genetic disorders that encompass various genetically distinct diseases, with a combined prevalence of 1:5000 births. They are characterized by a deficiency in lysosomal enzymes responsible for breaking down and recycling cellular waste. The enzyme deficiency disrupts the lysosomal function, leading to the accumulation of toxic substances. This accumulation triggers an inflammatory response, characterized by the overproduction of pro-inflammatory cytokines, which contributes to cellular damage and dysfunction.

Over the last years extracellular vesicles (EVs) have been described as key mediators in the inflammatory cascade in which they play a dual role. Depending on their origin, EVs can promote or suppress the inflammatory process. This project aims to understand the role of EVs, and its crosstalk with peripheral blood mononuclear cells (PBMCs), as inflammation drivers in LSDs. For that, plasma samples from LSD patients will be used and EVs and PBMCs will be isolated, quantified, and phenotypically characterized, with a focus on their immunological and inflammatory profiles. Furthermore, in vitro culture of the isolated PBMCs with stimulation of cytokine production and subsequent analysis of secreted EVs will allow the assessment of their inflammatory profile and compare with the previous collected EVs from the plasma.

Thus, a deeper understanding of the involvement of EVs-PBMCs in LSD-associated inflammation may reveal novel therapeutic targets and facilitate the development of innovative biomarkers for early diagnosis and disease monitoring.

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Tissue-specific somatic mosaicism in Machado-Joseph disease

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Machado-Joseph Disease (MJD), or Spinocerebellar Ataxia Type 3 (SCA3), is a progressive neurodegenerative disorder caused by an expanded CAG repeat in the *ATXN3* gene, primarily characterized by motor dysfunction. This CAG expansion is highly unstable, both during transmission from parent to child and within somatic tissues, where distinct cell populations may contain different repeat lengths. Larger expansions are associated with earlier disease onset and increased severity.

In this study, we investigated somatic mosaicism in the CMVMJD135 mouse model of MJD, focusing on how tissue-specific differences in CAG repeat length may influence disease progression. DNA was extracted from five tissues—muscle, hippocampus, pons, spinal cord, and deep cerebellar nuclei—from one-year-old mice with advanced disease. The CAG repeat region was amplified by PCR using fluorescently labeled primers and analyzed by fragment analysis via capillary electrophoresis. GeneMarker software was used to assess mosaicism and somatic expansion indexes.

Our results showed that the pons, a region severely affected in MJD, exhibited the highest level of CAG repeat instability, including both expansions and contractions. In contrast, the spinal cord, also strongly affected, displayed lower levels of instability, possibly due to advanced neuronal loss, with highly expanded cells already degenerated. Similar patterns were seen in the muscle and deep cerebellar nuclei. Interestingly, the hippocampus—a region generally considered spared in MJD—showed instability levels comparable to the pons. This may be due to ongoing neurogenesis in the hippocampus, making it more susceptible to genetic instability, or it may suggest that somatic CAG repeat instability is not exclusive to regions affected by neurodegeneration.

In conclusion, our study shows that the CMVMJD135 mouse model exhibits significant somatic CAG repeat instability in both affected and unaffected brain regions, providing a valuable tool for future investigations into repeat expansion dynamics, including longitudinal and high-resolution sequencing studies.

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Re-activation of a cocaine-responsive ensemble but not a shock-responsive ensemble in the nucleus accumbens controls behavioral preference

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The nucleus accumbens (NAc) plays a critical role in processing both rewarding and aversive experiences, shaping behavior through experience-dependent neural plasticity. The NAc is primarily composed of GABAergic medium spiny neurons (MSNs) expressing either dopamine receptor D1 (D1-MSNs) or D2 (D2-MSNs). While both cocaine and shock alter NAc neuronal activity, it remains unclear whether these ensembles are segregated or not, and how it affects behavior. Here, we investigate how cocaine- or shock-responsive neuronal ensembles in the NAc are reactivated by motivationally salient stimuli and how their reactivation influences behavioral preference.

We used a transgenic mouse model of targeted recombination technique in active populations (TRAP2;Ai14) to label NAc ensembles activated by either cocaine or foot shock stimuli. We found that single exposure to either cocaine or foot shock significantly recruited NAc neurons, with minimal overlap between the ensembles. Calcium imaging of individual NAc MSNs in freely behaving mice showed excitatory or inhibitory responses to either cocaine or shock. Similarly to the anatomical data, when tracking the activity of the same neuron in both sessions, a small overlap in types of response to the different stimuli was observed, confirming distinct encoding populations.

To determine the causal role of these ensembles, we used TRAP2;Ai32 mice, that allows opsin expression in TRAped cells, enabling optogenetic re-activation of cocaine- or shock-responsive ensembles during behavior. Re-activation of cocaine-responsive ensembles elicited place preference for the stimulation-paired side in a conditioned place preference test and increased lever-pressing for food paired with optical stimulation in a two-choice task. Curiously, re-activating shock-responsive ensembles did not significantly affect preference.

These findings suggest that NAc cocaine-responsive ensembles directly influence choice behavior, possibly by encoding reward-related memory traces that influence long-term reward-seeking behavior. This work enhances our understanding of how neural mechanisms differentiate reward and aversion and their implications for addiction and decision-making.

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Understanding the regulation of mood and stress responses in a mouse model of SCA3

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Machado-Joseph disease (MJD) is a rare inherited neurodegenerative disease caused by a CAG repeat expansion in the *ATXN3* gene. While loss of motor function is a key feature in MJD, anxiety and depressive symptoms are frequently reported by these patients. Additionally, recent studies reflect on the presence of executive dysfunction in MJD, as part of the so called – Cerebellar cognitive affective syndrome. Previous studies from our lab revealed a decrease in the glucocorticoid receptor (GR) expression in the brainstem and an elevation of corticosterone (CORT) in the blood of MJD animals at late stages of the disease which could also be mood-related. To address the intricate etiology of these mood alterations, animal models could be of great value, as they lack awareness of their progressive disease condition. Thus, we further investigate the presence of non-motor symptoms in MJD by performing longitudinal behavior analyses using our in-lab generated CMVMJD135 mouse model, that closely mimics MJD both phenotypical and pathologically. Our results showed that MJD mice are not cognitively affected but there is a progressive anxious phenotype, assessed in the elevated plus maze. Moreover, given the importance of GR in the corticoid-dependent feedback loops of the hypothalamus-pituitary-adrenal (HPA)-axis we aimed to study the effects of stressful events in CORT variations and on motor function in the MJD mouse. Importantly, the late-stage elevation of CORT was not determinant for a functional HPA-axis activity while responding to acute stressors as well as recovering from a suppression test using dexamethasone. Also, chronic stress exposure did not have a major impact on MJD motor phenotype. Nevertheless, our findings indicate that beyond its well-known effects on the motor systems, MJD appears to influence emotion regulation processes. The multidimensional symptoms of MJD mirror its complexity, reinforcing the need for integrated care approaches to address its broad spectrum of manifestations.

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Cytokine Profilling in Fabry Disease: Insights from Free and EV- Associated Profiles

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Introduction: Fabry Disease (FD) is an X-linked lysosomal storage disorder caused by deficient or absent activity of the enzyme alpha-galactosidase A (α-Gal A). This enzymatic defect leads to the progressive accumulation of globotriaosylceramide (Gb3) within cells, resulting in widespread, multisystemic dysfunction. The buildup of Gb3 contributes to chronic inflammation and cellular damage. Small extracellular vesicles (EVs) involved in long-distance intercellular communication, carry molecular cargo that reflects the physiological or pathological state of their cell of origin. These vesicles can influence recipient cells by transferring bioactive molecules, potentially reprogramming their function and contributing to disease progression. This project investigates cytokine dysregulation in patients with FD, examining both freely circulating cytokines and those associated with EVs, to evaluate their potential diagnostic and clinical significance.

Methods: Cytokine profiles were assessed in PBMCs and EVs from 60 FD patients and 40 healthy controls. PBMCs were isolated via Ficoll gradient centrifugation, and cytokine levels were measured using a bead-based multiplex flow cytometry assay. EVs were isolated from plasma by size exclusion chromatography, and exosome- associated cytokines were quantified using ELISA.

Results: A consistent imbalance in the ratio of pro- to anti-inflammatory cytokines was observed in FD patients, indicative of an inflammatory state. This proinflammatory alteration was evident both in circulating and EVs-associated cytokine profile. Notably, this imbalance varied according to clinical phenotype. Asymptomatic individuals exhibited a coordinated early proinflammatory signature detected even before clinical manifestations, while patients with renal involvement showed a sustained and systemic proinflammatory state. In contrast, those with cardiac manifestations presented a more compartmentalized profile, suggesting potential compensatory regulatory mechanisms.

Conclusions: These findings underscore phenotype-specific immune alterations in FD, with the integration of free and EV-associated cytokine analysis. Moreover, here we identified potential non-invasive biomarkers for early detection, phenotype- specific monitoring, and a deeper understanding of the immunopathogenesis of FD.

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Skeletal muscle alterations as an early pathological manifestation in a mouse model of Machado-Joseph disease

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Machado-Joseph disease (MJD) is a rare monogenic neurodegenerative disease caused by an expanded CAG trinucleotide repeat within the gene encoding the protein ataxin-3. The resulting elongated glutamine abnormally accumulates and forms neuronal nuclear inclusions that can lead to neuronal dysfunction and death. While MJD is primarily known for its effects on the central nervous system, peripheral neuropathy and muscle alterations are also an important aspect of the disease. Muscle cramps and fasciculations are persistent disabling events, and 82% of patients can also experience changes in muscle excitability. In addition, patients and animal models of MJD experience weight loss, which can aggravate the disease progression. As in other neurodegenerative diseases, it is most often assumed that loss of muscle mass and weight is a secondary consequence of neurological dysfunction. In this study, we aimed to characterize in detail the skeletal muscle alterations in MJD progression, using a well-established mouse model of the disease – the CMVMJD135 mice. Our morphometric analysis of quadriceps muscle from MJD mice indicates a progressive decrease in muscle fiber diameters, accompanied by nuclei internalization at the late stages of the disease. Interestingly, the human-expanded ataxin-3 is highly expressed in the quadriceps muscle of 34-week-old CMVMJD135 mice. In addition, transcriptional alterations in key genes related to skeletal muscle wasting and atrophy were detected in the skeletal muscle of these mice, as were metabolic changes, including a decrease in ATP levels, present since the early stages of the disease. Overall, our findings indicate that the CMVMJD135 mouse model of MJD exhibits early and significant skeletal muscle pathology. This study provides further evidence for peripheral involvement in MJD and highlights the importance of considering skeletal muscle pathology as a potential therapeutic target in disease management and treatment.

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Endogenous dynorphin signaling in the nucleus accumbens in motivated behaviors

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Research using mostly pharmacological manipulation has shown that the endogenous opioid circuit is a powerful pre- and post-synaptic modulator, influencing reward and aversion. The nucleus accumbens (NAc), a key brain region in rewarding and aversive behaviors, is mainly composed of GABAergic medium spiny neurons (MSNs) expressing dopamine receptor D1 and the endogenous opioid dynorphin, and another subset of cells expressing D2 and the endogenous opioid enkephalin. Initial data from our team (and others) suggest that endogenous opioids produced and released by NAc MSNs play a critical role in motivated behaviors. Dynorphin is typically associated with aversive effects, but emerging evidence suggests that its behavioral impact may differ depending on the release site. Despite its recognized importance, the lack of appropriate tools hindered real-time measurement of their release during behavior.

To address this, we took advantage of a newly-developed dynorphin sensor to record temporal- and spatially- resolved opioidergic transmission in the NAc during motivated behaviors using fiber photometry. First, we validated the use of this sensor *in vivo* by measuring its response to an agonist (U50,488). Then, we measured the temporal dynamics of dynorphin transients during appetitive/aversive associative learning.

Our results revealed that, in an initial phase of Pavlovian learning when the cue is still not predictive of reward delivery, animals present an increase in dynorphin signal to the cue, but when learning is established, the opposite is seen, and we observed a decrease in the dynorphin signal to the cue (day 7). In the case of the reward consumption, decreased dynorphin signals are observed in the initial phase, becoming more prominent throughout learning. Concerning the aversive associative learning, we observed that, at cue onset, the dynorphin signal increases, but after shock delivery, a slight drop is observed.

These findings indicate that dynorphin is dynamically released during associative learning tasks, highlighting the need for further studies on its role in motivated behavior.

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The role of astrocytic metabotropic glutamate receptor 5 in social behavior

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Introduction
Astrocytes were seen for a long time as supportive cells in the central nervous system. However, evidence points to the pivotal role of these cells in brain function, including the modulation of synaptic transmission. Astrocytes express neurotransmitter receptors at the cellular membrane, whose activation may cause somatic calcium elevations and consequently release signaling molecules in the synaptic cleft. The impact of astrocytic signaling in the modulation of neuronal circuits and behavior is still poorly understood. Social behavior results from a complex interaction between brain regions. It is promoted by a variety of sensory cues, modulated by social internal states such as motivation, emotion, reward, or memory, and lastly affected by decision-making integration. Cortico-limbic regions in the brain are responsible for the integration between cognition and emotion and are crucial for the behavioral output as social behaviors. In those regions where glutamate is the primary fast excitatory neurotransmitter, astrocytes express glutamate receptors, such as the metabotropic glutamate receptor 5 (mGluR5).
Methods
In this work, we generated a mouse line lacking the mGluR5 in astrocytes and tested social behavior in these mice in an optimized three-chamber sociability and social novelty test.
Results
The astrocytic mGluR5 levels modulation promoted a modulation of sociability and social memory.
Conclusions
This work provided further information on the involvement of astrocytes in different forms of behavior.

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Untangling Motor Control Dynamics: A Neural Manifold-Based Analysis of Cortical Activity During Human Movement

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Introduction: Motor control is governed by complex cortical dynamics that remain poorly integrated across theoretical perspectives. Representational models highlight direct neural encoding of motor variables, while dynamical systems emphasize population-level activity trajectories. This study seeks to reconcile these approaches through neural manifold analysis of electroencephalographic (EEG) data acquired during goal-directed movement.

Methods: A methodological validation phase will precede data collection to test EEG acquisition, preprocessing, and analysis pipelines. Participants (n = 15–20) will perform two motor tasks—a simple reaching action and a more complex object manipulation task—while 32-channel EEG is recorded. Movements will be self-paced following visual cues, with synchronized video recording for movement phase segmentation (preparatory, initial execution, divergent execution). EEG preprocessing will involve filtering, artifact rejection (ICA), and segmentation. Dimensionality reduction techniques—Principal Component Analysis (PCA), Uniform Manifold Approximation and Projection (UMAP), and Independent Component Analysis (ICA)—will be applied to extract structured cortical activity patterns. Frequency-band (theta to gamma) and topographic analyses will aid in interpreting component relevance.

Results: The analysis will primarily explore how neural dimensionality varies across task complexity and movement phases within individuals. Cross-subject comparisons will be used to examine generalizability. PCA will quantify global variance, UMAP will reveal nonlinear cluster structures, and ICA will identify potential source-level contributions. Interpretations will focus on how emergent low-dimensional structures reflect coordinated cortical strategies, rather than isolated variable encoding.

Conclusion: This exploratory study aims to identify recurring patterns in EEG-based neural manifolds during motor behavior, contributing to a more unified framework of motor control. By linking cortical dimensionality to task demands, it may offer new insights for neurorehabilitation, brain-machine interface development, and the theoretical refinement of motor control models.

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Dynamic representation of second-order association in nucleus accumbens D1- and D2-medium spiny neurons

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The nucleus accumbens (NAc) is a central hub for integrating motivationally relevant information and shaping adaptive behavior. While its role in first-order appetitive and aversive learning is well established, how specific neuronal populations contribute to higher-order associations remains unclear. Here, we used single-cell calcium imaging in freely moving mice to monitor the activity of dopamine receptor D1- and D2-expressing medium spiny neurons (MSNs) in the NAc medial shell during a discriminative second-order fear conditioning task.

In this multi-day paradigm, mice first learned a first-order association between an auditory cue (CS_1) and a foot-shock. During the second-order conditioning phase, a novel cue (CS_2) was repeatedly paired with CS_1 in the absence of reinforcement. A third cue (CS_m), never associated with an aversive outcome, served as a control across all sessions. Retrieval and extinction sessions tested the persistence and flexibility of these learned associations.

Our results reveal that mice freeze in response to both CS_1 and CS_2 following second-order conditioning, indicating successful acquisition of the CS_2–CS_1 association. Freezing levels to CS_2 were comparable to those elicited by CS_1 confirming that animals generalized fear to CS_2 via its predictive relationship with the aversive CS_1.

When looking at D1- and D2-MSNs activity, we observed that both populations encode second-order associations and aversive outcomes, but with distinct temporal and functional dynamics. D1-MSNs were preferentially recruited during the acquisition of the CS_2–CS_1 association, suggesting a role in linking neutral cues to predictive value. In contrast, D2-MSNs exhibited stronger responses to the directly conditioned CS_1, consistent with a role in encoding aversive predictions.

These findings demonstrate that D1- and D2-MSNs contribute differentially to higher-order associative learning, extending their established roles in direct stimulus-outcome encoding. By revealing how inferred threat value is dynamically represented in the NAc, this work offers new insight into the neural basis of complex associative learning and fear generalization.

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Low-calorie sweeteners impact yeast chronological lifespan

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Introduction. Low- and no-calorie sweeteners have been increasingly incorporated into foods, beverages, medicines, and personal care products to provide sweetness while reducing the health risks associated with added sugars. The Food and Drug Administration (FDA) and the European Food Safety Authority (EFSA) have approved the consumption of several low-calorie sweeteners, including steviol glycosides (rebaudioside A) from the leaves of *Stevia rebaudiana* as a natural sweetener, and aspartame and acesulfame potassium (currently under safety review by EFSA) as chemically synthesized or artificial sweeteners. However, their long-term health effects, particularly on aging, remain unclear. This study aimed to investigate the effects of these sweeteners on cellular aging, using *Saccharomyces cerevisiae* as a model organism.
Methods. Chronological lifespan (CLS) assay and growth curves were performed on wild-type *S. cerevisiae* BY4741 and the isogenic mutant strains *hog1*Δ and *sch9*Δ. Cells were cultured in media supplemented with glucose or one of the selected sweeteners (acesulfame potassium, aspartame or rebaudioside A). Osmolarity was assessed, and reactive oxygen species (ROS) accumulation, mainly superoxide anion and hydrogen peroxide, were evaluated by flow cytometry.
Results. Acesulfame potassium increased medium osmolarity and ROS accumulation, extended CLS and promoted growth, except in *hog1*Δ cells. Aspartame reduced osmolarity, enhanced growth and longevity. Rebaudioside A decreased osmolarity, impaired growth; however, extended CLS in wild-type and *sch9*Δ cells, despite increasing ROS levels. In *hog1*Δ cells, however, it increased ROS accumulation, except hydrogen peroxide on the tenth day of CLS, when it caused a negative effect on longevity.
Conclusions. These sweeteners may have differing effects on cellular aging pathways and stress responses in yeast through distinct signalling pathways. Acesulfame and rebaudioside A appear to affect HOG1 mediated osmotic and oxidative stress responses, while SCH9 deletions reveals its impact on TORC1 associated longevity pathways. Given their widespread use in human diet, more research is needed to elucidate their long-term effects.

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Dynamics of cholinergic projections from Laterodorsal Tegmentum to the Nucleus Accumbens in associative learning

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To survive, animals must form new associations between initially neutral cues and specific outcomes, to anticipate critical events and manage resources. The Nucleus Accumbens (NAc), a crucial component of the brain's reward circuitry plays an important role in encoding these associations. Studies have reported that NAc neuronal activity is regulated by dopamine but is also influenced by cholinergic signals. Considering that the NAc receives cholinergic inputs from the Laterodorsal Tegmentum (LDT), this study aims to investigate the role and dynamics of LDT-to-NAc projections in modulating associative learning.

We first optogenetically inhibited LDT cholinergic neurons during distinct behavioral phases while simultaneously recording acetylcholine (ACh) dynamics in the NAc. To this end, we expressed the inhibitory opsin NpHR in the LDT of ChAT-Cre mice and a green fluorescent ACh sensor (gACh3.0) in the NAc. This approach allows to inhibit LDT cholinergic cells and evaluate the impact in cholinergic levels in the NAc during behavior.

To complement this experiment, we used another approach to modulate acetylcholine release from the LDT in the NAc by expressing an inhibitory presynaptic GPCRs to suppress synaptic transmission – PPO. PPO was injected in the LDT and in the NAc we injected a red ACh sensor (rACh1.7).

Finally, to monitor the activity of LDT cholinergic neurons, we expressed a calcium indicator (jGCaMP8m) in all cholinergic cells and a retrograde calcium indicator (jRGECO1a) in LDT-NAc-projecting neurons, enabling real-time imaging of both neuronal populations during behavior.

We observed that across different behavioral tasks, both the optogenetic inhibition of LDT cholinergic neurons and presynaptic blockade of LDT cholinergic neurons altered ACh signaling dynamics in the NAc.

Overall, our results suggest that LDT cholinergic projections to the NAc modulate local cholinergic tone and may play a role in shaping associative learning.

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Unraveling the role of long non-coding RNAs in Plasmodium falciparum: Implications for malaria therapeutics

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The extensive transcriptional regulation of *Plasmodium falciparum* underlies its remarkable ability to adapt to diverse host environments and evade therapeutic interventions. However, these regulatory networks remain largely unexplored, limiting our ability to pave new avenues for antimalarial drug development. Long non-coding RNAs (lncRNAs), renowned for their role in transcriptional and post-transcriptional regulation in eukaryotes, have shown to play a central role in mediating drug resistance mechanisms in various cancers. The similarity between these resistance mechanisms and those of the deadly malaria parasite suggests that lncRNAs may underscore antimalarial resistance as well. This project aims to elucidate the role of lncRNAs in modulating drug response and resistance mechanisms in *P. falciparum.* First, through a *de novo* identification of lncRNAs differentially expressed under stress conditions, such as exposure to relevant antimalarials. Secondly, through loss of function using antisense oligonucleotides, custom designed for each candidate lncRNA. Functional assays will follow, namely ChIP-Seq, RNA-seq and ring-stage survival assays which will aim to explore chromatin states, transcriptomic profiles and drug susceptibilities of treated parasites, respectively, allowing for a complete characterization of candidate lncRNAs. The possibility of targeting these non-coding elements may not only pose a novel therapeutic strategy, but also allow for the disruption of existing resistance pathways when combined with existing antimalarials, prolonging the efficacy of current treatment regimens.

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Compound Library Screening for Neural Repair: Finding New Drugs for Old Problems

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Traumatic central nervous system (CNS) injuries, including traumatic brain injury (TBI) and spinal cord injury (SCI), lead to severe and often irreversible physical, cognitive, and psychological impairments. Despite ongoing research, no effective therapeutic strategies have been developed to address the complex pathophysiological and inhibitory microenvironments that restrain neuroplasticity and tissue repair. In this study, we aimed to explore novel therapeutic approaches by screening a recently synthesized compound library for its neural repair capabilities.

This library was screened in cortex and spinal cord cell cultures derived from rats, employing the MTS assay to identify compound-induced metabolic alterations. This initial screening led to the identification of seven promising compounds (ST1059, ST1055, ST673, ST656, ST149, ST666, and ST18). Further characterization using immunocytochemistry revealed that three of these compounds promoted enhanced oligodendrocyte branching, suggesting possible potentiation of remyelination. Additionally, three compounds were able to induce significant increases in microglia area which can indicate possible microglial activation.

Molecular analysis of our cultures did not reveal significant changes in the mRNA expression levels of genes associated with oligodendrocyte maturation, though there was a trend towards increased expression of oligodendrocyte progenitor markers. Analysis of the Rho/ROCK signaling pathway, known to play an important role in CNS injury and repair, did not show alterations in RhoA mRNA expression, however, one compound significantly upregulated ROCK1 mRNA expression.

In summary, this study identified three compounds that promote oligodendrocyte branching, which may enhance myelination and provide structural and functional support to neurons. These findings suggest that these compounds may possess neuroregenerative properties, however more studies are needed to understand their real therapeutic value.

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The ACOD1-Itaconate axis is detrimental for antifungal immunity against the fungal pathogen *Aspergillus fumigatus,* promoting host susceptibility to invasive pulmonary aspergillosis

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Introduction: Recent progress in medical care has, paradoxically, contributed to an increasing prevalence of severe fungal infections in immunocompromised hosts, such as invasive pulmonary aspergillosis (IPA). Treatment is often unsuccessful, but our limited understanding of the molecular mechanisms leading to IPA has hampered the rational design of new therapies. The reprogramming of cellular metabolism is a fundamental mechanism whereby immune cells respond to infection. How these processes are coordinated and crosstalk during fungal infection remains to be elucidated. Upon infection, activated myeloid cells upregulate aconitate decarboxylase 1 (ACOD1) to produce itaconate, a metabolite known for its several immunoregulatory and antimicrobial properties. In this study, we proposed to elucidate the contribution of the ACOD1/Itaconate axis to host innate defense against *A. fumigatus*, contributing to the dissection of novel metabolic circuits involved in the pathogenesis of IPA.
Methods: WT and *Acod1*$^{-/-}$ mice were infected with *A. fumigatus* and 3 days post-infection, experimental aspergillosis progression and cell effector functions were evaluated. Bone-marrow derived macrophages (BMDMs) were isolated and infected with *A. fumigatus in vitro* for further characterization and functional analysis evaluation.
Results: BMDMs from *Acod1*$^{-/-}$ mice displayed enhanced ability to phagocytose and eliminate fungal conidia compared to WT macrophages. *Acod1*$^{-/-}$ macrophages also exhibited an increased production of reactive oxygen species (ROS), which we propose to be associated with the enhanced fungal clearance observed in itaconate-deficient macrophages. Accordingly, *in vivo* analysis showed that *Acod1*$^{-/-}$ mice exhibit enhanced capacity for fungal killing and a faster rate of infection resolution. The exact mechanisms through which itaconate promote susceptibility are still not fully understood. Currently, we are exploring the underlying mechanisms of its deleterious effects by depicting its putative role in modulating ROS and lipid metabolism and determining the possible crosstalk between these mechanisms.
Conclusions: Our results highlight a detrimental role of the ACOD1/itaconate axis in antifungal immunity, shedding light on a possible new mechanism involved in IPA pathogenesis.

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HLA-A2-Restricted CD8+ T Cells specific to Epstein-Barr Virus in Multiple Sclerosis patients: evaluation of function and T Cell Receptor Repertoire

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Multiple Sclerosis (MS) is a chronic autoimmune disease that disrupts the central nervous system. Epstein-Barr virus (EBV) infection has emerged as a leading candidate in MS development, particularly in genetically predisposed individuals. MS patients often exhibit reduced control over EBV infection, contributing to disease progression and relapses, highlighting the need for effective immune control. Human Leukocyte Antigen (HLA) molecules, particularly HLA-A2, play a critical role in antiviral responses by presenting EBV-derived peptides to CD8+ T cells, enhancing their recognition and activation. The presence of HLA-A2 is associated with a lower risk of MS, suggesting a protective effect. However, MS patients display reduced CD8+ T cell levels, indicating impaired viral control. This study aims to analyse EBV-specific CD8+ T cells restricted to HLA-A2 regarding their T cell receptor (TCR) repertoire and function in MS patients and healthy controls. Identifying EBV-specific TCRs could be relevant for EBV control and enable targeted therapies.

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Sleep and MCH-neuron impact on microglia-Aβ plaque dynamics

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Background: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of amyloid-β (Aβ) plaques and disruption of brain homeostasis, particularly affecting memory and sleep. Sleep disturbances are a hallmark of early AD and are tightly linked to the regulation of neuronal and immune functions. The melanin concentrating hormone (MCH) system, involved in sleep regulation and hippocampal memory processing, has recently been shown to be impaired in the early stages of AD. MCH neurons, located in the lateral hypothalamic area, modulate sleep biology and hippocampal activity. In parallel, microglia plays a central role in maintaining neuronal homeostasis by remodeling synapses and clearing Aβ plaques, processes influenced by sleep-wake states.

We hypothesized that the MCH system, through its regulation of sleep and neuronal homeostasis, modulates microglial activity, affecting synaptic pruning and Aβ phagocytosis.

Methods: To investigate this, we used App^{NL-G-F} and App^{NL-G-F}.$Pmch^{cre}$ mice and assessed microglia-plaque interactions across the circadian cycle (ZT0, ZT6, ZT6 with sleep deprivation [ZT6SD], ZT10, and ZT10 with rebound sleep [ZT10RB]). Additionally, manipulation of the MCH system was achieved using chemogenetics and 3 experimental groups were generated: Cherry (control group), Gi (MCH inhibition) and Gq (MCH activation). Immunohistochemistry for Iba1 and Aβ was performed to assess microglial interactions with plaques.

Results: Our results revealed circadian variations in microglia-Aβ interactions. Notably, sleep disturbances increased Aβ plaque size, suggesting a complex relationship between sleep and Alzheimer's disease. Activation or inhibition of the MCH neurons during 4 days was not sufficient to have an impact on these parameters.

These findings underscore the importance of sleep in regulating microglial function and Aβ pathology in AD. While brief MCH modulation was insufficient to alter microglial behavior, further studies are needed to explore the impact of prolonged MCH manipulation in AD.

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AMP-activated protein kinase deficiency confers resistance to aspergillosis by modulating macrophage effector functions

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Introduction: Invasive pulmonary aspergillosis (IPA), a life-threatening fungal infection mainly caused by the opportunistic fungus *Aspergillus fumigatus*, represents the most severe form of pulmonary aspergillosis. It is estimated that more than two million individuals develop IPA annually, with a crude mortality rate of approximately 85%. This epidemiology is mainly attributed to the increased clinical use of cancer chemotherapy and immunosuppressive therapies, resulting in an expanding population of patients at-risk. AMP-activated protein kinase (AMPK) has been shown to play a pivotal role in regulating cellular energy homeostasis and multiple aspects of cell metabolism. AMPK is central for the metabolic reprogramming of macrophages, and it has been shown to regulate their inflammatory phenotypes. However, the function of this regulatory mechanism in the immune response to *A. fumigatus* infection remains to be elucidated.
Methods: In this study, we employed an *in vivo* infection model in WT and *Ampkα1*$^{flox/flox}$*LysM Cre*$^{+/-}$(*Ampk*$^{-/-}$) mice. On day 1 and day 3 after infection, mice were euthanized and the progression of the disease, immune effector functions, as well as leukocyte recruitment to the site of inflammation were assessed.
Results: Our data demonstrate that following experimental fungal infection, mice with a myeloid cell- specific deletion of AMPK (*Ampk*$^{-/-}$) exhibit a reduced fungal burden in the lung, along with diminished inflammatory cell recruitment and cytokine production. Nevertheless, although no significant differences were observed in the production of reactive oxygen species between the two genotypes, iNOS production by alveolar macrophages was significantly increased. Upon iNOS inhibition, the effector functions of immune cells were impaired, increasing the severity of infection.
Conclusions: Collectively, our findings underscore the importance of AMPK in immunity against *A. fumigatus* and warrant future studies aimed at elucidating the molecular mechanisms driving this phenotype.

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Decoding the Functional and Transcriptomic Effects of Stem Cell Secretome: insights from *Caenorhabditis elegans* and Immunocompetent Human-Derived Assembloids for Parkinson's Disease

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to dopamine deficiency and α-synuclein aggregation. Current treatments provide symptomatic relief but do not halt disease progression, highlighting the need for innovative, disease-modifying therapies. Mesenchymal stem cell (MSC)-based therapies have emerged as a promising alternative due to their neuroprotective and immunomodulatory properties. Rather than relying solely on cell replacement, recent evidence points to the therapeutic potential of the MSC-derived secretome—a collection of bioactive molecules capable of modulating inflammation, oxidative stress, and cellular homeostasis.

Our research focuses on exploring the therapeutic potential of MSC-derived secretome in *Caenorhabditis elegans* (C. elegans) models. *C. elegans* offers a powerful, genetically tractable system to study neurodegeneration *in vivo*, enabling rapid, cost-effective, and high-throughput assessments of candidate therapies. Using transgenic *C. elegans* strains expressing human α- synuclein, we demonstrate that MSCs secretome reduces α-synuclein aggregation and prevents dopaminergic degeneration. Moreover, our studies reveal that the secretome modulates conserved oxidative stress response pathways in *C. elegans*, providing insights into its mechanistic role in neuroprotection.

To complement and translate these findings into human-relevant models, we aim to develop immunocompetent midbrain-striatum assembloids (PD-IMSAs) incorporating microglia. These 3D organoid-based models will enable the study of complex human PD pathology, including neuroinflammation, and will be generated using both toxin-induced and genetic approaches to mimic both oxidative stress lesions and a-synuclein pathology. RNA sequencing will be employed to dissect the molecular effects of secretome treatment, identify therapeutic targets, and uncover biomarkers of response. By integrating *C. elegans* and human assembloids models, our study bridges invertebrate and human systems to comprehensively investigate MSC-derived secretome therapy for PD, advancing the search for effective, disease-modifying treatments.

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The endolysosomal pathway in Alzheimer's disease: the interplay between Amyloid Precursor Protein processing and lipid metabolism

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Alzheimer's disease (AD) is the most common cause of dementia, characterized by the accumulation of extracellular amyloid plaques that cause neurotoxicity1. Amyloid plaques are built upon Amyloid Precursor Protein (APP) cleavage in the endolysosomal pathway by b- and g-secretases, generating toxic APP C-terminal fragments (b-CTFs) and Ab fragments. In multifactorial sporadic AD, Ab production is altered without genetic mutations. APOE4 variant is the best described genetic risk factor of AD sporadic form, and is involved in the ineffective clearance of Aβ. In this project we hypothesize that the underlying cause of altered APP fragment production and clearance lies in the endolysosomal pathway, which affects trafficking of APP fragments, secretases, or enzyme processivity. We aim to understand the precise mechanisms causing toxicity by blocking the production of the phosphatidylinositol 3-phosphate (PI3P), a glycerophospholipid involved in endosomal sorting, that is deficient in the brains of AD patients and mouse models, and analyze trafficking and processing alterations. Previous studies showed that PI3P deficiency recapitulates endolysosomal defects observed in AD, increases b-CTFs levels, and affects lipid metabolism. Preliminary results confirmed an increase in the levels of the autophagy adaptor p62 and in the fluorescence of p62-positive structures, as well as increased GSK-3b levels, a kinase linked to Rab5 overactivation and endocytic dysfunction, that contributes to tau hyperphosphorylation and that has been linked to sporadic and familial AD. Additionally, despite the accumulation of b-CTFs, we found an increase in the levels of nicastrin and presinilin-1, important subunits of g-secretase, suggesting possible mislocalization of the proteins within the endolysosomal system, or alterations in its environment that may affect processivity. We are currently investigating alterations in the lipid composition and pH within the endolysosomal system. With this work, we hope to find a target within the endolysosomal pathway to prevent the detrimental effects observed in AD.

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A whole-brain and spinal cord approach to study Machado-Joseph Disease: 3D analysis of neuropathological hallmarks in mouse and human tissues

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Machado-Joseph Disease (MJD) is caused by a polyglutamine expansion in the ataxin-3 (ATXN3) protein, leading to its misfolding and aggregation, causing neurodegeneration in the central nervous system. Traditional immunohistochemical methods have limited our understanding of the origin, progression, role and interactions of MJD neuropathological hallmarks (NHs) within their complex 3D micro-environment. Here, we propose a 3D analysis of MJD NHs in archival human brains and transgenic mice.

Employing targeted molecular labelling with tissue clearing techniques and high-resolution microscopy (confocal and light-sheet microscopy), we will analyse entire spinal cord and whole-brain ataxin-3 aggregation, along with tau, alpha-synuclein and TDP-43 proteins, as well as neuronal demise, axonal dystrophy and vasculature, using specific markers. Volume imaging, combined with automated detection and mapping, will allow rapid quantification of NHs throughout the entire brain and spinal cord. The optical tissue clearing protocol is currently being optimized to achieve refractive index-matched spinal cord and brain tissue blocks. Simultaneously, the multiplexed immunofluorescence technique is being refined for specific antibodies (namely for ATXN3) to determine the optimal imaging conditions for 3D reconstruction and data analysis, including single-cell counting, signal intensity assessment and morphometric evaluation.

The ability to visualize ATXN3 aggregates in 3D, especially in the context of their micro-environment, may yield new medical insights with significant therapeutic implications for MJD.

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Extracellular Vesicle miRNA Signatures in Fabry Disease

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Introduction. Anderson-Fabry disease is a rare lysosomal storage disorder characterized by a progressive accumulation of globotriaosylceramide (Gb-3). This toxin buildup disrupts the endosomal-lysosomal system, leading to cell death and organ dysfunction. However, the initial symptoms are often non-specific, complicating early diagnosis. This study investigated micro RNAs (miRNAs) derived from plasma extracellular vesicle (pEV) as potential biomarkers for Fabry Disease (FD) progression across asymptomatic, cardiac, and renal clinical forms.

Methods. Peripheral blood samples were collected from 40 healthy controls and 60 FD patients carrying a pathogenic mutation in the GLA gene. Patients were stratified into three groups: asymptomatic (n=20), with cardiac manifestations (n=30), and with renal manifestations (n=10). pEVs were isolated from plasma using size exclusion chromatography (SEC), and miRNAs were extracted for small RNA sequencing.

Results. A total of 69 differentially expressed transcripts were identified. The majority were downregulated in asymptomatic (88.4%) and cardiac (88.6%) patients, suggesting underlying repressive molecular mechanisms. In contrast, only 8.6% of transcripts were downregulated in renal FD, indicating a more active and distinct transcriptional profile. Functional analysis revealed dysregulation of protein-binding pathways in asymptomatic patients, immune-related pathways in cardiac FD, and cytoplasmic processes in renal FD. Among the 382 differentially expressed miRNAs, MIR375 and MIR124-1 were significantly downregulated.

Conclusions. Profile of pEV miRNA could become a minimally invasive strategy for early FD detection and differentially expressed miRNAs showed potential to be worthwhile biomarkers of FD progression to different clinical manifestations.

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Disclosing the benefits of a marine macroalgae dietary supplementation to the fish (*Diplodus sargus*) brain: a morphometric approach

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Introduction. Marine algae are an important source of biologically active metabolites, which have been studied for their health-promoting properties. However, despite their numerous advantages, the potential benefits of marine algae supplementation in fish health remain to be understood. The optic tectum is a brain region described to be particularly vulnerable to inorganic mercury (iHg) toxicity, and the main goal of this study was to explore the protection provided by marine microalgae to iHg toxicity in the white seabream (*Diplodus sargus*).

Methods. Fish were fed with a marine macroalgae-enriched feed (total incorporation of 5%, with *Ulva rigida*, *Fucus vesiculosus* and *Gracilaria gracilis* in equal proportions) for three months. Control animals were fed with a standard diet. Then, the animals were exposed to waterborne iHg (2 μg/L) for 7 days, followed by 14 days post-exposure recovery. Brain tissue was collected at three timepoints: before exposure to iHg (T0), after 7 days of exposure (E7) and at the end of the recovery period (PE14). Through a stereological approach, we performed a morphometric analysis of the optic tectum and respective layers, to quantify changes in volume, cell density, as well as the number of neuronal and glial cells.

Results. The stereological analysis of the optic tectum is currently in progress. Preliminary data from the pre-exposure group (T0), established baseline measurements and suggests a macroalgae-enriched diet did not influence the optic tectum structural and cellular composition. The ongoing analysis of E7 and PE14 groups will allow the assessment of the potential neuroprotective effects of mare marine macroalgae supplementation against iHg-induced toxicity, but initial data points to a significant impact of iHg on glial cell density.

Conclusions. Overall, this work has the potential to unveil novel applications of marine algae in aquaculture, improving fish health and resilience to environmental toxins.

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Towards whole-heart cardiac function quantification from 2D echocardiographic sequences

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Introduction: Echocardiography is the primary imaging modality for cardiac morphology and function evaluation in clinical practice. Its viability for whole-heart three-dimensional assessment -essential for early detection of cardiac conditions and treatment planning- is, however, limited by its 2D nature, variable image quality, and operator dependence. The advent of 3D ultrasound technology, despite promising, has not achieved widespread use due to the necessity for specialized, higher-cost equipment, technical and operational challenges, and inferior image quality. Despite notable advancements, there is still no solution capable of achieving accurate 3D whole-heart quantification through echo without sacrificing accessibility, temporal resolution, or requiring modifications to existing hardware, clinical workflows, or training. This PhD project proposes the development of a novel framework for whole-heart three-dimensional analysis from standard 2D+t echocardiographic sequences. Central to this effort is the need for a large, high-quality dataset of synchronized multi-view echocardiographic sequences with corresponding 3D pose and anatomical labels. Acquiring such data clinically is challenging due to logistical, ethical, and regulatory constraints.

Methods: To address this, we propose generating a synthetic echocardiographic dataset by combining dynamic whole-heart models, ultrasound simulation, and diffusion-based unpaired image translation with video- level consistency. This approach aims to produce anatomically accurate and temporally consistent ultrasound sequences that replicate realistic speckle patterns and imaging artifacts, while also providing all necessary labels for training/validating the target modules.

Results: We evaluated our method on publicly available apical 4-chamber images. Preliminary results indicate that the diffusion-based model can produce anatomically plausible and visually realistic synthetic echocardiographic images, demonstrating its potential for data augmentation and algorithm training.

Conclusion: In conclusion, we introduce a novel pipeline for generating synthetic echocardiographic data that supports the development of machine learning algorithms for 3D whole-heart reconstruction. Future work will focus on extending the framework to multiple cardiac views and full video sequences.

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mTOR Inhibition mitigates stress-Induced Tau pathology and synaptic dysfunction in a Tauopathy mouse model

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Tauopathies are a group of neurodegenerative disorders characterized by the abnormal accumulation of tau protein in the brain, with Alzheimer's disease (AD) being the most prevalent. Chronic stress and elevated glucocorticoid levels have been associated with disease progression and predisposition, as they trigger Tau hyperphosphorylation, leading to its accumulation and formation of neurotoxic Tau aggregates, with the dysregulation of autophagy being described to be an important contributor to stress-related Tau accumulation. Furthermore, autophagic dysfunction in synapses has been shown to be involved in synaptic failure, which is strongly correlated with cognitive decline. This project aimed to focus on autophagy and mTOR-related pathways, clarifying how they can regulate synaptic function through Tau pathology modulation, while understanding mTOR modulation therapeutic potential for stress-induced neuronal dysfunction and spine morphology.

We used CCI-779, an mTOR inhibitor, during 5 weeks along chronic stress protocol in P301L-Tg mice and wild-type littermates, followed by behavioural, molecular and neurostructural analysis. While we observe that CCI-779 induction leads to mTOR inhibition and autophagy modulation in the hippocampus of WT mice, our behaviour data suggests that mTOR inhibition has an anxiolytic effect and improves the cognitive function on stressed P301L-Tg mice; while presenting improvements in depressive-like behaviour in WT. We also observed that autophagy induction during chronic stress, was protective for both neuronal structure and spine density of both P301L and WT DG neurons. Additionally, hippocampal proteomic analysis shows changes in proteins in synaptic-related pathways and, importantly, altered mTOR and autophagy pathways, biological processes, and molecular components. We are now studying the effect of mTOR inhibition at the synaptic levels, studying autophagy markers at neuronal synaptosomes.

So far, our results highlight the importance of mTOR signalling for the interplay of chronic stress, autophagy dysfunction, and tau aggregation, in the preservation of synaptic integrity, representing a potential therapeutic approach for tauopathies and other Tau-related brain disorders.

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Itaconate is a key regulator of immune cell metabolic activation during granuloma formation

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Introduction. Sarcoidosis is a multiorgan disease of unknown etiology characterized by the formation of non-caseating granulomas, predominantly in the lungs. While the histological landscape of granulomas in sarcoidosis is well understood, the factors that trigger cell aggregation and initiate/sustain granulomatous inflammation remain unclear at the genetic, molecular and inflammatory levels. The aconitate decarboxylase 1 (*Acod1*) gene encodes an enzyme that is highly responsive to pro-inflammatory stimuli. Acod1 converts cis- aconitate, a tricarboxylic acid (TCA) cycle intermediate, to itaconate, which plays a critical role in the regulation of inflammatory responses. Therefore, we developed an integrative approach to investigate its immunometabolic role during granulomatous inflammation.

Methods. We took advantage of an *in vivo* model of granuloma formation on both WT and *Acod1*$^{-/-}$ mice, where mice were euthanized at day 2 and 4 after sodA-coated beads embolization. Moreover, we implemented an *in vitro* model of multinucleated giant cells (MGCs) formation from bone marrow precursors to assess their contribution to granuloma formation.

Results. Using the *in vivo* model, we identified itaconate as an important player in granuloma formation, as evidenced by the larger granuloma-like structures exhibited by *Acod1*$^{-/-}$ mice compared to their wild-type counterparts. Despite the larger granuloma area, *Acod1*$^{-/-}$ mice did not show increased immune cell recruitment or proliferation. We thus analyzed the cellular area of both WT and *Acod1*$^{-/-}$ granulomas, and demonstrated that *Acod1*-deficient cells occupied a larger area within them. Consequently, we postulated that itaconate-deficient macrophages might undergo epithelization and transformation into MGCs. In support of this, cytokeratin, an epithelial marker, was also increased in *Acod1*$^{-/-}$ mice. Furthermore, the IL- 4/JAK1/STAT6 axis, previously implicated in the polarization of M2-like macrophages and their transformation into MGCs was inhibited by itaconate in our experimental setting.

Conclusions. Collectively, these findings emphasize the pivotal role of itaconate in granuloma formation in sarcoidosis and lay the foundation for a novel immunometabolic-based intervention for the management of patients with sarcoidosis.

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Plasmodium falciparum Ubiquitin-proteasome system (UPS) modulating antimalarial resistance

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The emergence of drug resistance in malaria parasites remains a major obstacle to malaria control. While artemisinin resistance has been mainly linked to mutations in the *k13* gene, other components of the ubiquitin-proteasome system (UPS) have also been implicated in modulating parasite susceptibility. Artemisinin acts by inducing protein damage, which is managed by the UPS through ubiquitination and degradation via the 26S proteasome. Given its essential role in protein homeostasis, the UPS has become a promising drug target. Notably, proteasome inhibitors show synergy with artemisinin, supporting their potential use as partner drugs. Ubiquitin receptors, such as the RPN2 subunit of the 19S regulatory particle, are crucial in the recognition of damaged proteins.

Previously, we investigated the functional relevance of an E738K variant in the *rpn2* gene, previously identified in an artemisinin-resistant *Plasmodium chabaudi* line. Using *Plasmodium falciparum* as a model, we demonstrate that the 738K mutation confers resistance to dihydroartemisinin (DHA) by stabilizing proteasome activity during drug exposure. Herein, we want to understand how the *rpn2* mutation modulates ARTs response in *P. falciparum* parasites with a resistance genetic background and its implication towards other antimalarial susceptibility. First, we introduced both E738K variants through the Selection-Linked Integration technique into a *k13*-mutant background (C580Y), and performed drug susceptibility and proteasome function assays.

Interestingly, the resistance phenotype of rpn2^{738K} is partially reduced. Despite the increased survival of *k13*-mutant parasites, functional tests revealed no changes in the proteasome function, confirming that *k13* acts upstream of the proteasome. Additionally, we observed a pleiotropic effect of this mutation on the resistance to other antimalarial drugs, highlighting its broader implications in parasite drug resistance.

These findings offer new insights into the role of the UPS in antimalarial action and resistance in *Plasmodium* spp. This work underscores the therapeutic potential of targeting the UPS in malaria treatment strategies.

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Cholesterol homeostasis regulates myeloid cell function in response to fungal infection

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Introduction: Invasive pulmonary aspergillosis (IPA) is a severe infection that affects immunocompromised patients and is mainly caused by the opportunistic fungal pathogen *Aspergillus fumigatus*. Over 2 million people develop IPA each year with a crude mortality of approximately 85%, as the result of limited diagnostic and therapeutic options. The immunometabolic dynamics of immune cells play a crucial role in shaping immunity against various pathogens. Our research has revealed that macrophages rapidly adapt their metabolic programs upon *A. fumigatus* infection to sustain specialized effector functions. In particular, we found that macrophages exhibit a significant upregulation of lipid metabolism genes, namely cholesterol synthesis pathway genes.

Methods: To investigate the relevance of this pathway to antifungal immunity, we employed pharmacological interventions, using fluvastatin to inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR1), the rate-limiting enzyme of cholesterol synthesis, and zaragozic acid to block farnesyl-diphosphate farnesyltransferase 1 (FDFT1), a key enzyme in sterol and cholesterol synthesis.

Results: Our results revealed that fungal infection increased the intracellular levels of free cholesterol in macrophages, a result that supports the genetic data and highlights cholesterol synthesis as an important immunometabolic pathway in response to *A. fumigatus* infection. Inhibiting either HMGCR1 or FDFT1 enhanced the fungicidal activity of macrophages. At the molecular level, this phenotype was correlated with a negative effect of cholesterol synthesis in the maturation of functional phagolysosomes, ultimately influencing the control of fungal growth and susceptibility to infection. In addition, supplementing macrophages with exogenous cholesterol resulted in a decreased capacity of these cells to eliminate the fungus. Studies are ongoing to understand how cholesterol homeostasis, dynamics, and trafficking converge to modulate the antifungal immune response of macrophages.

Conclusions: In summary, our results put forward a new pathogenetic model for IPA, in which lipid metabolism, by regulating innate antifungal immunity, regulates susceptibility to fungal infection. This hypothesis highlights the manipulation of cholesterol homeostasis as a potential strategy for preventing or treating IPA.

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Therapeutic Failure in Uncomplicated Malaria in Luanda

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Introduction. Despite remarkable progress in recent years, malaria, primarily caused by Plasmodium falciparum (*P. falciparum*), remains a significant public health challenge. It continues to be one of the leading causes of morbidity and mortality, with a prevalence of 17% in Angola in 2024. The current treatment for uncomplicated *P. falciparum* malaria involves using artemisinin-based combination therapies (ACTs), with Angola adopting artemether-lumefantrine (AL) as a first-line therapy for uncomplicated *P. falciparum*. However, therapeutic efficacy trials have reported AL effectiveness below the 90% threshold set by WHO in several locations across the continent, including Angola. Artemisinin resistance and delayed parasite clearance (i.e., patients positive for parasitemia on day 3), has emerged as a serious concern and poses a growing threat to the effectiveness of ACTs. This development could reverse the substantial progress achieved in malaria control and elimination.

Methods. A clinical trial was carried out to evaluate the efficacy of AL in hospitalized patients in Luanda diagnosed with *P. falciparum* malaria, all presenting with parasitemia levels exceeding 1000 parasites/mm³ at the time of admission. A total of 120 subjects were enrolled in the study. At the baseline visit, biochemical analyses were performed, and demographic data were collected. During follow-up visits, blood samples were taken to assess parasitemia and gametocyte presence, posteriorly used to monitor treatment response, including early treatment failure and adequate clinical and parasitological response.

Results. This study assessed the prevalence of day 3 *P. falciparum* in patients with uncomplicated malaria treated with AL. A high prevalence of 15% of *P. falciparum* was found, reinforcing the urgency of regular surveillance of the effectiveness and efficacy of AL in the country.

Conclusions. This work aims to support and encourage future research on the urgency and importance of monitoring and updating information on anti-malarial drug efficacy, a critical factor in achieving malaria elimination goals by 2030.

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Long Pentraxin-3 reduces the interaction between Alveolar Epithelial Cells and *Aspergillus fumigatus*: Evidence from *In vivo* and *In vivo* Models

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Introduction. Fungal diseases cause approximately 3 million deaths annually. *Aspergillus fumigatus*, classified by the WHO as a critical fungal pathogen, triggers invasive pulmonary aspergillosis (IPA) with high mortality in immunocompromised individuals, highlighting the need for better therapies. Alveolar epithelial cells (AECs) are not mere barriers; they can engulf and destroy inhaled conidia. The surfactant-associated pattern-recognition molecule long pentraxin-3 (PTX3) is thought to strengthen this epithelial defense but the underlying mechanism remains unclear.
Methods. *In vitro*, *A. fumigatus* conidia were incubated with A549 and PC9 AEC-like cell lines with or without recombinant human PTX3. Adhesion, internalization and germination were quantified by ELISA and flow cytometry. *In vivo* (on going), wild-type (WT) and $Ptx3^{-/-}$ mice were intratracheally challenged with *Aspergillus fumigatus* conidia; lungs were harvested 4 h and 12 h post-infection. Epithelial fungal burden was measured by CFU counting. Immune and epithelial populations plus fungal internalization and viability were assessed by flow cytometry; pulmonary PTX3 concentration was determined by ELISA and its distribution examined histologically.
Results. Exogenous PTX3 markedly reduced AEC-conidial contact and intracellular germ-tube formation *in vitro*. Dexamethasone treatment did not affect AEC viability at any tested concentration. *In vivo*, AECs from WT mice internalized few conidia at either time point, while immune cells phagocytosed and killed efficiently *Aspergillus* conidia by 12 h.
Conclusions. PTX3 limits the earliest interaction between *A. fumigatus* and the alveolar epithelium, impeding fungal establishment before classical innate immunity is activated. In immunocompetent hosts, resident and recruited leukocytes eliminate most conidia before epithelial invasion. Ultimately our project could provide insights guiding surfactant-based prophylaxis and PTX3-boosting interventions against IPA.

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The Secretome of induced pluripotent stem cell derived mesenchymal stem cells cultured in normoxic and hypoxic environments under dynamic conditions induces dopaminergic differentiation and neurite outgrowth in SH-SY5Y Cells

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Introduction: Parkinson's disease (PD) is a chronic degenerative disease of the central nervous system that results from the death of dopamine neurons in the substantia nigra. In PD, oxidative stress plays a crucial role in the degeneration of dopaminergic neurons. The accumulation of reactive oxygen species leads to cellular damage, mitochondrial dysfunction, and inflammation[2]. It was demonstrated that the administration of induced pluripotent stem cell derived mesenchymal stem cells (iMSCs) secretome is able to promote significant motor function and histological improvements in in vivo models of PD.
Methods: This work aims to understand the effect of normoxia and hypoxia preconditioning on iMSs and to investigate the neuroprotective and neurogenic properties of the secretome of iMSCs cultured in spinner flasks biorreactors under normoxic or hypoxic conditions using the SH-SY5Y as an in vitro model for PD.
Results: Real-time RT-PCR results from iMSCs demonstrate that the expression of some genes that are related to protection against oxidative stress such as PARK7, HSP27, Thioredoxin, PRDX1, Cyclophilin A and PRI-miR-17-92 are upregulated in iMSCs cultured under hypoxic conditions. Furthermore, the expression of both the neuronal (NGF) and vascular endothelial (VEGF) growth factors were upregulated in iMSCs cultured under hypoxic conditions. Neurite area assessment demonstrated that both secretomes induced neurite outgrowth by SH-SY5Y cells. In particular for the neurodifferentiation assay, incubation with the secretome of iMSCs cultured under hypoxic conditions resulted in greater neurite outgrowth compared to all other groups. Furthermore, gene expression analysis showed an increase in the expression of genes related to differentiation towards neuronal phenotype in the groups treated with both iMSCs secretomes. Specifically, we were able to observe an greater increase in the expression of TH in SH-SY5Y cells treated with the secretome of iMSCs cultured under hypoxic conditions. Finally, after exposure to 6-OHDA, treatment with both secretomes promoted an increase in the expression of genes related neuron survival and protection against oxidative stress.
Conclusions: The results obtained demonstrate that the that secretome of both iMSCs cultured under normoxic or hypoxic conditions was capable of inducing neuronal differentiation of SH-SY5Y cells towards a dopaminergic phenotype and had a neuroprotective effect after chemical lesion with 6-OHDA.

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Breaking Barriers: A Multidisciplinary Therapeutic Approach for Spinal Cord Injury Repair

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Spinal cord injury (SCI) represents a dramatic neurological condition that leads to severe motor, sensory and social impairments. Currently, effective treatments remain elusive due to the complexity of SCI pathophysiology. After SCI there is a severe loss of neuronal and non-neural cells. Besides, the chronic stage of SCI is characterized by the formation of a glial scar, that hamper neuronal regeneration. In order to overcome this intricate injury, it is imperative to integrate different therapeutic approaches that can address multiple facets of SCI pathology simultaneously.

We will employ a multidisciplinary approach that combines cell and molecular therapies with a neuromodulatory approach aimed to repair the spinal cord. This approach will consist in transplantation of induced Neural Stem Cells (iNSCs) committed for spinal cord differentiation, capable of secret chondroitinase ABC (ChABC) using a dox-inducible transgene. Physical rehabilitation and neuroplasticity will be promoted using epidural electrical stimulation (EES). We aim to replace the neural cells lost after injury using iNSCs transplantation, increase their integration into the spinal cord tissue by degrading the glial scar with the ChABC, and finally restore synaptic connectivity, promoting relevant locomotor improvements using EES.

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Electrophysiological Characterization of Astrocytic mGluR5 Signaling in Corticolimbic Circuits: Insights from a Conditional Knockout Mouse Model

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Recent studies have elucidated the pivotal role of astrocytes in neural function, particularly in synaptic regulation, circuit dynamics, and behavioral outcomes, challenging the traditional paradigm of astrocytes as passive support cells. Astrocytes are now recognized as integral components of the tripartite synapse, where they actively participate in bidirectional communication with neurons, detecting, processing, and modulating synaptic signals. For instance, astrocytes have been shown to respond to glutamate, the predominant excitatory neurotransmitter in the central nervous system, via metabotropic glutamate receptor 5 (mGluR5) activation. This activation triggers intracellular calcium (Ca2+) signaling cascades in astrocytes, which subsequently influence synaptic function in cortico-limbic regions critical for cognitive processes.
Sardinha et al. (2017) demonstrated that astrocytic signaling, mediated by the dominant negative SNARE (dnSNARE) mouse model, plays a crucial role in hippocampal-prefrontal theta synchronization and cognitive function. However, the specific contributions of astrocyte-derived signaling in corticolimbic circuits, particularly those mediated by mGluR5, remain to unfold.
The present study aims to characterize a novel mouse model lacking astrocytic mGluR5. We will implement high-density electrophysiological recordings using 32-channel probes in the medial prefrontal cortex and dorsal hippocampus to elucidate the electrophysiological signature of astrocytic mGluR5-mediated signaling within these networks. This approach will provide insights into the functional impact of astrocytic mGluR5 on neural circuit dynamics and cognitive processes.

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Zinc transporter-3 as a novel target for fast and long-lasting antidepressant actions

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Major Depressive Disorder (MDD) remains one of the leading causes of disability worldwide, with a significant clinical challenge presented by treatment-resistant depression (TRD), affecting approximately 30% of patients. This substantial treatment gap underscores the urgent necessity for developing novel therapeutic interventions that offer rapid onset of action and sustained efficacy. Current pharmacological approaches typically require weeks to achieve therapeutic effects and are often accompanied by problematic side effects and limited long-term outcomes.

Zinc transporter-3 (ZnT3), encoded by the SLC30A3 gene, represents a promising yet relatively unexplored molecular target in depression research. This transporter plays a crucial role in regulating synaptic zinc levels, particularly at glutamatergic synapses in the hippocampus. ZnT3 is integrally involved in fundamental neurobiological processes disrupted in MDD, including synaptic plasticity, adult hippocampal neurogenesis, and the balance of zinc-glutamate signaling. Notably, genetic association studies have identified specific variants in SLC30A3, particularly the single-nucleotide polymorphism rs11126936, as significantly associated with increased risk for developing MDD.

Recent findings from our laboratory have revealed that both ZnT3 function and the activity of adult-born hippocampal neurons are essential components in mediating the sustained antidepressant effects of psilocybin. Therefore, this project aims to comprehensively validate ZnT3 expression specifically within adult-born neurons as a potential therapeutic target capable of inducing rapid and enduring antidepressant outcomes. We will systematically examine its role in neuroplastic and synaptic mechanisms using established animal models of depression. Our experimental approach will assess whether selective modulation of ZnT3 expression in adult- born neurons can elicit fast-acting and persistent antidepressant-like effects. By targeting this specific molecular mechanism, we anticipate developing novel therapeutic strategies for patients who remain unresponsive to conventional antidepressant treatments.

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Unlocking the neuro-regenerative potential of Adipose Stem Cell secretome: a novel target treatment for spinal cord injury

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Spinal cord injury (SCI) affects over 250,000 individuals annually, leading to lifelong disability and a significant societal burden. Despite decades of research, no effective clinical treatments exist. One of the most extensively explored interventions over the past 50 years has been stem cell- based therapy, with mesenchymal stem cells (MSCs) among the most promising candidates. Although technical limitations still hinder clinical translation, recent studies have revealed that the therapeutic effect of transplanted MSCs relies mainly on their potent paracrine activity. This finding has led to the exploration of the MSC secretome (bioactive molecules and exosomes) as a cell-free alternative for treating SCI.

So far, intravenous administration of the MSC secretome has shown functional improvements in animal models of SCI. However, this delivery route leads to the predominant accumulation of exosomes in the liver, spleen, and kidneys, with limited presence in the central nervous system (CNS). For that reason, we believe that further refinement of the therapy, consisting of the specific guidance of the secretome to the CNS after intravenous administration, is needed to fully exploit the potential of MSCs for neuronal-related outcomes.

We propose engineering MSC-derived exosomes to specifically target and be internalized by neuronal populations along the CNS using exosome surface modification with a CNS-targeting peptide from rabies virus glycoprotein (RVG). RVG is a 29-amino acid peptide that interacts with the acetylcholine receptor, enabling specific exosome uptake by neurons. RVG-mediated CNS targeting has already proven efficient and safe in a mouse model of Alzheimer's disease, but its therapeutic potential in SCI still remains unexplored. This innovative strategy aims to combine systemic immunomodulation with targeted neuronal regeneration to enhance motor and sensory recovery in preclinical SCI models. If successful, it could lead to minimally invasive, clinically translatable treatments for SCI and other neurodegenerative conditions.

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Baclofen based CSPG-targeted Nanotherapy as a Novel Approach for Spinal Cord Injury

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Spinal cord injury (SCI) induces a cascade of pro-inflammatory mechanisms that significantly contribute to functional deficits. A key component of this cascade of events is the excessive release of excitatory neurotransmitters, particularly glutamate, which triggers pathological calcium (Ca^{2+}) influx into neurons, leading to excitotoxicity and cell death. Evidence suggests that upregulation of GABAB receptors in the mammalian central nervous system (CNS) exerts neuroprotective effects post-injury by inhibiting voltage-dependent calcium channels, resulting in neuronal hyperpolarization and reduced glutamate release. This mechanism holds promise for limiting further neuronal damage following SCI.

Recent studies demonstrated that Baclofen, a GABAB receptor agonist, promotes both motor and non-motor functional recovery in animal models of SCI. Baclofen acts by attenuating excitatory neurotransmitter release, thereby reducing secondary damage and preventing neuronal loss at the lesion site. Moreover, emerging evidence indicates that Baclofen may modulate the immune response, as shown by a marked reduction in the inflammatory profile in SCI mice following treatment.

Despite these encouraging findings, systemic administration of Baclofen lacks specificity and may lead to off-target effects. To address this limitation, we aim to development a nanoparticle-based delivery system using biocompatible polymers such as PLLA and PDLG for the targeted release of Baclofen at the injury site, particularly in CSPG-enriched regions known to impede regeneration. This strategy has potential to enhance the neuroprotective potential of Baclofen while minimizing systemic exposure, thereby contributing to the advancement of targeted therapies for SCI.

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Alveolar macrophages modulate BCG-Induced protection against Mycobacterium tuberculosis

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Despite its widespread use, Bacille Calmette-Guérin (BCG) vaccine fails to consistently confer protection against pulmonary tuberculosis. Understanding the immune mechanisms limiting its effectiveness is therefore essential. Recent insights suggest that heterogeneity in the immune profile of lung-macrophages and the inflammatory microenvironment may shape the adaptive immune response contributing to the levels of protection conferred by BCG vaccination.

Herein, we aim to investigate the contribution of alveolar macrophages (AMs) to the differences in protection against *Mycobacterium tuberculosis* (Mtb) infection induced by BCG vaccination using two mouse strains with different susceptibility to Mtb infection: the resistant C57BL/6 (B6), and the susceptible C3HeB/FeJ (FeJ) strain. Mice were vaccinated with BCG for two months at which point they were challenged with Mtb through the aerosol route. Bacterial burdens and the profile of the immune response were analyzed at different time-points.

Our results show that BCG vaccination promotes early bacterial control in both B6 and FeJ mice. However, this control is delayed and short-lived in FeJ mice. Prior to Mtb challenge, we found that BCG vaccination enhances the activation of AMs in B6 mice to a higher extent than in FeJ mice. Following Mtb infection, this heightened activation state promoted an earlier dissemination of Mtb to other lung-myeloid cells, resulting in an early expression of adaptive immunity. Importantly, depleting AMs with clodronate liposomes further enhanced the Ag-specific recall response and bacterial control in both strain of mice. Our most recent data further suggest that BCG vaccination influences directly AM activation and the alveolar microenvironment.

Altogether, our data suggest that the response of AMs plays a pivotal role in the protection conferred by BCG vaccination. As depletion of AMs further enhances vaccine-induced control, including in susceptible hosts, understanding the mechanisms whereby AMs impact vaccine-induced protection will be crucial to improve upon BCG or develop novel vaccines.

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A Novel Therapy for Spinal Cord Injury Repair Based on Regenerative Macrophages Extracellular Vesicles

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In response to spinal cord injury (SCI), a persistent inflammatory reaction follows, driven by pro- inflammatory mediators secreted by local infiltrating cells. The microenvironment at SCI site favors predominant macrophage polarization towards a pro-inflammatory M1 phenotype, which is one of the reasons why macrophage transplantation has failed. Our research focuses on leveraging extracellular vesicles (EVs) from anti-inflammatory M2c macrophages to modify this harmful milieu and enhance neural repair. Preliminary data show that M2c-conditioned media promotes axonal growth in vitro, induces a pro-regenerative phenotype in vivo, and improves functional outcomes. However, the precise therapeutic components within the secretome remains unknown. EVs, in particular, have shown better therapeutic effects in several diseases, offering a promising, easily translatable therapy compared to the entire secretome. Our study aims to characterize M2c macrophage-derived EVs, optimize dosing and delivery methods, and elucidate the underlying mechanisms. Success could profoundly impact SCI treatment, offering new hope for recovery.

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Defining novel behavioral traits, brain proteomic signatures and new therapeutic opportunities in a clinically relevant mouse model of Rett Syndrome

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Introduction: Rett Syndrome (RTT) is an X-linked neurodevelopmental disorder caused by mutations in the gene encoding the methyl CpG-binding protein 2, a key regulator of gene transcription. Although it affects almost exclusively female patients, a thorough analysis of the phenotype and pathology of female mouse models of RTT is still missing. Likewise, therapies that can meaningfully improve patient's symptoms are not yet available. In this context, serotonergic dysfunction has emerged as a potential therapeutic route, found to be altered in both patients and animal models.

Methodology: Here, we performed an extensive longitudinal phenotypic characterization of the Mecp2$^{tm1.1Bird}$ heterozygous female mice, followed by proteomic studies on a brain region critical for the behavioral deficits observed, the prefrontal cortex (PFC). In different cohorts of animals, we evaluated the therapeutic potential of NLX-101, a highly selective post-synaptic 5-HT$_{1A}$R agonist, against the described behavioral impairments and breathing dysfunction present in this model.

Results: Our data indicates the presence of severe motor, cognitive and social memory deficits, along with alterations in animals' circadian profile. Proteomic analysis of the PFC uncovered alterations of several proteins related to neuronal signaling and synaptic function, including proteins related to excitatory (E) and inhibitory (I) signaling, pointing towards a possible pathophysiological role of E/I ratio underlying some of the behavioral deficits found. This was further confirmed by an increased expression of parvalbumin and vasoactive intestinal peptide-positive in this region. At the therapeutic level, our preclinical study revealed that targeting serotonergic signaling with NLX-101, largely corrects the breathing abnormalities and prevents the cognitive deficits present in these mice.

Conclusion: Overall, our studies demonstrate that Mecp2$^{tm1.1Bird}$ female mice mimic core clinical aspects of RTT and uncover several potential interventional targets, while providing compelling evidence on the therapeutic potential of targeting post-synaptic 5-HT$_{1A}$R to improve breathing and cognitive function in RTT.

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In Search for Depression Biomarkers: Unveiling the Impact of Adult-born Astrocytes Modulation in Extracellular Vesicles Signatures

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Depression is a prevalent neuropsychiatric disorder, featuring emotional and cognitive impairments, disproportionately affecting women at twice the rate of men. Major challenges include ineffective treatment responses and the absence of specific biomarkers for accurate diagnosis.

Opposed to earlier beliefs, the adult brain is a dynamic structure capable of remarkable plasticity, due to the generation of new neurons and glial cells in adulthood, a process known as neuro- and astrogliogenesis, respectively, which occurs predominantly in the dentate gyrus region of the hippocampus. Moving beyond neuron-centric paradigms, recent evidence highlights the critical role of astrocyte-neuron communication in regulating neurogenesis and its disruption in depressive states. Our laboratory demonstrated that antidepressant treatment enhances hippocampal astrogliogenesis and improves depressive-like behaviours. Furthermore, ablation of hippocampal adult-born astrocytes (hABAs) impairs mood, cognition and alters hippocampal electrophysiological signatures, underscoring their potential relevance in the pathophysiology of depression.

Extracellular vesicles (EVs) are nanoparticles secreted by most brain cells capable of carrying molecular cargo that can serve as disease biomarkers and have been associated with neuroinflammation and neurogenesis in major depressive disorder.

Using adult male and female Nestin-Cre mice, this project aims to elucidate the role of hABAs in depression pathophysiology and investigate sex-related differences through advanced optogenetic tools for hABAs activation or inhibition. Proteomic profiling of human and mouse plasma and cerebrospinal fluid samples from astrocyte-, neuron- and microglia-enriched EVs populations will be conducted. Afterwards, a comparative analysis of human and mouse datasets will be performed using AI-driven machine learning tools to identify 3-5 clinically relevant targets with potential as novel biomarkers for depression. These findings will support the development of a PET sensor for their non-invasive detection, paving the way for earlier diagnosis and personalized treatment strategies.

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Social cognition deficits in the J20 mouse model of Alzheimer's disease

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The hippocampal CA2 subregion plays important roles in regulating social behavior. It is required for the encoding, consolidation, and recall of social novelty recognition memory, which is essential to discriminate a novel from a familiar conspecific, and acts to promote social aggression. CA2 dysfunction has been implicated in a number of neurological and psychiatric disorders associated with abnormal social behavior, including epilepsy, schizophrenia, and different forms of dementia. Recent studies of the Tg2576 mouse genetic model of Alzheimer's disease (AD) reported a decrease in inhibitory synaptic transmission and synaptic plasticity that contributes to social memory deficits. Here we explore a second genetic model of AD, the J20 mouse line, to determine whether social memory deficits and altered CA2 function are a common feature of AD mouse models.

In our preliminary results, we found that male and female J20 mice show increased mobility in an open field test, in line with previously reported hyperactivity phenotype observed in these mice. Moreover, male and female J20 mice show relatively normal levels of sociability, the preference to explore a novel mouse inside a wire cup cage compared to an empty cup. We then subjected the mice to a CA2-dependent social novelty recognition task, in which a subject mouse explores an open arena containing a novel conspecific and a previously encountered conspecific confined to separate wire cup cages. Social memory is manifested by the preference of the subject to explore the novel mouse. Male J20 mice showed a significant impairment in this task. Strikingly, age-matched female J20 mice showed a normal social memory behavior.

In preliminary electrophysiological experiments in ex vivo hippocampal slices, we have so far failed to observe a significant difference in either intrinsic electrophysiological properties of CA2 pyramidal neurons or in the amplitude of excitatory or inhibitory synaptic potentials evoked by electrical stimulation of the hippocampal or cortical inputs to CA2 in J20 compared to wild-type mice. Future experiments will explore whether the deficit in social memory is associated with altered in vivo activity of CA2 neurons in this AD mouse model.

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Unravelling the Impact of Sleep-related MCH Neurons on the Molecular Substrates of Memory

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Sleep is essential for converting short-term memories into long-term ones. Melanin-concentrating hormone (MCH)-producing neurons – highly active during REM sleep – consolidate hippocampal memory engrams by refining synaptic connections. Our group found that MCH peptide decreases hippocampal neuronal firing rates and synaptic strengths, while regulating gene expression linked to neuronal excitability and synaptic plasticity; we propose this stabilises engrams by strengthening potentiated synapses and eliminating interfering signals. However, the precise synaptic changes remain unknown. MCH neurons are also implicated in Alzheimer's disease (AD), characterised by hippocampal-dependent memory impairment and sleep disturbances. Our previous findings identified early-stage MCH system dysfunction in an AD mouse model and patients' postmortem hippocampal tissue, highlighting the need to decode MCH's role in hippocampal function.

Therefore, this study aims to uncover MCH's role in memory processing under physiological and AD conditions by elucidating its impact on hippocampal synapse composition and morphology, and investigating its role in organising hippocampus-dependent memory engrams.

We will use unbiased proximity-dependent TurboID labelling to identify the pre- and postsynaptic proteome in WT mice across circadian phases, and following 6h sleep deprivation (SD). We will also assess MCH-induced synaptic modifications in chemogenetically-manipulated *Pmch*^{cre} mice, alongside spine density and morphology using immunofluorescence and Neurolucida software. Chronic MCH-neuron activation in *App*$^{NL\text{-}G\text{-}F}$(AD mouse model)×*Pmch*^{cre} mice will be assessed for its potential to rescue sleep defects (EEG/EMG), spine density alterations, and the MCH-induced synaptic proteome (immunofluorescence, RNAscope). Engrams will be labelled in TRAP2xAi14 mice performing novel object recognition (NOR) and context-dependent fear conditioning (CFC) via c-Fos immunostaining and tdTomato expression; mice will undergo chemogenetic MCH-neural activation during consolidation or 6h SD. Lastly, we will assess MCH-circuit activation's impact on NOR, CFC, and Barnes Maze performance in 9- month-old *App*$^{NL\text{-}G\text{-}F}$×*Pmch*^{cre} and *App*^{WT}×*Pmch*^{cre} mice.

By decoding MCH-driven hippocampal synaptic changes, this study advances memory research while exploring MCH's therapeutic potential for AD.

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The therapeutic potential of TUDCA in stress-related brain pathologies

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Tauroursodeoxycholic acid (TUDCA), an endogenous bile acid, exerts neuroprotective effects in neurodegenerative models by reducing oxidative stress, apoptosis, inflammation, and ER stress. Given its interaction with the Glucocorticoid Receptor and its modulation of stress-related pathways, this study explores TUDCA's potential to counteract chronic stress-induced deficits. Elevated glucocorticoids contribute to Tau accumulation, hyperphosphorylation and mislocalization, leading to synaptic dysfunction and neuronal atrophy, warranting evaluation of TUDCA's protective effects. 3-month-old Wild-Type mice underwent a 7-week Chronic Unpredictable Stress (CUS) protocol, receiving intraperitoneally injections of TUDCA or Fluoxetine in the last 15 days. Behavioral tests assessed anxiety (EPM and OF), depression (TST, SPT and Splash) and cognition (Y-maze, NOR and CFC), followed by molecular and cellular analyses.

TUDCA mitigated CUS-induced depressive-like behavior and cognitive deficits, with effects comparable to Fluoxetine. It modulated pathways involved in inflammation, synaptic plasticity, Tau phosphorylation, and GR signaling, reversing CUS-induced changes in the PFC. Notably, TUDCA induced previously unreported cell- and sex-specific effects on microglia and astrocytes, altering their proliferation, morphology, and activation in the PFC and hippocampus. Neurostructural analysis revealed TUDCA-induced changes in neuronal morphology and spine density in the prelimbic PFC.

These findings highlight TUDCA's potential as a modulator of neuronal, synaptic, and glial function in stress-related brain pathologies, underscoring its therapeutic potential.

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The role of macrophage heterogeneity in the outcome of *Mycobacterium tuberculosis* infection

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Macrophages play a pivotal role in the control of *Mycobacterium tuberculosis* (Mtb) infection. Resident alveolar macrophages are the first cells to encounter bacteria, and their response can shape of the outcome of infection, by modulating the initiation and expression of the adaptive immune response. Understanding how macrophage heterogeneity impacts Mtb control and shapes the adaptive immune response is essential for developing improved therapeutic strategies.

Herein, we used bone marrow-derived macrophages (BMDMs) from two strains of mice that differ in their ability to control to Mtb infection: the resistant C57BL/6 and the susceptible C3HeB/FeJ. BMDMs were differentiated using either granulocyte-macrophage colony-stimulating factor (GM-BMDMs) or macrophage colony-stimulating factor (M-BMDMs) to assess how their distinct phenotypes influence their response to Mtb and ability to control infection.

Our results show higher accumulation of lipid droplets in C3HeB/FeJ M-BMDMs and in C57BL/6 GM-BMDMs when compared to their respective counterparts, indicating that both genetic background and differentiation conditions shape the metabolic profile of macrophages. Despite this, GM-BMDMs adopt a more pro-inflammatory phenotype and exhibit superior bacterial control, irrespective of the background origin. This phenotype is further enhanced by IFNγ and is associated with an early expression of microbicidal molecules, namely nitric oxide and inflammatory mediators.

These findings demonstrate that macrophage responses to Mtb are influenced by both differentiation conditions and genetic background. However, IFNg activation appears to enhance the bactericidal mechanisms of macrophages, independently of the background of the host. Future data assessing the ability of primary macrophages to control Mtb, both *in vitro* and *in vivo* will clarify this subject.

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REAL-TIME COLOR IMAGING FOR NERVE IDENTIFICATION DURING SURGERY

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Optical molecular imaging (OMI) in surgical practice has given rise to innovative concepts involving the use of fluorescent dyes to mark target areas, allowing real-time color contrast during surgery by irradiating tissues with light sources of different wavelength, but peripheral nerve identification using these techniques is progressing slowly. This project aims to accelerate the development and validation of this reality into surgical practice, more specifically for the identification of periprostatic nerves - avoiding their injury - during Radical Prostatectomy (RP). This work pretends to develop a rat model to (i)validate organ-target injection techniques for retrograde periprostatic nerve labeling; (ii)characterize periprostatic neuroanatomy, including the pelvic ganglion; and (iii)evaluate real-time fluorescence emission during minimally invasive surgery. This model will be further validated using cavernous nerve injury approaches. For this, one group of animals were injected with fluorescent agent in organ-target (penis) to label nerves retrogradely, and in another two groups, the pelvic nerve was injured by (i)compression and (ii)cutting to assess retrograde transport of the injected agent. Post- mortem immunofluorescence analysis was performed to identify the fluorescently labeled neurons. The results show that there is retrograde transport of the fluorescent agents in the group without nerve lesion, thus marking the pelvic ganglion neurons. In injected animals with nerve lesion there are differences between the two groups, with the compression model presenting some retrograde neuronal labeling while in the cutting model no retrograde labeling was observed. By immunofluorescence analysis was observed that labeled neurons are exclusively parasympathetic besides both types - sympathetic and parasympathetic - are present within the pelvic ganglia. Regarding the real-time fluorescence emission, we observed fluorescence emission by naked eye when samples were exposed to focused laser beam while with laparoscopic surgical equipment no emission was observed. This project represents a step forward the real- time color imaging during surgery.

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IDO1-driven immunometabolic modulation of granulomatous inflammation in sarcoidosis

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Introduction: Granuloma formation is a central feature of sarcoidosis, marked by the organized accumulation and transformation of activated macrophages and T cells, and sustained by a proinflammatory cytokine milieu. Increasing evidence points to a multifactorial etiology involving genetic susceptibility, microbial agents, and environmental triggers. Indoleamine 2,3-dioxygenase 1 (IDO1) is an immunoregulatory enzyme that catalyzes the rate-limiting step of tryptophan catabolism along the kynurenine pathway to produce immunomodulatory metabolites, being mostly expressed in antigen-presenting cells and associated with the generation of tolerogenic T-cells. This study aimed to determine whether the immunometabolic role of IDO1 could serve as a critical link underlying the pathological mechanisms of granuloma formation in sarcoidosis.
Methods: To achieve this, we applied different experimental *in vitro* and *in vivo* models of granuloma formation and cellular transformation, by using peripheral blood mononuclear cells (PBMCs) obtained from sarcoidosis patients harboring loss-of-function single nucleotide polymorphisms (SNPs) and gene-deleted mice, respectively.
Results: Our findings reveal an association between the rs7820268 SNP in the *IDO1* locus and the risk of developing sarcoidosis. Beyond disease susceptibility, this variant also correlates with distinct immunological profiles, influencing both disease trajectory and the composition of immune cell infiltrates in bronchoalveolar lavage fluids of patients at diagnosis. PBMCs from sarcoidosis patients carrying the risk genotype exhibit a defective IFN-γ response, characterized by reduced IDO1 transcript levels and protein expression. As a proxy for clinical observations, the *in vitro* granuloma model demonstrated that risk carriers exhibit reduced hIDO1 expression accompanied by the formation of larger granuloma-like structures. Consistently, our *in vivo* model of granuloma formation showed that mice lacking IDO1 exhibited enhanced recruitment of leukocytes and overall increased inflammation.
Conclusions: The consistent findings across patient samples, *in vitro* granuloma models, and *in vivo* mouse models support a critical role for *IDO1* in modulating the intensity of granulomatous inflammation and immune cell recruitment.

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TLR7 activation via Gardiquimod-loaded lipid nanoparticles drives a profound macrophage reprogramming and delays *Leishmania* infection in mice

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Macrophages are the final host cells of the protozoan parasite *Leishmania*. Despite their microbicidal capacity (M1 phenotype), the parasite can subvert this activation and redirect it toward an M2 phenotype that supports its survival by exploiting the macrophage's remarkable functional plasticity. Since M1 macrophages can effectively control the infection, promoting this phenotype represents a promising therapeutic strategy, especially in the current context, where drug resistance threatens treatment efficacy. Gardiquimod (GDQM) is a TLR7 agonist known to promote M1 polarization. However, its high lipophilicity compromises systemic bioavailability and is associated with toxicity issues. Our aim is to develop a lipid-based formulation of GDQM to overcome these limitations, evaluate its immunomodulatory capacity *in vitro*, and assess its efficacy in a murine model of cutaneous leishmaniasis.

Lipid-based nanoparticles (NPs) were prepared by hot ultrasonication and characterized in terms of size, polydispersity index, stability, and drug encapsulation efficiency. Their ability to modulate macrophage phenotype was evaluated using BMDM, assessing gene expression (qPCR), cytokine production (ELISA, flow cytometry), and metabolic profile (glucose consumption and lactate production, NMR-metabolomics). For *in vivo* studies, BALB/c mice were infected in the ear with a fluorescent strain of *Leishmania major*, to allow real-time monitoring of infection progression. Therapeutic efficacy was assessed by administering GDQM-loaded NPs intravenously (1.5 mg/kg) once weekly for 7 weeks.

We developed a biocompatible lipid-based formulation with an average particle size of 40 nm and an encapsulation efficiency of 20%. *In vitro* assays demonstrated that GDQM-loaded NPs effectively reprogrammed BMDM toward a pro-inflammatory M1 phenotype, as evidenced by increased expression of pro-inflammatory-related genes. Regarding *in vivo* results, NPs successfully co-localized with *Leishmania* parasites within the dermal macrophage population of the ears. Therapeutically, the treatment slowed infection progression, resulting in significantly reduced parasite burden at the study endpoint compared to untreated controls. These findings indicate that NP-GDQM delivery effectively promotes M1 macrophage activation and controls infection, supporting their potential as a treatment for cutaneous leishmaniasis.

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Molecular and Phenotypic Characterization of Antimalarial Drugs Resistance in *Plasmodium falciparum* from Angola

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Malaria remains a major public health concern in Angola, where artemether-lumefantrine (AL) is the first-line therapy. Preliminary findings from our clinical trial (PACTR202405595426468) and other published data, suggest emerging partial resistance to AL. We focus on characterizing resistance through molecular markers and in vitro drug susceptibility testing to understand this phenomenon better.

We analyse *Plasmodium falciparum* isolates from Angolan patients to identify known resistance molecular markers associated and other antimalarial drug resistance. Target genes include pfcrt, pfmdr1, and kelch13, with sequencing performed to detect polymorphisms linked to different drug susceptibility. At the same time, we are adapting patient-derived parasites for in vitro culture to measure IC_{50} values for the known antimalarial drugs using standardized drug sensitivity assays. These two approaches will allow us to correlate genotypic data with phenotypic resistance profiles.

This study aims to generate robust evidence of AL resistance by linking molecular and phenotypic indicators. These insights are critical for guiding malaria treatment policies and understanding emergent resistance dynamics in Angola. Ultimately, this work supports regional surveillance efforts and the global fight against antimalarial drug resistance.

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Exploring TRPV4 as a molecular mechanism driving inflammatory cells' infiltration into the spinal cord after injury

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Spinal cord injury (SCI) ignites excessive inflammation via pro-inflammatory microglia and infiltration of peripheral inflammatory cells. While acute inflammation is needed for recovery, excessive responses worsens damage. The spleen, via sympathetic signals, promotes myeloid cell infiltration into the spinal cord after SCI, though the mechanisms remains unclear. RNAseq data indicate that sympathetic activation upregulates ion channels-related genes in splenic myeloid cells, with Transient Receptor Potential Cation Channel Subfamily V member 4 (TRPV4) showing the highest expression. This project aims to assess TRPV4's role in SCI-associated immune cell infiltration.

To assess whether norepinephrine (NE) modulates TRPV4 expression, total splenocytes were stimulated *in vitro* with increasing NE concentrations, and TRPV4 expression analysed by flow cytometry. In parallel, TRPV4 expression and immune cell infiltration were evaluated *in vivo*, by analysing the spleen and spinal cord at 3, 6, and 24h post-injury using flow cytometry.

Our results show that NE stimulation increased TRPV4 expression pro-inflammatory monocytes and neutrophils 9h post stimulation at 10^{-9} M NE. Interestingly, a lower concentration (10^{-12} M) also increased TRPV4 expression, but only on neutrophils.

In vivo, TRPV4 expression was increased in pro-inflammatory monocytes and $Ly6G^{low}$ neutrophils 3 hours post-injury in both SCI and sham-operated animals (LAM) relative to naive controls. At 6 hours, expression levels decreased but remained elevated in $Ly6G^{low}$ neutrophils in SCI and LAM animals, whereas pro-inflammatory monocytes and $Ly6G^{+}$ neutrophils showed reduced expression.

At the lesion site, SCI animals exhibited an increased percentage of infiltrative myeloid cells - specifically pro-inflammatory monocytes, $Ly6G^{low}$ and $Ly6G^{+}$ neutrophils – at 6 and 24h post- injury compared to LAM and controls.

These results suggest that TRPV4 may contribute to early mobilization and infiltration of myeloid cells to the spinal cord after injury. Ongoing experiments will explore the impact of TRPV4 pharmacological modulation on immune infiltration and neuroinflammation after SCI.

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Dissecting T cell responses in Multiple Sclerosis: A focus on T cell senescence, exhaustion, and the inflammatory environment

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Autoreactive T cells are central to multiple sclerosis (MS) pathophysiology, though triggers are unclear. Our group showed that newly diagnosed, treatment-naïve MS patients in remission have fewer memory T cells than healthy controls (HC), likely due to lower T cell proliferation. Elevated senescence-associated cytokines in their plasma suggest T cell senescence and/or exhaustion. However, the contribution of senescent/exhausted T cells in MS remains poorly understood, as does the inflammatory milieu's impact on T cell function and phenotype.

We aim to investigate if immune dysfunction in MS is linked to T cell exhaustion and/or senescence, and to assess how MS patients' plasma affects T cell function and phenotype. To address this, we will perform in vitro assays using peripheral blood mononuclear cells and plasma from newly diagnosed, treatment-naïve MS patients in relapse and in remission, and from HC. This study may help clarify disease progression mechanisms and identify potential therapeutic targets.

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The role of phospholipase D pathway in lipid droplet accumulation

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Emerging evidence highlights the disruption of lipid homeostasis and metabolism as a key factor in the pathophysiology of neurodegenerative disorders, such as Alzheimer's Disease (AD). Phospholipase D (PLD) enzymes, particularly the mammal canonical isoforms PLD1 and PLD2, are key regulators of lipid signaling. These enzymes hydrolyze phosphatidylcholine (PC), generating choline and phosphatidic acid (PA), a lipid second messenger that regulates signaling and membrane dynamics making it a crucial molecule in neurodegeneration processes.

Although PLD1 and PLD2 share structural and enzymatic similarities, they differ in subcellular localization and regulatory mechanisms. Both isoenzymes are vital for neuronal function, where lipid homeostasis is tightly regulated, and disruptions may contribute to neurodegeneration. Interestingly, PLD1 ablation impairs hippocampal function and organization, whereas PLD2 ablation has been shown to rescue synaptic and memory deficits in an AD model, underscoring their differential roles in AD.

Lipid droplets (LDs), intracellular organelles that function as storage for neutral lipids, have been recognized for their involvement in inflammation, oxidative stress, and aging—all hallmarks of AD. LD accumulation in microglia has been directly linked to AD´s progression, with LD- accumulating microglia exhibiting disrupted lipid metabolism and increased pro-inflammatory activity. Notably, PC is the main component in the LD monolayer, while PA is essential for synthesizing neutral lipids—suggesting a direct link between PLD activity and LD biology.

We hypothesize that the distinct localization and enzymatic activity of PLD1 and PLD2 differentially influence LD dynamics and lipid homeostasis, contributing to AD pathology. To investigate this, we employed a cross-species approach using yeast, nematodes, and a mammalian neuronal cell line to assess the impact of PLD inhibition or knockout on LD accumulation. Preliminary results, supported by confocal microscopy and quantitative image analysis, reveal increased LD accumulation following PLD modulation.

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Host genetic variant in galectin-3 impairs phagolysosome function and fungal clearance during *Aspergillus fumigatus* infection

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Introduction: Invasive pulmonary aspergillosis (IPA) poses a severe threat to immunocompromised individuals, affecting over 2 million patients annually. Patients with severe respiratory viral infections, particularly those in intensive care, are at greatest risk. Between 10-20% of ICU patients with severe influenza develop influenza-associated pulmonary aspergillosis (IAPA), linked to a 50% mortality rate. Galectin-3, encoded by *LGALS3*, is a critical regulator of innate antifungal immunity, which mediates several key cellular mechanisms, including phagocytosis, cell signaling, and lysosomal repair. Transcriptomic analysis of BAL samples from IAPA patients identified *LGALS3* expression as a mortality predictor among IAPA cases. In this study, we aimed to investigate how the single nucleotide polymorphism (SNP) rs4644 in *LGALS3* affects macrophage responses to fungal infections.

Materials & Methods: DNA from patients with severe influenza was genotyped for the rs4644 SNP in *LGALS3,* and 30-day cumulative incidence of IAPA was assessed according to the different genotypes. Additionally, functional analyses were performed using human monocyte-derived macrophages (MDMs) from healthy donors carrying different rs4644 genotypes after *in vitro* infection with *A. fumigatus*.

Results: Our findings demonstrated a significant association between the rs4644 SNP in *LGALS3* and susceptibility to IAPA. MDMs from donors carrying the risk genotype (AA) exhibited significantly reduced galectin-3 levels in both the cell surface and intracellular compartments compared to wild-type (CC) counterparts. Mechanistically, macrophages with the AA genotype displayed impaired phagocytic and fungicidal activities against *A. fumigatus.* Our results further demonstrated that galectin-3 co-localizes with *A. fumigatus* conidia in the phagosomal compartment, and that the AA genotype was linked to decreased phagolysosomal fusion and increased fungal germination, suggesting that galectin-3 is essential for fungal clearance.

Conclusions: These results offer novel insights into the pathogenesis of IAPA, suggesting that genetic defects in the expression of galectin-3 compromise the activation of innate antifungal immunity, thereby predisposing individuals to fungal infections.

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Correlates of Spinal Cord Injury-Induced Immune Dysfunction: From Preclinical Models to Human Studies

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Traumatic spinal cord injury (SCI) is a devastating condition with complex pathophysiology. Beyond motor and sensory deficits, SCI causes systemic immune changes ranging from subtle shifts in immune cell activation to harmful pro-inflammatory responses, autoimmunity, and immunosuppression. This immune dysregulation increases infection risk, damages peripheral organs, and worsens secondary injury, ultimately impairing patient outcomes. Although research mostly focuses on spinal inflammation, evidence shows peripheral immune system involvement, indicating broader dysfunction. Our preliminary data reveal that SCI causes progressive morphological and secretory changes in sympathetic nerve endings innervating lymphoid organs. These changes lead to immune alterations dependent on sympathetic signaling and injury severity. Within 24 hours post-injury, norepinephrine (NE) levels rise in the spleen, producing concentration-dependent effects: high NE causes splenocyte apoptosis, while lower levels—typical in acute SCI—enhance neutrophil activation, mobilization, and trained immunity in monocytes. These shifts coincide with early myeloid skewing, increased inflammation, and reduced neutrophil spinal infiltration when sympathetic signaling is disrupted. Further studies are necessary to clarify the timing and dynamics of both innate and adaptive immune responses. This project aims to comprehensively characterize SCI-induced immune dysfunction in mouse models by examining lymphoid organs, immune cell populations, cytokine profiles, and cell function to gain insights into post-injury immune responses with translational potential. Additionally, we will investigate molecular, metabolic, and epigenetic changes to identify biomarkers and predictors of outcomes, develop a predictive immune dysfunction signature relevant to patients, and validate these markers clinically. Ultimately, this research intends to bridge preclinical findings with patient care by developing a treatment stratification system to improve diagnosis, enable earlier interventions, and transform recovery and prognosis for SCI patients. These predictive markers could pave the way for innovative diagnostic and monitoring tools, enhancing the assessment of immunodeficiency and infection susceptibility, guiding treatment decisions, and refining patient management strategies to improve outcomes.

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Assessing the Potential of Combined Antibiotic–Bacteriophage Therapy Against *Mycobacterium ulcerans* Infections

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Buruli ulcer (BU) is a neglected tropical disease caused by *Mycobacterium ulcerans* (*M. ulcerans),* characterised by extensive skin necrosis. The treatment requires an 8-week course of antibiotics, often associated with many refractory and paradoxical events. The long-term treatment and the prevalence of the disease in underdeveloped countries highlight the need for improved therapeutic strategies.

In this project, we will study the potential of combining conventional antibiotic treatment with bacteriophages – viruses that infect bacteria. We will use 3 bacteriophages previously shown to eliminate *M. ulcerans* in vitro and assess their efficacy in a mouse model of *M. ulcerans* infection. The most effective bacteriophage(s) will be tested for antibiotic interactions through an in vitro isobologram analysis. Lastly, a compatible bacteriophage-antibiotic treatment will be assessed for efficacy in the mouse infection model. Ultimately, this work will outline the feasibility of future clinical testing of bacteriophage-antibiotic treatments for BU.

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Spatiotemporal uncertainty-enhanced diffusion models for automatic detection of extracardiac findings in cardiac MRI

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Anatomical plane sequences serve as reference for initiating the acquisition of cardiac views during cardiac MRI examinations. Because of its auxiliary role, clinicians often overlook potential non-cardiac abnormalities visible in these sequences, known as extracardiac findings (ECFs). This gap opens space for the implementation of automatic methods to detect them. Anomaly detection (AD) approaches appear to be the most promising to solve this problem; however, current state-of-the-art AD methods lack the robustness needed to handle the complexity and particularities associated with this task. Diffusion models are a recent class of AD methods with interesting modelling capabilities but remain limited by the trade-off between high and low regularization regimes. To address this limitation, we propose a spatiotemporal uncertainty framework that leverages the outputs of diffusion models.

Diffusion models are methods that learn to reconstruct images from their noisy versions. Naturally, applying different noise masks and/or levels of noise to the original image results in different reconstructions. To avoid dependence on a single noise configuration and, consequently, on a fixed regularization level, the proposed framework computes the residuals between the original image and its multi-time-step representation, defined as the average of the ensemble of reconstructions obtained with different noise masks and noise levels, which is weighted by an uncertainty measurement, calculated with the Mahalanobis distance between the input image and the distribution of all reconstructions.

This methodology was validated on a dataset comprising 690 cases, retrospectively acquired at *Unidade Local de Saúde de Braga*, demonstrating an improved specificity and robustness in ECFs detection when compared to both baseline diffusion model and a broad set of state-of-the-art AD methods. These results represent a step toward clinical integration, enabling early detection of abnormalities and supporting the timely referral of patients to relevant medical specialties, improving overall patient care.

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SPINT2 Expression in Cancer: Focus on Its Role and Therapeutic Impact in *BRAF*-Mutated Melanoma

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Serine protease inhibitor Kunitz-type 2 (SPINT2) inhibits serine proteases like hepsin, matriptase, and hepatocyte growth factor activator (HGFA). These proteases interact with the extracellular matrix and influence signaling pathways that affect tumor progression. HGFA activates HGF, the receptor for c-Met, which is implicated in BRAF inhibitors resistance in *BRAF*-mutated melanoma. This study aims to investigate SPINT2 expression in cancer and its functional and therapeutic impact in *BRAF*-mutated melanoma.

SPINT2 expression and association with survival in tumors were analyzed using bioinformatic tools. SPINT2 protein expression and gene promoter methylation were evaluated in formalin-fixed paraffin-embedded (FFPE) samples from different solid tumors. SPINT2 overexpressing transfectants were generated in melanoma cells, and effects were assessed using 2D and 3D cell culture models (CCM). SPINT2 impact on tumor growth and angiogenesis was explored through Chick Chorioallantoic Membrane (CAM) assay. SPINT2's biological role was analyzed using Nanostring's PanCancer Progression panel and Proteome Profiler Human XL Oncology array. SPINT2's effect on melanoma cell sensitivity to BRAF/MEK inhibitors, and cumulative effect on spheroid growth and migration was evaluated.

SPINT2 expression was lower in melanoma, liver, and glioma compared to other cancers. *In silico* analysis linked low *SPINT2* levels to increased tumor aggressiveness. In FFPE melanoma samples, SPINT2 downregulation was associated to promoter hypermethylation. Overexpression in melanoma cells decreased cell viability, migration, and proliferation in 2D and 3D CCM. Additionally, SPINT2 inhibited tumor growth and angiogenesis in CAM assay, suggesting a tumor-suppressive role. At the molecular level, SPINT2 influenced genes and proteins involved in extracellular matrix remodeling, angiogenesis, and migration. This gene also increased melanoma cell sensitivity to BRAF and MEK inhibitors, enhancing therapy efficacy in spheroid growth and migration.

SPINT2 downregulation is linked to increased cancer aggressiveness. In melanoma, this protein functions as a tumor suppressor and holds potential as a therapeutic biomarker for *BRAF*-mutated melanoma patients.

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Unveiling CD147 as a modulator of immunosurveillance in Colorectal Cancer

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Colorectal cancer (CRC) is one of the most diagnosed cancers and a leading cause of cancer-related deaths globally. In Portugal, it ranks second in incidence and first in mortality for both men and women. Despite advances in screening programs, early diagnosis remains challenging, and many patients present with metastatic disease, which has poor survival outcomes. Immunotherapy has emerged as a breakthrough in CRC treatment, yet only a small subset of patients responds to these therapies, underscoring the need for predictive biomarkers and combination strategies to enhance efficacy.

CD147, a transmembrane glycoprotein enriched in cancer cells, has been identified as a key player in tumor progression and immune modulation. Recent studies suggest that CD147 promotes an immunosuppressive tumor microenvironment (TME) by influencing tumor- infiltrating immune cells, contributing to immunotherapeutic resistance. Preliminary data from our group reveal that CD147 knockout (KO) in cancer cell lines reduces tumor growth and shifts the immune landscape by decreasing anti-inflammatory immune cells (M2-like macrophages, regulatory T cells) and increasing pro-inflammatory immune cells (M1-like macrophages, CD8+ T cells). These findings suggest that targeting CD147 may reprogram the TME and enhance responses to immune checkpoint inhibitors (ICIs).

Thus, this project aims to evaluate CD147 as a prognostic and therapeutic biomarker in CRC and develop novel therapeutic strategies by: 1) determining its clinical relevance, 2) establishing its role as a predictive marker for ICI therapy, and 3) combining ICIs with CD147 inhibitors. Using syngeneic mouse models, we will explore synergistic effects of these treatments. This innovative approach seeks to provide preclinical evidence supporting personalized therapies for CRC, addressing critical challenges in diagnosis and treatment.

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The Role of Phospholipase D in APOE4-Driven Pathogenesis in Alzheimer's Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder marked by the accumulation of extracellular amyloid-β plaques and intracellular hyperphosphorylated Tau tangles. The strongest genetic risk factor for AD is the ε4 allele of the human apolipoprotein E gene (APOE), which encodes a glycoprotein essential for lipid transport between astrocytes and neurons. Research has increasingly linked disruptions in lipid metabolism with the development of AD. Lipidomic analyses of astrocytes expressing APOE4 have revealed an increased accumulation of lipid droplets compared to those expressing APOE3. A similar phenotype, along with a growth defect, has also been observed in yeast models expressing APOE4. A genetic screen aimed at identifying suppressors of the APOE4-induced defects highlighted OPI1—a phosphatidic acid (PA)- binding regulator of inositol synthesis—as a key candidate. Enzymes responsible for PA production, including PLD1 and PLD2, and their yeast homolog SPO14, have also been implicated in lipid-driven mechanisms underlying AD. Given the evidence pointing to the role of lipid signaling in AD, we hypothesize that PLD1 or PLD2, via their product PA, may be critical modulators of AD pathophysiology.

Using a yeast model, stable expression of human APOE3 and APOE4 was established in various strains. Gene knockouts for SPO14, OPI1, and SPO14+OPI1 were generated through gap-repair. Growth was assessed in liquid culture and on solid media. Preliminary findings confirm the APOE4-associated growth defect in wild-type strains and its reversal upon deletion of SPO14, in liquid and solid media. Consistent with previous reports, deletion of OPI1 also rescued the APOE4-induced growth impairment. The combined deletion of SPO14+OPI1 also alleviated the growth defect.

Additional phenotypes are being evaluated in these strains, including lipid droplet accumulation and vacuolar dynamics, as well as evaluation of the equivalent phenotypes in a mammalian cell line. Our preliminary results support a role for phospholipase D in APOE4-related AD mechanisms.

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Immune response and functional recovery after Spinal Cord Injury: does biological sex matters?

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Spinal cord injury (SCI) is a devastating neurological condition that disrupts motor, sensory and autonomic functions, and is often accompanied by profound immune system dysfunction. Following SCI, a sustained and unresolved inflammatory response develops at the lesion site, exacerbating tissue damage and impairing healing. Concurrently, systemic immune alterations may increase susceptibility to infections or trigger pro-inflammatory responses in unaffected organs, contributing to long-term complications and morbidity. Although growing evidence highlights significant sexual dimorphism in both immune and nervous system function, preclinical SCI research has historically relied on female-only rodent models. This sex bias limits the translational value of findings, particularly given that approximately 80% of human SCI cases occur in males.

In this study, we investigated how biological sex influences the immune response and functional recovery following SCI, focusing specifically on myeloid cell dynamics during both acute and chronic phases of injury. Using flow cytometry and behavioral assessments in male and female mouse models, we identified notable sex differences in the progression and nature of the immune response. Males exhibited delayed infiltration of myeloid cells into the injured spinal cord, whereas females displayed a more complex acute response, combining not only the pro- inflammatory activation profile observed in males, but also a reparative profile. Interestingly, this reparative signature in females vanishes at the chronic phase, leading to a convergence of immune profiles between sexes over time. Despite these early differences, which translated into slower post-operative recovery in males, no significant sex differences were observed in motor and sensory recovery at later stages.

These preliminary findings underscore the critical importance of including both sexes in SCI research to accurately capture variability in immune responses and recovery trajectories. Understanding sex-specific immune mechanisms following SCI is essential for developing personalized, more effective therapeutic strategies to improve clinical outcomes for individuals affected by this condition.

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AstroMap: First Glimpses of Adult Astrogliogenesis in Health and Depression

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Major depressive disorder is a neuropsychiatric condition with a highly heterogeneous pathophysiology. Currently, approximately 280 million people worldwide (8% of the portuguese population) suffer from depression, with women experiencing it at twofold higher rates than men. Furthermore, approximately 70% of patients do not respond to current first-line therapies, underscoring depression treatment as a major unmet medical need.

Emerging evidence highlights an astrocytic dysfunction in the hippocampus of both human patients and animal models of depression, disrupting neuron-astrocyte communication and affecting cellular and circuitry level processes in this region.Notably, previous data from our lab showed that the generation of hippocampal adult-born astrocytes (hABA) in the dentate gyrus is impaired in stress-induced depression, while a specific class of antidepressants significantly enhances hippocampal astrogliogenesis, suggesting these cells as a promising therapeutic target for depression. However, despite this compelling evidence, the role of hABA in adult brain function remains poorly understood.

In this project, using the Cytbow-Nucbow mouse model in combination with targeted cell ablation tools, electrophysiological techniques, and transcriptomics, we aim to functionally characterize hABA and assess their role in the molecular mechanisms of stress-induced depression.

Preliminary data from extracellular electrophysiological recordings show that ablating hABA leads to increased basal synaptic transmission and decreased long-term potentiation of the CA3-CA1 Schaffer collateral fibers, suggesting that hABA are key modulators of intra-hippocampal circuitry.

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White matter microstructure is differentially impacted by cerebral amyloid angiopathy, neurofibrillary tangles and neuritic plaques co-pathology

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White matter (WM) is affected by and serves as a pathway to neurofibrillary tangles (NFTs) propagation in Alzheimer's disease (AD). Additional neuropathological changes frequently coexist in AD, alongside NFTs and neuritic plaques (NPs) accumulation, further accelerating the progression of dementia. For instance, cerebral amyloid angiopathy (CAA) associates with NPs to exacerbate NFTs accumulation. We aim to study how these co-pathologies differently affect WM integrity in AD.

We performed a cross-sectional study of antemortem diffusion tensor imaging (DTI) data according to participants' postmortem NFTs, NPs and CAA neuropathology, from the National Alzheimer's Coordinating Center dataset. Our study cohort comprised 26 AD participants, with MRI and autopsy data collected within a maximum interval of four years. DTI metrics were compared between neuropathology-defined groups and correlated to Clinical Dementia Rating (CDR) scores and hippocampal volumes.

We found statistically significant asymmetric DTI changes in several WM regions between Braak NFTs stages III/IV and V/VI, and across CAA pathological burden, with increased mean, radial and axial diffusivities. CAA demonstrated a greater WM impact on the posterior right hemisphere while NFTs had greater impact on the left hemisphere. This posterior predominance of CAA effects has also been observed in previous studies. CAA-NFTs co-pathology effects were observed in the splenium of the corpus callosum, as significant changes were observed in both CAA and NFTs analyses but disappeared after correction for the co-pathology. DTI metrics correlated significantly with CDR scores and hippocampal volumes across multiple regions among the ones identified as exhibiting neuropathology-related diffusivity changes, supporting the concept that these WM changes may reflect a worsening of disease outcomes. Our results suggest that WM integrity is differentially impacted by AD neuropathology, with CAA and NFTs influencing each other's effects on WM microstructure, highlighting the importance of considering the influence of CAA co-pathology on WM degeneration in AD.

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Studying the impact of the melanin-concentrating hormone producing neurons on the lipid dynamics in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is the leading cause of dementia, yet there are no effective treatments to stop the disease progression. Sleep disturbances are a risk factor for AD and exacerbate amyloid beta (Aβ) pathology, which in turn further impair sleep. This suggests a bidirectional relationship between sleep and AD, where sleep disturbances increase Aβ burden and inflammation, while Aβ accumulation disrupts sleep. The melanin-concentrating hormone (MCH) system regulates REM sleep and hippocampal activity, and recent findings implicate its dysfunction in early AD stages. Furthermore, MCH downregulates the *Ptgs2* gene, which encodes COX-2, a key enzyme in prostaglandin signaling. Lipid droplets (LD) are prostaglandins producing sites and its accumulation is associated with neuroinflammation and microglial dysfunction in AD. We hypothesize that MCH modulates neuronal-glial lipid dynamics via COX-2 regulation during sleep, and that this dynamic is impaired in early AD. Here, we addressed the expression levels of Ptgs2 in a mouse model of AD, and further characterized the capacity of MCH neurons to regulate it *in vivo*. Moreover, we are charactering LD accumulation in neurons and microglia using BODIPY 493 staining in *App*$^{NL-G-F}$ mice, a mouse model of early AD, and in controls. Overall, our work will determine the potential of MCH as a modulator of neuron lipid biology and LD accumulation dynamics in control and *App*$^{NL-G-F}$ mouse model.

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The role of Tau in the regulation of translational stress response and its importance for brain pathology

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Clinical evidence links chronic stress and high glucocorticoids(GCs) to Alzheimer's disease(AD). Our previous findings suggest that Tau mediates stress-related pathology, likely through GC signaling, but the underlying molecular mechanisms remain unclear. Given that GC receptors function as transcription factors and RNA-binding proteins(RBPs) regulate mRNA translation under stress, we investigated how Tau influences stress-induced translational dysfunction, RBP dynamics, and nuclear stability. Using P301L-Tg, Tau-KO, and WT mice subjected to chronic unpredictable stress, we analyzed cognitive and emotional outcomes, RBP localization, and nuclear integrity. Consistent with prior findings, Tau deletion was protective against stress-induced deficits, whereas P301L-Tau exacerbated them. We observed that CUS triggered perinuclear RBP redistribution and stress granule(SG) formation (e.g.,TIA-1+,TDP-43+), while Tau deletion partially prevented this response. Notably, some RBPs, such as G3BP, exhibited stress-induced movement independent of Tau, indicating selective Tau involvement in SG dynamics.

Further analysis of Tau and TIA-1 interactions revealed that stress disrupted TIA-1 binding to key synaptic proteins in WT mice, suggesting a novel role for TIA-1 in synaptic regulation. In Tau-KO mice, TIA-1's interactome was profoundly altered, underscoring Tau's essential role in its normal function. Moreover, both P301L-Tau expression and Tau deletion led to nuclear structure and chromatin, paralleling AD-related nuclear dysfunction. Increased nuclear p-Tau levels in stressed WT mice further support a link between Tau pathology and nuclear dysregulation.

Overall, our findings suggest that Tau plays a critical role in nuclear organization and perinuclear RBP transport. Under chronic stress, mRNA translation dysregulation is mediated by Tau-TIA-1 interactions, contributing to AD-related synaptic and nuclear dysfunction.

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Targeting KDM4C Epigenetic Regulation as a Therapeutic Strategy for Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is considered the most aggressive breast cancer subtype, where patients are mainly treated with conventional chemotherapy, associated to poor efficacy and severe side-effects, highlighting the need for new therapies. Lysine-specific demethylase 4C (KDM4C) has been identified as a promising epigenetic target for TNBC, since overexpression has been associated to the initiation and progression of tumorigenic processes that contribute to the aggressive phenotype. The combination of computational biochemistry, organic synthesis and molecular biology techniques has proven to be an effective approach to the rational design of novel therapies. This study presents the initial two phases and preliminary findings of a third phase in a multidisciplinary study to develop selective and potent KMD4C inhibitors.

For the virtual screening, we began by selecting six structures of KDM4C from the Protein Data Bank. The effectiveness of four scoring functions in reproducing experimental binding poses was assessed using protein-ligand docking with the GOLD software. A training set was created with 30 active molecules from ChEMBL database and 2000 generated decoys. The performance of different docking protocols in distinguishing active molecules from decoys was measured by calculating the enrichment factor at 1% (EF1%) and the area under the curve (AUC). Among the tested combinations, 5FJK/ChemPLP presented an AUC of 70.2% and an EF1% of 6.8, while 5FJH/ASP and 5FJH/ChemPLP presented AUC values of 83.5% and 74.7%, respectively, both with an EF1% of 6.8.

Several libraries were screened with the selected protocol-structure combinations leading to the selection of a set of top-performing molecules *in silico*. These selected compounds have been synthesized, and their biological evaluation in triple-negative cancer cells is currently in progress.

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Impact of Lipid Modulation on Macrophages Infected with *Mycobacterium ulcerans*

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Buruli ulcer (BU) is a chronic, necrotizing skin disease caused by *Mycobacterium ulcerans*, a pathogen that produces mycolactone—a lipid toxin and the key virulence factor responsible for tissue damage. Classified as a neglected tropical disease, BU is predominantly associated with stagnant water sources, and their mechanism of transmission remains unclear. Despite effective treatments, delays in seeking medical care often lead to permanent disability and substantial socioeconomic burden. These challenges underscore the urgent need for innovative therapeutic strategies to improve clinical outcomes and mitigate long-term impact. Recent advancements in immunometabolism suggest that infection-driven metabolic alterations may offer promising therapeutic targets. In this study, we aimed to explore host metabolic alterations during *M. ulcerans* infection.

For that, two distinct strains of *M. ulcerans* were used to infect macrophages: a mycolactone-producing strain (mycoMu) and a non-mycolactone-producing strain (ΔmycoMu). Our RNA sequencing data revealed an upregulation of lipid metabolism pathways in myco*Mu*-infected macrophages, while inflammatory-related genes were upregulated in ΔmycoMu-infected macrophages. To further explore this data, we inhibited fatty acid synthesis using two different inhibitors: C75 or SorA. Interestingly, while no differences were found for myco*Mu*-infected macrophages upon treatment with C75, we did find an increased bacterial burden in Δmyco*Mu*-infected macrophages, associated with an increase in IL-β production. The addition of SorA did not result in any alterations in bacterial burden or in reactive oxygen species and cytokine production.

These findings suggest that modulation of host lipid metabolism may influence the outcome of *M. ulcerans* infection. Targeting lipid synthesis pathways could represent a novel therapeutic approach for BU. Further investigation is necessary to elucidate the underlying mechanisms and optimize intervention strategies.

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Artificial Intelligence-Driven Protein Language Modelling to Map Emergent Drug Resistance Pathways in HIV-1

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Introduction: Antiretroviral therapy (ART) has transformed HIV-1 into a manageable chronic infection, yet the continuous emergence of resistance-associated mutations (RAMs) in viral enzymes such as protease (PR) and reverse transcriptase (RT) poses clinical challenges. In the era of large-scale protein language modelling powered by artificial intelligence (AI), we investigate the potential of a lightweight PLM to identify and characterize mutational patterns associated with drug resistance directly from sequence data.

Methods: A comprehensive dataset of HIV-1 PR and RT protein sequences was retrieved from the NCBI Protein database. The ESM-2 t6_8M model was fine-tuned using masked language modelling on a curated subset of the dataset. Mutation impact was estimated via single residue Δlog probability (Δlog P) scans, and preliminary comparisons were made against known RAMs listed in the Stanford HIV Drug Resistance Database.

Results: Initial results suggest a reduction in model perplexity following fine-tuning, alongside emerging patterns in Δlog P values that may reflect biological relevance. Known resistance mutations appear to cluster within characteristic Δlog P ranges, and several novel substitutions of potential interest have been identified. Further analysis is underway to confirm these trends and evaluate positional effects in greater detail.

Conclusions: Preliminary findings support the feasibility of using AI-based PLMs to explore evolutionary constraints and mutation tolerance in HIV-1. The approach provides an alignment-free framework that could complement phenotypic assays in resistance monitoring. Ongoing work will refine the model, expand to additional viral genes, and integrate mutational data from longitudinal patient cohorts.

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Are there valence-specific ensembles in the Laterodorsal Tegmentum?

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The Laterodorsal Tegmental Nucleus (LDT) is a heterogeneous brainstem region containing cholinergic, glutamatergic, and GABAergic populations playing distinct roles in reward and aversion. For example, cholinergic LDT projections to the nucleus accumbens (NAc) support reinforcement learning and preference formation, while GABAergic projections are associated with aversion. However, how specific LDT ensembles respond to and discriminate between stimuli of opposing valence remains poorly understood.

To address this, we used fibre photometry to monitor real-time calcium events as a measure of neural activity, during exposure to valenced stimuli and in a classical fear conditioning paradigm. We used the TRAP2 mouse model to genetically tag LDT neurons activated by cocaine (reward) or foot shock (aversion). This mouse model enables selective expression of the calcium indicator GCaMP8m (green) in stimulus-specific neuronal ensembles, together with the expression of a general sensor in all LDT cells (jRGECO1a; red).

Our results revealed that overall LDT neuronal activity increased in response to aversive events and valenced odours but decreased following rewarding liquid administration. Cocaine- and shock-tagged LDT ensembles showed strong activation to aversive stimuli, odours, and shock- predictive cues. These findings indicate that the LDT dynamically responds to different modalities of valence stimuli and is particularly responsive to aversive signals during associative learning.

Overall, our work complements the gathered evidence on how this brainstem nucleus integrates reward and aversion processing in the brain, identifying potential circuit-level targets for modulating dysregulated valence processing.

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The role of astrocytic metabotropic glutamate receptor 5 in the basolateral amygdala

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Astrocytes regulate brain homeostasis and can modulate synaptic activity. They sense and respond to neuronal activity through activation by neurotransmitter receptors. Specifically, they sense glutamate, the primary excitatory neurotransmitter, through the activation of the metabotropic glutamate receptor 5 (mGluR5). Work from our laboratory showed that whole-brain deletion of mGluR5 in astrocytes induces an anxious-like phenotype, learned helplessness, social deficits, and modulates cognition. Furthermore, by targeting astrocytic mGluR5 expression in the hippocampus, the laboratory validated the cognitive phenotype. However, which brain regions are involved in the emotional phenotype is still unclear, which urges us to study the role of astrocytic mGluR5 in emotion-related regions. The basolateral amygdala (BLA), which is interconnected to the hippocampus, plays a key role in emotional processing. Thus, in this dissertation, we propose to perform a study of loss Vs. gain of astrocytic mGluR5 function through a viral approach, followed by a behavioral, structural, and molecular characterization.

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Optimizing the Cryopreservation of Induced Mesenchymal Stem Cell-Like Cells Secretome: Exploring the Efficacy of Lyophilization

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The secretome released by induced mesenchymal stem cell-like cells (iMSCs), comprising both soluble and vesicular fractions, represents a promising cell-free alternative to the paracrine effects of tissue-derived mesenchymal stem cells (MSCs). iMSCs offer reduced heterogeneity and higher proliferation rates, making them an attractive source for regenerative medicine and neurodegenerative disease therapies. Preserving the structural and functional integrity of the iMSCs-derived secretome is essential for its therapeutic potential. Lyophilization, a technique widely used in pharmacological formulations, has emerged as a candidate strategy for secretome stabilization.

This study aimed to evaluate the impact of lyophilization on the iMSCs-derived secretome, comparing it to a laboratory-established concentration method using a 5 kDa molecular weight cut-off filter.

Structural analyses included quantification of proteins, lipids, and extracellular vesicles (EVs) using Bradford and Nile Red assays, nanoparticle tracking analysis (NTA), immunoblot, and semi-quantitative proteomics.

Functional bioactivity was conducted in vitro using SH-SY5Y neuroblastoma cells and a 3D dopaminergic model derived from mouse embryonic stem cells (mESCs), employing flow cytometry, immunocytochemistry, and both bright-field and confocal microscopy.

Preliminary data indicate no significant differences between the lyophilization and filtration-based concentration methods in terms of secretome composition or bioactivity. These findings highlight lyophilization as a promising, scalable approach for the preservation of iMSCs-derived secretome, advancing its potential for clinical translation in cell-free regenerative therapies.

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Predictive Capacity of Brain and Blood Lipid Biomarkers in the Diagnosis and Progression of Alzheimer's Disease

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Structural brain imaging can help identify progressive neurodegeneration, a hallmark of Alzheimer's disease (AD). Gray-white matter contrast (GWC), obtained from MRI, has become a promising biomarker of cortical microstructural integrity, although cortical thickness and volume are more frequently used as structural metrics.

This study focuses on GWC's usefulness as an imaging biomarker for ageing and AD progression, its behaviour compared to more traditional structural metrics like cortical thickness and volume, and its relationship with blood lipidomic.

The values for all three metrics studied in this project (GWC, thickness and volume) were calculated using FreeSurfer, for 35 different regions of the Desikan-Killiany atlas and compiled in a dataset with 2754 individual records, alongside demographic data and blood lipidomic from ADNI (Alzheimer's Disease Neuroimaging Initiative) in an earlier study.

Spearman partial correlations, adjusted for sex, were used to assess correlations between structural metrics and age and adjusted for both sex and age for the case of studying metric-metric correlations. In most cortical regions, there were significant negative correlations between age and all three structural metrics, although GWC was found to share the highest correlation with most areas. GWC also provided complementary information to the other metrics, evidenced by its lower correlation. The orbitofrontal and temporal pole regions showed the most significant decline in GWC values and age. In contrast, cortical thickness and volume showed the highest change in their values in the temporal and parietal lobes.

Until now, the results have corroborated with most recent literature, suggesting that GWC can differentiate itself as a structural imaging metric for neurodegeneration from more common metrics, confirming its potential utility as an imaging biomarker. Therefore, these findings justify the continuation of the studies on GWC in a more in-depth investigation of GWC in the context of AD.

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Regional roles of astrocytic Foxo1 in the modulation of behavior

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Introduction: Astrocytes are crucial for modulating synaptic activity and behavior through bidirectional communication with neurons. They sense and respond to neuronal activity by elevating intracellular calcium levels, which display complex spatiotemporal properties. To disclose its implications for behavior, we studied the IP3 receptor type 2 knockout (IP3R2 KO) mouse model that lacks global calcium elevations in astrocytes. We found that mice lacking IP3R2 display enhanced fear memory. Molecular analysis of hippocampal tissues revealed hundreds of up- or down-regulated genes, among which 76 are modulated by the transcription factor Foxo1. Given the involvement of other cortico-limbic regions in emotional processing and fear memory, the main aim of this work was to assess the impact of region-specific astrocytic Foxo1 overexpression on cognition and emotion.

Methods: We generated mouse models overexpressing Foxo1 in astrocytes of the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC). To evaluate the impact of astrocytic Foxo1 overexpression on cognition, mice underwent the Morris Water Maze and Y-Maze Two-Trial Place recognition tests. Mice performed the Elevated Plus Maze, Light/Dark Box, Tail Suspension, and Contextual Fear Conditioning tests to assess the emotional dimension.

Results: Mice with Foxo1 overexpression in astrocytes from the mPFC present alterations in cognition, namely decreased cognitive flexibility. Mice with Foxo1 overexpression in astrocytes from the BLA display increased anxiety-like behavior and decreased depressive-like behavior.

Conclusions: Astrocytic Foxo1 function appears to be involved in cognitive and emotional behaviors in a region-specific manner.

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Neuroanatomical and Functional Study of Serotonergic Circuits in MJD Mice

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The cerebellum is primarily involved in maintaining balance and posture, coordinating voluntary movements, and facilitating motor learning. Dysfunction of the cerebellum leads to uncoordinated movement or ataxia. Machado-Joseph disease (MJD) or Spinocerebellar Ataxia type 3 (SCA3) is the most common form of dominant ataxia, associated with an expansion of the CAG repeat tract in the coding region of the causative gene, ATXN3. Among the most affected brain regions are the deep cerebellar nuclei (DCN). Currently, there is no therapy available to stop or delay disease progression.

Previously, using a hypothesis-free approach, we found that activation of the serotonergic (5-HT) system suppressed MJD-associated proteotoxicity. Importantly, we demonstrated early dysfunction of the DCN in MJD mice, which was restored upon administration of the 5-HT modulator citalopram. However, the role of 5-HT in MJD remains unclear. We hypothesize that the serotonergic neural circuitry linking the dorsal raphe nucleus (DRN)—the site of 5-HT production—to the DCN is dysfunctional in MJD. To test this hypothesis, we will measure 5-HT levels and release patterns in 5-HT terminals of the DCN of rodents performing motor tasks, combining neural activity recordings with 5- HT sensors of DRN-to-DCN projections. Additionally, we aim to develop a protocol for deep brain stimulation mimetics using chemogenetics, with the prospect of translating such an approach to the clinic.

Knowing that there is a huge gap in the field, we are exploring the integrity of the 5-HT circuitry and 5-HT-related markers (e.g. expression of serotonin transporter, Sert) in MJD. We also crossed Sert-Cre (expressing Cre recombinase in 5-HT neurons) with Ai14 mice (which express tdTomato in a Cre-dependent manner) to evaluate the 5-HT circuitry. Additionally, we are using a chemogenetic approach and testing if injection of hM3Dq into DRN neurons can activate serotonergic neurons and assess its effects on MJD-related motor impairments and neuropathology. We are also optimizing the use of the serotonin sensor iSeroSnFR in the cerebellar nuclei.

This cutting-edge approach will unravel with unprecedented detail how serotonergic neurotransmission modulates MJD progression at the behavioral and pathological levels, using techniques that have not previously been applied in the ataxia field.

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Macrophage response against *Leishmania infantum* involves HIF-1α and HIF1A antisense long noncoding RNA

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Transcription factors regulate metabolism against infectious agents. In macrophages, HIF-1α contributes to a proinflammatory profile by promoting a metabolic switch from oxidative phosphorylation to glycolysis. The interplay between HIF-1α and the HIF1A antisense long noncoding RNA (HIF1A-AS3), a non-protein-coding RNA forms complexes with other factors, supports the transcription of glycolysis-related genes. However, the mechanisms underlying the role of this long noncoding RNA in such regulatory complexes remain unclear. In this work, we aim to elucidate the interaction between HIF-1α and the HIF1A- AS3 in the metabolic context in macrophages infected with Leishmania infantum. We quantified gene and protein expression by RT-qPCR and flow-cytometry of glycolytic-related targets downstream of HIF-1α in human THP-1 macrophages infected with L. infantum or stimulated by LPS. Our results showed that L. infantum infection downregulates HIF1A gene expression but upregulates GLUT1 in THP1 macrophages. Stimulation of TLR4 pathway with LPS upregulated both HIF1A and the lncRNA HIF1A-AS3, while stimulation of TLR3 and TLR7 had no effect. Knockout of HIF1A or HIF1A-AS3 reduced parasite burden at early time points but increased it at 24h post-infection. Notably, HIF1A-AS3 knockout also reduced HIF-1α levels in infected macrophages, whereas HIF1A-AS3 overexpression increased HIF- 1α levels at 24h post-infection. In conclusion, our findings suggest that HIF1A and HIFA- AS3 lncRNA contribute to the control of L. infantum infection in human macrophages by modulating HIF-1α expression and associated metabolic responses.

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Impact of exposure to stimuli of opposing valence in the nucleus accumbens proteome

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The mammalian brain has developed to assign valence to environmental stimuli, which represents the inherent positive/negative value of a situation/object. While many stimuli have innate valence, eliciting approach or avoidance, most stimuli acquire valence with learning. Brain regions such as the nucleus accumbens (NAc) are crucial in processing these rewarding/unpleasant stimuli and associated behaviors. However, the molecules and/or molecular pathways involved in valence encoding in the NAc are not known.

Our goal was to characterize the NAc proteome after exposure to stimuli of opposing valence to find valence-specific targets. C57BL/6J mice were exposed to three stimuli with distinct valence – sucrose (natural reward; positive); cocaine (drug of abuse; positive), and footshock (negative). Proteome characterization was done by using bulk protein extraction of macrodissected NAc tissue in brain slices, later processed via mass spectrophotometry.

Our results show that sucrose exposure had the lowest impact in proteome changes out of the three stimuli (660 proteins up or down regulated). While having considerable overlap with cocaine, it affected less pathways, which may be linked with fundamentally varying natures of different rewards. Cocaine exposure (4707 proteins up or down regulated) caused changes in general metabolic processes (i.e. mRNA processing, mitochondrial cascades), but also in more specific pathways related with transmembrane glutamate, GABA and dopamine transport. Shock exposure prompted significant proteome changes (1489 proteins up or down regulated); notably basal processes like glycolysis, but also neurotransmitter-related pathways (for example, cholinergic) were shown to be affected by exposure to this aversive stimulus.

Intriguingly, many proteins presented opposite tendencies in shock when compared to cocaine (upregulated in one, downregulated in the other), such as, extracellular vesicle- and exosome-related proteins, and proteins associated with the zinc ion binding pathway.

Here, we present some insights regarding NAc proteome/pathway enrichment after specific-valence stimuli, which might facilitate future studies in valence/internal states.


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