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ABSTRACT BOOKLET

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ABSTRACT BOOKLET

CONTENTS

Bachelard Lecture

A JOURNEY FROM NEUROPEPTIDES TO COVID-19 - A SERENDIPITOUS NEUROCHEMICAL TRAVEL

Turner Anthony John

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As we approach the 50th Anniversary of the European Society for Neurochemistry (ESN), it is appropriate to reflect on the impact of the Society in promoting not only the rapid development of the science but the integration of scientific collaborations across Europe during times of huge political changes. Foremost among the pioneers of neurochemistry, and European scientific collaborations, is Herman Bachelard to whom this Award Lecture is dedicated. I thank him for his friendship and leadership in the field. The Society, along with ISN, has provided the backdrop for my own research directions, collaborations and scientific friendships. Our discovery in Leeds of a cell-surface neutral endopeptidase, neprilysin (NEP), led to the concept of the "neuropeptidase" terminating the action of a variety of regulatory peptides, and subsequently of a key role in the metabolism of the amyloid β-peptide (Aβ) in the brain. In parallel, the vasopeptidase angiotensin converting enzyme (ACE) may also regulate amyloid metabolism in addition to its cardiovascular role. Our subsequent search for human homologues of ACE led to our discovery in 2000 of a related gene and protein now termed ACE2. Since 2020, ACE2 is better known as the cell-surface receptor for the coronavirus initiating COVID-19. ACE2 primarily plays cardio-regulatory roles in the brain and periphery counterbalancing the actions of ACE but can also participate in Aβ metabolism. Together, the peptidases of my research interest are valid therapeutic targets for neurodegenerative and other major diseases. I sincerely hope that ESN will continue to flourish and provide support for neurochemists young and old, as it reaches its major Anniversary. I am grateful to the various granting bodies (UK MRC, BBSRC, ARUK, BHF, the Royal Society UK, INTAS and others) for generous support of my research.

Special Lecture

SITE ACTIVATED MULTI TARGET IRON CHELATOR-ANTIOXIDANT WITH CHOLINESTERASE AND MONOAMINE OXIDASE INHIBITORY MOIETIES FOR ALZHEIMER'S AND PARKINSON'S DISEASES

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Novel therapeutic approaches for the treatment of Alzheimer's (AD) and Parkinson's Diseases (PD) comprise drug candidates designed specifically to act on multiple CNS targets, rather than a single "receptor' as has been done with cholinesterase inhibitors and monoamine oxidase B (MAO-B) inhibitors. One major pathology of AD is the accumulation of iron in nucleus basilus, dentate gyrus, amyloid plaques, and tangles and with PD in substantia nigra pars compacta where the melanized dopamine neuros degenerate. The iron contributes to the onset of oxidative stress and glutaminergic excitotoxicity via interaction with hydrogen peroxide generated by the increased reaction of mitochondrial monoamine oxidase (MAO). We have synthesized several multi target non-toxic, brain permeable site activated iron chelator drugs, pro chelator M30P, M-30 and HLA-20, containing propargyl MAO inhibitory and carbamate pseudo cholinesterase inhibitory moieties. These drugs possess anti apoptotic neuroprotective, and pro-survival neuro rescue activities. They induce neuronal differentiation by the outgrowth of neurites in neuroblastoma, NCS-34 and PC12 cell cultures, via the increase of cell cycle arrest in G0/G1 phase and enhancement of the expression of growth associated protein-43, HIF (Hypoxia Inducing Factor) and the increase of brain levels of BDNF, GDNF, VGEF and erythropoietin. This has been shown to be associated with the inhibition of iron dependent prolyl-4-hydroxylase, which is the cell iron and oxygen sensor, regulating HIF. Both M30 and HLA-20 possess neurorestorative activity in four animal models of PD and restore the cognitive deficit in APP/PSI double transgenic mice, rat transgenic and the streptozotocin (STZ) models of AD. The dual control of mitochondrial biogenesis and energy metabolism is regulated by silent information regulator-1 and -3 (SIRT1 and SIRT3), which deacetylate the peroxisome proliferator activated receptor γ co-activator 1α (PGC-1α), a transcriptional co-activator, which is a central inducer of mitochondrial biogenesis in cells. SIRT1 is necessary for HIF-1α protein accumulation and activation of HIF-1 target genes further activating PGC-1α-mediated transcription of nuclear factor (Tfam) and mitochondrial genes encoding for proteins promoting mitochondrial proliferation. We have demonstrated that M30 and HLA-20 activate SIRT1, PGC-1α, and Tfam in cell cultures and thus consider them as a novel therapeutic approach for neurodegenerative disorders.

Young ScientistLectureship Award I

THE ROLE OF PROTEIN QUALITY CONTROL SYSTEM IN REPEAT EXPANSION NEURODEGENERATIVE DISEASES

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Repeat expansion neurodegenerative diseases (RENDS) are characterized by degeneration of specific neuronal populations. The presence of expanded repeat sequence in C9orf72 and in TATA-binding protein (TBP) genes are responsible for amyotrophic lateral sclerosis (ALS) and spinocerebellar ataxia 17 (SCA17). By repeat-associated non-ATG (RAN) translation, the G4C2 noncoding repeat expansions may also be translated in toxic di-peptide responsible for ALS. Intronic GGGGCC (G4C2) hexanucleotide repeat expansions within the human C9orf72 gene represents the most common cause of familial forms of amyotrophic lateral sclerosis (fALS) and frontotemporal dementia (FTD). Repeat-associated non-AUG (RAN) translation of resulting RNA leads to the production of neurotoxic dipeptide-repeat (DPR) proteins. DPR proteins aggregate into cytoplasm or nuclei of motor neurons, altering the proteotoxic response machinery. The protein quality control (PQC) system maintains protein homeostasis by re-folding or degradation (autophagy or proteasome) of misfolded proteins to counteract proteotoxic events. In parallel, mutations in the ubiquitin ligase STUB1, a gene involved in chaperone-assisted selective autophagy, explain the incomplete penetrance of SCA17 in patients carrying intermediate expansions contributing to misfolded protein accumulation. In C9 ALS models we identified i) forskolin (FSK, a cAMP-elevating compounds) as DPR protein levels enhancer, and ii) geldanamycin (GELD, an HSP90 inhibitor) and spironolactone (SPL, an aldosterone antagonist), as reducer of DPR protein levels. Interestingly, FSK-increased cAMP levels may activate PKA. We demonstrated that PKA blockage (by H89 treatment) or knockdown reduced translation efficiency (polyribosome profile) of DPRs in neuronal cells overexpressing DPR proteins, and in C9ALS/FTD patient-derived iPSC motor neurons with endogenous DPR protein levels. In neurons, we demonstrated that proteasome and autophagy pathways are responsible for proteins degradation in cells treated with GELD and with SPL suggesting that degradative systems and selective modulation of RAN translation can be molecular targets to reduce toxic protein in REND.

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Young Scientist Lectureship Award II

TYPE 2 NEUROIMMUNE DYSFUNCTION CONTRIBUTES TO NEURODEVELOPMENTAL DISORDERS

Guedes Joana [1,2], Ferreira P [1,2], Parker H [3], Soares-Cunha C [4], Reis T [1,2], Costa J [1,2], Lebre C [1,2], Vogelaar C [5], Hepworth M [3], Allen J [3], Cardoso AL [1,2], Rodrigues AJ [4], Zipp F [5], Greenhalgh A [3], Peça J [1,2,6]

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The immune system plays a fundamental role in neuronal development. While microglia are the most abundant immune cells in the brain, other immune populations also play relevant roles in brain development. Although type 1 immune dysfunction has been consistently associated with neurodevelopmental disorders, much less is known on the impact of type 2 inflammation.

We recently showed that low levels of interleukin (IL)-4, a type 2 cytokine that drives allergies, delineates a critical window of postnatal development of the cerebellum during which microglia extensively prune neurons. Elevating the levels of this cytokine in early life was sufficient to block microglia-mediated neuronal pruning leading to lasting microglia alterations, cerebellar circuit deficits, and behaviors compatible with Attention-Deficit/Hyperactivity Disorder (ADHD). Interestingly, an increase in IL-4 later in development had no consequences in these phenotypes.

Knowing that human studies have also strongly linked allergies with ADHD, we are now exploring how allergy-induced IL-4 increase in the cerebellum may lead to hyperactivity via dopaminergic dysfunction in the prefrontal cortex (PFC), a feature of ADHD. Our preliminary data shows that upon airway allergen exposure in early life, a large population of innate lymphoid cells type 2 in the meninges starts producing IL-4 in the critical period of cerebellar development. This population may serve as a bridge between signals from the periphery and the activation of microglia in the cerebellum. Importantly, IL-4-induced cerebellar dysfunction impinges on the activity of dopaminergic neurons in the ventral tegmental area with consequences for *in vivo* release of dopamine in the PFC. Altogether, our work hints at a type 2 neuroimmune dysfunction contributing to ADHD.

S1-01

NEURO-GLIA-IMMUNE MECHANISMS OF SYNAPSE LOSS IN NEURODEGENERATION

Soyon Hong
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Microglia are critical contributors to synapse function and health. One important question is how microglia detect and determine which synapses to eliminate and which ones to spare. Emerging data suggest that microglial cell states, including the synapse phagocytosing ones, are influenced not only by changes in neuronal activity but also by surrounding astrocytes and perivascular macrophages. Further, cell-cell crosstalk influencing synaptic fate can also involve adaptive immune signalling along brain borders. I will discuss potential modulators of microglia-synapse interactions with relevance in neurodegenerative diseases.

S1-02

Mitochondrial complex I activity in microglia sustains neuroinflammation and neurotoxic damage: a novel therapeutic target for multiple sclerosis

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Currently, there are no approved therapies to mitigate the accumulation of neurological disabilities occurring independently of relapses in multiple sclerosis (MS). Chronic, low-grade activation of myeloid cells, particularly microglia, plays a critical role in the pathogenesis of MS. Our research focuses on how these immune cells maintain their inflammatory state through distinct metabolic features, specifically mitochondrial complex I activity, which drives reverse electron transport and reactive oxygen species production. By employing state-of-the-art single cell analyses and multiomics approaches, we identified a molecular signature that sustains microglial activation, perpetuating central nervous system (CNS) inflammation.

Our findings demonstrate that inhibiting mitochondrial complex I in pro-inflammatory microglia protects the CNS from neurotoxic damage and enhances functional recovery in rodent models of MS. Understanding how myeloid cells communicate with and influence brain structure and function underpins our strategy to develop novel therapeutic approaches aiming to slow disease progression and promote tissue healing through enhanced brain plasticity. By targeting mitochondrial complex I activity in microglia, we propose a transformative therapeutic approach that could pave the way for effective interventions against the neuroinflammatory processes underlying progressive MS, ultimately leading to significant neuroprotection and restoration of critical functions in affected individuals.

Selected References

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Disclosure

SP is founder, CSO and shareholder (>5%) of CITC Ltd.

S1-03

THE YIN AND YANG OF ASTROCYTE-NEURON INTERACTIONS IN PARKINSON'S DISEASE: IMPLICATIONS FOR PATHOLOGY AND TREATMENT STRATEGIES

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Alpha-Synuclein (αSyn) plays a central role in Parkinson's disease (PD) and the p.A53T mutation causes an early-onset familial PD form with severe manifestations. The pathological effects of p.A53T-αSyn have been extensively studied in neurons, yet its consequences on astrocytes and their contribution to PD pathology are understudied. We differentiated induced pluripotent stem cells (iPSC) from PD patients carrying the mutation to ventral midbrain astrocytes, and characterized them through comprehensive molecular, biochemical, and functional analyses along with proteome profiling. Astrocytes derived from healthy and gene-corrected iPSCs served as controls. Further, we established neuron-astrocyte co-cultures comprising iPSC-derived control and mutant cells at all possible combinations. Our analyses uncovered cell-intrinsic pathological phenotypes in p.A53T-αSyn astrocytes, including calcium dyshomeostasis, and accumulation of protein aggregates. Proteomic and mechanistic studies revealed perturbed protein catabolic processes, involving proteasome and autophagy pathways, with lysosomal dysfunction and disturbance of mTOR signaling. These impairments affected the endocytic clearance capacity of p.A53T-αSyn astrocytes compromising their ability to process exogenous αSyn cargo. Also, p.A53T-αSyn dopamine neurons co-cultured with p.A53T-αSyn astrocytes displayed hallmark Lewy-like pathologies, similar to those identified in human post-mortem PD brains, and exhibited exacerbated neurodegeneration in both morphological and functional terms. The aggravated neuropathology was alleviated by control astrocytes, suggesting a prominent neuroprotective effect with prospective therapeutic implications. In contrast, p.A53T-αSyn astrocytes exerted a toxic influence on control neurons, inducing PD-relevant neuropathology. Our findings highlight astrocytes as important contributors to PD neuropathology. Additionally, our study presents a two-dimensional co-culture model that reliably reflects key aspects of PD pathology, offering a useful tool for mechanistic and drug discovery studies.

Supported by HFRI Project 1019 DiseasePhenoTarget and the General Secretariat for Research and Innovation action TAA TAEDR-0535850 - Brain Precision.

S1-04

OLIGODENDROCYTES AND NEURONS AS DRIVERS OF AMYLOID-BETA DEPOSITION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, but age as a risk factor is poorly understood. Brain ageing affects oligodendrocytes and the structural integrity of myelin, associated with secondary neuroinflammation. We found that age-associated myelin dysfunction and demyelinating injuries are drivers of amyloid deposition in mouse models of AD. Here, myelin dysfunction increases the accumulation of the Aβ-producing enzymes in axonal swellings and the processing rate of its precursor protein APP in myelinated axons. While amyloid-β (Aβ) plaques are of neuronal origin, APP transcripts and all processing enzymes are also abundant in oligodendrocytes. By cell-specific deletion of *Bace1* in a humanized AD mouse model (*APP*^{NLGF}), oligodendrocytes contribute some 30% of the total Aβ plaque burden in the mouse cortex. However, oligodendrocytes are more abundant in the subcortical white matter where plaques are hardly detectable and have an unusual morphology. This raises the question how oligodendroglial and axonal Aβ are transported in and cleared from myelinated fiber tracts. Ongoing experiments address the role of microglia, astrocytes and oligodendrocyte lineage cells in Aβ turnover and its possible decline in the aging brain.

S1-05

Microglial Trem2 controls neuronal bioenergetics and synaptic function: implications for neurodevelopmental/ neurodegenerative diseases

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Despite the traditional view of the brain as an immunological privileged organ, recent discoveries have revealed that a continuous crosstalk between microglia, the brain residential immune cells, and neurons is required for the maintenance of brain homeostasis and for the sculpting of neuronal connections during development. Indeed, defects in this bidirectional communication have been described in brain diseases, where altered microglia function, synaptic activity and plasticity may produce profound changes in nervous circuits and associated functions.

The Triggering receptor expressed on myeloid cells 2 (Trem2) is a myeloid cell-specific gene expressed in brain microglia, with variants that are associated with neurodegenerative diseases, including Alzheimer's disease. Trem2 is essential for microglia-mediated synaptic refinement, and plays a key role in controlling the bioenergetic profile of pyramidal neurons during development. Indeed, in the absence of Trem2, developing neurons in the hippocampal CA1 but not CA3 region, display compromised energetic metabolism, accompanied by a transcriptional rearrangement that include a pervasive alteration of metabolic and synaptic signatures, ultimately leading to a delay in the maturation of CA1 pyramidal neurons. Such early derangement is maintained at later developmental windows, leading to synaptic and circuitry alterations. How Trem2 modulates microglia-neuron communication, however, remains to be defined. Thus, we are addressing whether the lack of Trem2 and consequent modification of the microglial state result in altered secretome, which in turn would differentially affect neuronal trajectories. Also, microglia-to-neuron contacts occurring during early development may play a role, with Trem2 localized at the microglial membrane binding to immature neuronal partners and influencing their trajectories. Altogether, understanding the molecular mechanisms underlying TREM2 mediated regulation of neuronal functions will pave the way for future therapeutic strategies to tackle both neurodevelopmental and neurodegenerative pathologies.

S2-01

Modeling ADNP Syndrome: From Peripheral Biomarkers to Sex-Divergent Neurogenesis and Rescue

Karmon Gidon, Illana Gozes

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Activity-dependent neuroprotective protein (ADNP) is a master regulator of brain development, gene expression, autophagy, and synaptic integrity, first discovered in our laboratory, with de novo mutations in *ADNP* causing a syndromic form of autism. In our 2022 study (Karmon et al., *Biological Psychiatry*, 2022), we generated a novel CRISPR/Cas9-edited mouse model harboring the most prevalent human *ADNP* syndrome mutation, p.Tyr718* (Tyr), and revealed dramatic sex-dependent alterations in peripheral and central phenotypes. Tyr mice displayed developmental delays, autistic-like behaviors, gait abnormalities, dendritic spine deficits, and early-onset tauopathy. These were paralleled by profound sex-specific transcriptional changes in the spleen and gut microbiota composition. Notably, treatment with the ADNP-derived peptide NAP (davunetide) corrected many of these deficits, with FOXO3 identified as a key ADNP/NAP-regulated gene bridging autophagy, synaptic plasticity, and microbiome resilience.

Building upon these findings, our 2024 study (Shapira et al., *Molecular Psychiatry*, 2024) demonstrated that ADNP governs sex-dependent hippocampal neurogenesis. Using BrdU labeling, we showed that wild-type males exhibit significantly higher neurogenesis than females, a difference lost in both *Adnp* haploinsufficient and Tyr mutant mice. Tyr males showed marked downregulation of unfolded protein response (UPR) genes, while females exhibited impaired mitochondrial gene expression and reduced ADNP mitochondrial localization, correlating with decreased mt-ATP6 splicing. Again, NAP (davunetide) treatment reversed many of these transcriptional changes, promoting neurogenesis and suppressing pro-apoptotic and neuroinflammatory gene expression. Together, these studies establish ADNP as a critical sex-dependent regulator of neurodevelopment and neurogenesis and support NAP (davunetide) as a promising therapeutic agent for ADNP syndrome and related neurodevelopmental disorders, currently in active commercialization (ExoNavis).

S2-02

The Helsmoortel-Van der Aa syndrome: a molecular perspective bridging patient-derived cell models, mice, and patients.

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In 2014, our lab identified *de novo* variants in the *Activity-Dependent Neuroprotective Protein (ADNP)* gene as the cause of Helsmoortel-Van der Aa syndrome (OMIM, 615873) through molecular inversion probes and whole-exome sequencing. Patients present with complex clinical features, including facial dysmorphisms, neurological impairments, behavioral abnormalities, and systemic defects. Advances in diagnostic tools now enable detection via two distinct methylation signatures, correlating with variant location and clinical severity.

We generated a CRISPR/Cas9-based mouse model carrying the c.2463_2476del/p.Leu822hisfsX6 variant, mirroring a prevalent human frameshift variant. Analyses revealed hallmark behavioral phenotypes, mild neuroanatomical changes, and Adnp's pivotal role in chromatin regulation. Impaired WNT signaling and cytoskeletal defects were found to underlie synaptic dysfunction and neurodevelopmental delays.

Upon examinations of post-mortem cerebellar tissue from a 6-year-old patient with the c.1676dupA/p.His559GlnfsX3 variant, we observed conserved perturbations in WNT signaling and cytoskeletal regulation. ADNP was also shown to complex with SIRT1 via microtubule-end binding proteins, regulating mitochondrial gene expression and energy homeostasis. Further insights came from a girl diagnosed through EPISign with a *de novo* splice-acceptor site variant in *ADNP*, and dizygotic twins with one diagnosed with the syndrome. These cases reinforced findings from our murine model, highlighting disruptions in actin filament organization, WNT signaling, embryonic and cardiac development, and immune regulation.

This multidisciplinary approach validates our *Adnp* frameshift mouse model as a preclinical model of Helsmoortel-Van der Aa syndrome. Concordance between murine transcriptional profiles, patient-derived methylation signatures, and post-mortem neuropathology underscores ADNP's role as a central regulator of brain development, providing mechanistic insights for precision therapies in neurodevelopmental disorders.

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S2-03

DECODING A PIVOTAL ROLE OF ADNP IN MICROGLIAL POLARIZATION AND METABOLIC REPROGRAMMING: IMPLICATIONS FOR NEURODEGENERATION

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The ADNP protein (Activity-Dependent Neuroprotective Protein) has been identified as an essential factor throughout the embryonic development of the CNS as well as in cognitive function. Some studies have shown the high prevalence of ADNP mutations in certain groups of patients with syndromic autism spectrum disorders (ASD) and aberrant expression of ADNP seems to be related to the development of neurodegenerative diseases such as Alzheimer's or Parkinson's disease. ADNP regulates various biological processes chromatin architecture, gene expression and RNA maduration, axonal transport, and dendritic spines and actin filaments. However, it remains largely unclear how ADNP deficiency sustains microglia physiological functions and its link to brain dysfunctions. With this aim, we generated ADNP-KO microglial cells, which were found to exhibit key alterations in both MyD88-dependent and independent inflammatory pathways. ADNP-KO cells also displayed overactivation in TANK-binding kinase 1 signaling, pAMPK regulation and defective autophagy/mitophagy mediators as well as impairment in mitochondrial real-time metabolic features. Metabolomic analysis revealed key regulatory events in agreement with glycolytic rate assays and lipid metabolism. ChIPseq and RNAseq disclosed the role of ADNP as a chromatin remodeler involved in innate immune microglial responses. Conditional media from ADNP-KO microglial cells impaired viability of primary motoneuron cells affecting pKap1, pChk1 and p-gammaH2AX levels linking ADNP-mediated effects to genomic instability mechanisms in neurons. These results suggest a physiological role of ADNP as an inflammatory repressor protein in microglia involved in metabolic microglial rewiring. Moreover, our results in animal models might help reveal altered neuroinflammatory responses in neurological and neurodegenerative diseases associated to ADNP functional status.

S2-04
ADNP/DAVUNETIDE (NAP) PROTECTION OF THE EYE

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University of Catania

The eye is formed by three distinct layers: the fibrous, vascular, and nervous layers. The fibrous tunic comprises the sclera and the cornea. The nervous layer is the retina, where light signals are converted into action potentials that travel to the brain through the optic nerve.

Activity-dependent neuroprotective protein (ADNP) is expressed in the eye, in particular in the retina and cornea of different species, including humans. NAP (or davunetide), a short peptide derived from the ADNP sequence, plays a protective role in the eye. In particular, it counteracts retinal damage induced by diabetic retinopathy (DR), a microvascular complication of diabetes. The hyperglycemic/hypoxic microenvironment contributes to aberrant angiogenesis, which characterizes this pathology, and activates many downstream target genes, including interleukin-1β (IL-1β) and vascular endothelial growth factor (VEGF).

Especially, the hypoxic event induces an over-release of VEGF, which is responsible for aberrant neo-angiogenesis leading to blood-retinal barrier (BRB) breakdown. Treatment with NAP has been shown to preserve the integrity of the outer BRB by counteracting the expression of hypoxia-inducible factors (HIFs) as well as VEGF in human retinal pigment epithelium in both *in vivo* and *in vitro* models of DR. The hyperglycemic/hypoxic event also promotes the release of inflammatory cytokines, which contribute to the impairment of the BRB. NAP treatment has been shown to modulate the inflammatory response during the early phase of DR. In fact, the intravitreal administration of NAP interfered with the expression of IL-1 family members. Moreover, the peptide preserved the outer BRB integrity after hyperglycemic-inflammatory insult in an *in vitro* model of DR.

The expression of ADNP has also been observed in the human and rabbit corneal epithelium, which represents the outermost layer of the cornea and acts as a barrier to protect the eye against external insults such as ultraviolet B (UV-B) radiation. NAP treatment prevents UV-B-induced inflammatory processes by affecting IL-1β cytokine expression and oxidative stress, as well as maintaining corneal epithelial barrier integrity. All this evidence suggests the potential use of NAP for the treatment of certain ocular diseases.

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S3-01

Extracellular vesicle dependent regulation of synaptobrevin/VAMP recycling in synapses

Ege Kavalali, Vanderbilt University, USA

Our group studies mechanisms underlying synaptic function, signaling and plasticity in health and disease. We have particularly focused on the role of SNAREs (Soluble N-ethylmaleimide-Sensitive Factor Attachment Protein Receptors) in regulation of distinct forms of neurotransmission. We recently found that the critical synaptic vesicle associated SNARE molecule synaptobrevin (also called VAMP) can be trafficked across neurons via neuronally derived extracellular vesicles. Interestingly, this extracellular pool of synaptobrevin can be rapidly integrated into the synaptic vesicle cycle of host neurons and alter neurotransmission. In this presentation, I will discuss our insight into this finding and its implications for synaptic function.

S3-02

MICROGLIAL EXTRACELLULAR VESICLES PROMOTE SYNAPTIC PRUNING VIA C1Q DEPOSITION AT PRE-SYNAPSE

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The complement factor C1q is released by microglia, localizes on weak synapses and acts as a tag for microglia-mediated synaptic pruning, a fundamental process for proper circuit refinement. However, how C1q tags synapses at specific time points during development remains elusive. Our previous studies indicate that extracellular vesicles (EVs) released by microglia carry C1q and move at the neuron surface, more efficiently along axons than dendrites, where EVs often stop and may deliver their cargo. On this basis we asked whether microglial EVs may act as carriers for C1q deposition to synapses designated for removal.

We optimized a protocol to extract EVs from WT and C9orf72KO mouse brains, displaying excessive synaptic pruning, and by Western Blot, TRPS and SimoA we measured their amount, cell source and complement cargo. Furthermore, we quantified C1q synaptic deposition and microglial synaptic engulfment using approaches to increase or reduce microglial EVs production by EVs supplementation, C9orf72 silencing or pharmacological inhibition (GW4869).

We show that microglia release more EVs carrying C1q in C9orf72KO compared to WT adult mice and that C1q secretion by means of EVs physiologically peaks during the pruning period in the early postnatal hippocampus (P17). In neuron-microglia co-cultures, microglial EVs make preferential contacts with synapses, deliver C1q to pre-synapses that externalize phosphatidylserine, and promote synaptic engulfment. Finally, C9orf72 KO microglia engulf more synaptic terminals and decrease synaptic density to a greater extent compared to WT microglia whereas inhibition of EVs release by GW4869 restores normal pre-synaptic density, providing mechanistic evidence linking EVs release to synaptic remodeling.

This study identifies microglial EVs as delivery vehicles for C1q to synapses targeted for removal and implicates abnormal EVs production from microglia in neurodevelopmental and age-related disorders characterized by dysregulated synaptic pruning. Analysis of specific subpopulations of brain EVs opens new perspectives to the understanding of EVs roles in physiology and pathology.

this study is supported by PRIN 2022-3CHCYX

S3-03

Chronic inflammation in neurodegenerative and infectious diseases: extracellular vesicles as friend or foe?

Kenneth W. Witwer

Johns Hopkins University, United States

Extracellular vesicles are thought to serve as biomarkers and therapies for disease, but what are their native roles in neurodegeneration? In this presentation, we will examine EVs as both friend and foe in neurodegenerative diseases. Since much of what we know about EVs in the brain is derived from cell culture models and peripheral samples, it is important to understand what is present in tissues. Methods for separation and characterization of brain tissue EVs will be presented along with results from several diseases and disease models. We then turn to therapeutics: how can we deliver EVs to the brain? Do rodents adequately model human physiology? What might be needed to ensure efficient delivery of EV therapeutics to the central nervous system?

S3-04

Extracellular Vesicles as Biomarkers in Central Nervous System Diseases

Anja Schneider
German Center for Neurodegenerative Diseases (DZNE), Germany

Neurodegenerative diseases are characterized by pathological mislocalisation, misfolding and aggregation of different proteins. Extracellular vesicles (EVs) have been shown to propagate disease pathology, e.g. misfolded proteins or inflammatory mediators, between neurons. In addition, EVs can protect their protein content from degradation and CNS derived EVs have been identified in cerebrospinal fluid but also blood. Here, we discuss the potential of EVs and their protein content to serve as potential biomarkers in different neurodegenerative diseases, in particular amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD).

S4-01

THE INVOLVEMENT OF ASTROCYTE CALCIUM-DEPENDENT SIGNALING IN FEAR MEMORY

João Filipe Oliveira

Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Portugal

The advent of calcium imaging techniques revealed that astrocytes are dynamic cells and active players in regulating brain circuit function. They display a complex morphology that allows them to set fine processes close to synapses. Activation of astrocytic receptors by transmitters and other modulators triggers intracellular calcium elevations that are thought to underlie synaptic transmission, metabolism, and brain homeostasis modulation. However, the calcium-dependent signaling pathways involved in these processes are poorly understood, representing a critical knowledge gap in this field.

To reveal calcium-dependent signaling pathways involved in circuit modulation, we performed a multi-level analysis of the IP3 receptor 2 knockout (IP3R2 KO) mouse model, which lacks calcium elevations, specifically in astrocytes. We focused on the hippocampus, a brain region responsible for cognitive function and emotional behaviors. A transcriptomic analysis indicated alterations in Foxo1-regulated genes related to structural modulation. We found that hippocampal pyramidal neurons of the IP3R2 KO display dendrites with a shift to a more immature spine profile. This spine profile shift may underlie previously described reduction of long-term depression and performance in fear memory tasks. Indeed, we confirmed that this model displays an enhancement of long-term fear memory.

To verify a causal relationship between these structural, synaptic, and behavioral observations, we drove the over-expression of Foxo1 in hippocampal astrocytes. This manipulation replicated the shift to an immature spine profile in pyramidal neurons and led to the reduction of long-term depression in the same region. Finally, this manipulation was sufficient to enhance long-term fear memory.

The detailed characterization of the mouse model lacking astrocytic somatic calcium revealed that astrocytes modulate hippocampal circuit structure and function through Foxo1 signaling to enhance fear memory.

S4-02
CATCHING ASTROCYTE ENSEMBLES: THEIR ROLE IN MEMORY FORMATION AND EXPRESSION

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To understand how complex cell circuits process information, it is necessary to use techniques that can precisely target and modulate the activity of the involved elements. Neuro-astrocyte networks are highly complex, and understanding their involvement in circuit modulation and behavior requires state-of-the-art complementary tools. Although most genetic tools have focused on neuronal activity, my talk will introduce newly adapted techniques from our laboratory that can dissect active astrocyte circuits with spatio-temporal precision, such as CaMPARIGFAP and Astro-Light. Additionally, I will present our recent data on mapping the functional astrocytic circuitries in the Nucleus Accumbens (NAc), which reveal the existence of specific astrocyte circuits in this region. Finally, we will show that activating the astrocyte ensemble related to a specific reward can shift behavior towards that option through optogenetic/chemogenetic stimulation. Overall, the cutting-edge data I will present supports the idea that NAc astrocytic networks play a critical role in integrating information.

S4-03

Bridging layers: how astrocyte networks boost tactile encoding and sensory integration.

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Cortical sensory processing relies on information transfer across layers, following a pathway where thalamic input reaches L4, transfers to L2/3, and then to L5 before exiting the cortex. While neuronal architecture and synaptic strength regulates this process, astrocytes also may play a role by forming large, connected networks capable of influencing neuronal signalling. In this talk, I will present our recent on how cortical astrocytes contributes to information transfer across cortical circuits and its impact on sensory behaviour. Using in vivo miniscope imaging, electrophysiology and behavior assessment, our results reveal that astrocytes strongly influence encoding behaviour and tactile detection threshold. They do that by facilitating translaminar integration through a functional network spanning from L2/3 to L5 and by controlling the activation of tactile encoding neuronal ensembles. Therefore, astrocytes serve as a complementary mechanism for maintaining information transfer and neuronal activation, contributing to cortical computation and transformation across layers.

S4-04

ASTROCYTE TRANSCRIPTION FACTOR-BASED SIGNALING SHAPES MOUSE SOCIO-SEXUAL BEHAVIOR

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Social behaviors are crucial for the survival and health of many species including humans. These innate behaviors rely on the activation of well-described brain circuits where the role of astrocytes remains overlooked. Here, we showed that the transcription factor-based signaling JAK-STAT displays brain region-specific and activity-dependent activation in astrocytes after social interaction. Replicating behaviorally-induced JAK-STAT activation through injection of astrocyte-targeted viral vectors increased local neuronal excitability and brain-wide activation of limbic regions involved in social interaction. At the behavioral level, we found that the activation of JAK-STAT signaling in astrocytes selectively enhanced male behaviors toward females, including social preference and mating attempts. Together, these data define a role for astrocyte transcription factor-based signaling in shaping mouse socio-sexual behavior.

S5-01

NOVEL DNA SEQUENCING APPROACHES IN SYNUCLEINOPATHIES

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Synucleinopathies are neurodegenerative disorders characterised by aggregation of alpha-synuclein. They include Parkinson's disease (PD), the second commonest neurodegenerative disorder, and the closely related Lewy body dementia, where aggregation is predominantly in neurons, and Multiple System Atrophy (MSA), with predominantly oligodendroglial aggregation. PD is rarely familial, although even sporadic cases have significant heritability, while MSA is a sporadic disorder with apparently negligible heritability.

Traditional genetic approaches including linkage analysis, genome-wide association studies of common polymorphisms, and exome sequencing, have identified several causative PD genes, and over 90 risk loci. As the cost of whole genome sequencing (WGS) has decreased, short read WGS is being used in major national and international PD cohorts, still predominantly geared towards detecting single nucleotide variants (SNVs). Identification of genomic copy number variants (CNVs) and other structural variants (SVs), of which each individuals harbours >20,000, can also be attempted from such data. A more thorough picture of SVs, however, is obtained by long read WGS, which can also provide methylation data.

These approaches are focused on germline mutations, but the prevalence of brain mosaicism caused by somatic mutations is increasingly appreciated. Their study requires sequencing of brain tissue, either using high coverage, to detect mutations present only in a fraction of cells, or indeed of single cells after whole genome amplification. Strategies can be targeted or WGS, using short or long read approaches. With respect to somatic CNVs, gains of the alpha-synuclein gene have already been detected in PD and MSA brains using cytogenetic approaches, but large somatic CNVs can be detected genome-wide in single cells by low coverage short read WGS. My lab is compiling single cell CNV profiles from 4,000 PD and control cells, and the data will be presented. Long read single cell WGS also has the potential to detect all variant types in a single cell, but its current limitations due to chimeras which appear as true SV will be discussed.

S5-02

Human brain organoids: understanding the role of the “niche” in neuroregeneration

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Human brain organoids, three-dimensional cell culture models derived from human pluripotent stem cells, offer unique insights into brain development and regeneration. By mimicking key aspects of human brain development, these organoids provide a valuable platform to investigate the role of the neural stem cell "niche" composed of supporting cells, extracellular matrix and hormones. Placental hormones play a crucial role in guiding neural cell fate and promoting regenerative processes. Corticotropin-Releasing Hormone (CRH) is a placental hormone that is secreted in large amounts and in a time-dependent manner during pregnancy only in anthropoid primates. The developmental role of placental CRH as well as the biological significance of its unique expression pattern in anthropoid primates remains elusive. In order to investigate the effects of placental CRH on human brain development and to overcome the limitations raised in experimenting with human tissue, we have generated human 3D-neural spheroids and human cerebral organoids from human embryonic stem cells (hESCs). Exposure of neural spheroids or/and cortical organoids to CRH results in significant differences in their size and cellular composition. In addition, immunohistological analyses using cortical layer-specific antibodies, revealed differences in the cytoarchitecture of the organoids exposed to CRH as compared to control. Pharmacological disruption of CRH signaling using the specific CRH receptor 1 antagonist, NBI, reverses the effects of CRH. In addition, RNA sequencing analysis of the CRH and NBI exposed organoids revealed altered expression of genes related to neurodevelopmental processes such as HOXB9 and FOXG1, depicting CRH as an essential modulator of human brain development. Our findings suggest that this in vitro approach provides a unique tool for our understanding of the mechanisms underlying the role of placental hormones in human brain development and facilitates the study of neuro-regeneration strategies, allowing researchers to explore potential therapies for neurodegenerative diseases.

S5-03

Engineered microphysiological systems in the study of neurodegenerative diseases

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The goal of most biomedical research is to gain greater insight into mechanisms of human disease and to develop improved therapies or diagnostics. Despite the great advances in developing disease models in animals, the species differences result in limited translatability of experimental findings to successful therapies for fighting the brain diseases epidemic. Relevant human cell-based models in engineered microphysiological systems (MPS), such as Organ-Chips, are emerging as a promising approach to address these challenges. MPS combined with human inducible pluripotent stem cells and organoids enable new insights into cell-specific contributions and cell-cell underlying pathogenesis, while providing a test bed for screening of new therapies. We will review the basic principles of this preclinical approach, provide some examples of its potential based on published, relevant models, discuss benefits and challenges and finally discuss how they are positioned to support transition to minimal use of animals in drug discovery and development.

S5-04

PROTEOLYTIC ACTIVITIES OF EXTRACELLULAR VESICLES ATTENUATE A-SYNUCLEIN AGGREGATION

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Secreted α-synuclein (α-syn) has been implicated in the transmission of pathology in Parkinson's Disease (PD) brain in a manner that resembles prion protein transmission. Although the exact mechanisms that account for the clearance of extracellular α-syn species are not fully elucidated, there is accumulating evidence that these mechanisms include proteolysis by extracellular proteases, as well cell-mediated uptake and intracellular degradation. In this respect, we have previously shown that α-syn species can be secreted in association with extracellular nano-sized vesicles of endosomal origin termed extracellular vesicles (EVs). Nevertheless, the possible role(s) of EVs in neurodegenerative disorders remain elusive. Emerging evidence indicates that active proteases are associated with EVs. We focused on EVs as a potential modifier of extracellular α-syn and sought to assess their effect on α-syn degradation and fibrilization. We suggest that cathepsins present in brain EVs are most likely involved in α-syn cleavage. It is possible that the α-syn cleaving enzymes associated with EVs represent a novel mechanism for brain cells to control the α-syn levels in the extracellular space. In this regard, degradation of extracellular substrates by enzymes associated with intact EVs represents a new area of investigation and elucidating their role will shed light as to how they could regulate disease initiation and progression. Proteolytic activities could be stored in EVs and liberated with various stimuli during physiological tissue remodeling providing a novel way to regulate pericellular proteolysis Our data raise the possibility that cellular proteases associated with EVs may be a novel mechanism involved in defective clearance of extracellular α-syn, which opens the possibility to exploit EV proteases as novel therapeutic targets for synucleinopathies.

S6-01
Mitochondrial RNA hitch-hiking in neurons

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Local maintenance of mitochondria is a crucial mechanism to counteract insults to this key organelle in neurons. A central role in mitochondrial quality control is played by the Parkinson-related mitochondrial kinase PINK1, whose mRNA is transported in neurons by mitochondrial hitch-hiking. Using a live-cell imaging assay for the visualization of the localization of the PINK1 precursor, we show that local translation of PINK1 requires a concerted interplay between three organelles. While mitochondria carry the Pink1 mRNA and receive the final protein product, the mRNA can be untethered due to disassembly of the anchoring complex downstream of insulin signaling. The mRNA then relocates to the endolysosomal compartment, whose degradative function is needed for PINK1 synthesis. The endoplasmic reticulum at this contact site contributes membrane-tethered chaperones, enabling the transfer of the newly synthesized PINK1 precursor to mitochondria in the ER-SURF pathway. The entirety of this complex multi-organelle mechanism is necessary for PINK1's ability to demarcate damaged mitochondria and target them for mitophagy in neurons.

S6-02

Mechanisms supporting localised translation of mitochondrial proteins in axons

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Mitochondria are dynamic and plastic organelles, which flexibly adapt morphology and metabolic function to meet extrinsic challenges and demands. Regulation of mitochondrial biogenesis is essential during development and in adult life to survive stress and pathological insults. Post-transcriptional mechanisms to modulate mitochondrial function play crucial roles in neuronal homeostasis. Owing to the extraordinary architecture of neurons with long axonal projections, mitochondria face several challenges to maintain their quality and to adapt their function to changing metabolic demands. CLUH is an RNA-binding protein specific for a subset of transcripts encoding mitochondrial proteins involved in OXPHOS, amino acid degradation, TCA cycle, and ketogenesis. We will discuss the role of CLUH in axonal maintenance, and reveal novel post-transcriptional mechanisms controlling the fate of nuclear-encoded mRNAs for mitochondrial proteins in long motor axons.

S6-03

THE AXONAL ER IN LOCAL TRANSLATION AND UNCONVENTIONAL PROTEIN SECRETION

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Local mRNA translation in axons is critical for the spatiotemporal regulation of the axonal proteome. A wide variety of mRNAs are localized and translated in axons; however, how protein synthesis is regulated at specific subcellular sites in axons remains unclear. Here, we establish that the axonal endoplasmic reticulum (ER) supports axonal translation in developing rat hippocampal cultured neurons. Axonal ER tubule disruption impairs local translation and ribosome distribution. Using nanoscale resolution imaging, we find that ribosomes make frequent contacts with axonal ER tubules in a translation-dependent manner and are influenced by specific extrinsic cues. We identify P180/RRBP1 as an axonally distributed ribosome receptor that regulates local translation and binds to mRNAs enriched for axonal membrane proteins. Importantly, the impairment of axonal ER-ribosome interactions causes defects in axon morphology. In addition, we have recently found an important role of the axonal ER in unconventional axonal secretion of newly synthesized membrane proteins. Our results establish a role for the axonal ER in dynamically localizing mRNA translation for local protein secretion, which is important for proper neuron development.

S6-04

UNVEILING ASTROCYTE LOCAL TRANSLATION—A FUNDAMENTAL MECHANISM FOR ASTROCYTE FUNCTIONAL COMPARTMENTALIZATION

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Astrocytes are a type of glial cell in the brain characterized by pronounced morphological, molecular, and functional compartmentalization. Their extensive branching enables close interactions with both neurons and blood vessels. These contacts are equipped with specialized molecular repertoires, allowing astrocytes to regulate the development and function of both neurons and the brain vasculature. Despite growing interest in astrocytes within the fields of brain physiology and pathology, their molecular and functional organization remains poorly understood. Our lab recently demonstrated that astrocytes organize local translation in their distal processes and proposed that this mechanism is critical for establishing and maintaining their functional compartmentalization—similar to what has been well described in neurons, where mRNA trafficking and local translation enable rapid responses to stimuli. We identified nascent protein chains and polysomal mRNAs enriched in astrocytic perivascular processes. This work allowed us to identify and study several proteins that regulate critical vascular functions, such as maintaining blood-brain barrier (BBB) integrity and regulating drainage and blood flow. Additionally, we isolated a polysomal mRNA repertoire from astrocytic perisynaptic processes in the dorsal hippocampus and demonstrated its regulation in memory and learning. Furthermore, our exploration of ribosome diversity in astrocytes revealed that the ribosome-associated protein RACK1 regulates astrocytic potassium currents through the translational control of the potassium channel Kir4.1. These findings suggest that local translation underlies the molecular, morphological, and functional compartmentalization of astrocytic distal processes.

S7-01

UNLOCKING NEUROIMMUNE DRIVERS OF NEURODEGENERATION: MECHANISMS AND THERAPIES

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The communication between the brain, immune and vascular systems is a key contributor to the onset and progression of neurological diseases. We identified the coagulation factor fibrinogen as a blood-derived driver for neuroinflammation in a wide range of neurologic diseases, such as multiple sclerosis, Alzheimer's disease, COVID-19 neuropathology and brain trauma. By developing cutting-edge two-photon imaging tools and chemogenetic models, we discovered a role homeostatic role for microglia in brain network synchronization. We reported the transcriptomic and global phosphoproteomic landscape of blood-induced microglia polarization and the selectivity for fibrin driving neurotoxic innate immune responses. We developed a first-in-class fibrin-targeting immunotherapy to selectively target neurotoxic immune activation by neutralizing the fibrin inflammatory domain without adverse anticoagulant effects with efficacy in autoimmune- and amyloid- driven neurodegeneration, as well as infectious models of COVID-19 neuropathology. These findings could be a common thread for the understanding of the etiology, progression, and development of new treatments for neurologic diseases with neuroimmune and cerebrovascular dysfunction[1].

[1]Akassoglou et al. Pioneering discoveries and therapeutics at the Brain-Vascular-Immune interface **Cell** (2024) 187:5871-5876.

S7-02

Immunotherapy harnesses immune cells to defeat Alzheimer's disease and other forms of dementia

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We previously demonstrated that the brain is tightly dependent on innate and adaptive immune cells for its maintenance and repair. Deep understanding of these relationships led to the discovery that the brain's borders, including the blood-cerebrospinal fluid barrier and the meninges, provide niches for blood-borne immune cells, from which they remotely affect the brain, and provide a gateway for leukocyte entry to the brain, upon need. These immune cells, located in specialized niches at the brain's borders, along with neurons and non-neuronal cells within the brain parenchyma, create an ecosystem that enhances the brain's resilience to continuous and diverse perturbations. Accordingly, we proposed that symptom onset in Alzheimer's disease (AD) could be linked with dysfunction of brain-immune system communication. Thus, while immune dysfunction is not the primary cause of the disease, such a defect could promote the transition to symptom onset. We therefore proposed that activating the systemic immune system in a well-controlled way could help restore immunity to mitigate neurodegenerative diseases. We found that transiently blocking the inhibitory PD-1/PD-L1 immune checkpoint pathway initiates an immune response in the periphery that facilitates homing of immune-repairing cells to the brain, including monocyte-derived macrophages and FoxP3 regulatory T cells, together mitigating multiple pathologies within the diseased brain. The pathologies affected by the treatment include local inflammation, accumulation of toxic proteins, and neuronal loss, and their modification arrests cognitive deterioration regardless of the primary cause of the disease. Our studies demonstrate that targeting the immune system opens new avenues for understanding and treating neurodegenerative diseases. This approach is currently being evaluated in a clinical trial involving AD patients, utilizing a novel anti-PD-L1 therapy specifically tailored to AD, based on its mechanism of action.

S7-03

HIPSC-BASED MODELS TO INVESTIGATE THE CONTRIBUTION OF HUMAN ASTROCYTES IN AGE-RELATED BRAIN DISEASES

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The study of human astrocytes is essential for understanding brain diseases, as their unique complexity and human-specific molecular profiles may play critical roles in disease mechanisms that cannot be fully replicated in animal models. Human-induced pluripotent stem cell (hiPSC)-based models provide powerful platforms to investigate these mechanisms, offering an opportunity to explore astrocyte function and dysfunction in a physiologically relevant context.

To this end, we are developing a range of hiPSC-based models—including 2D cultures, brain-on-a-chip systems, and 3D organoids—to study astrocyte involvement in neuroinflammation and disease progression. Our work has led to the identification of *Transgelin 3* (TAGLN3) as a potential key regulator of astrocytic reactivity. We have uncovered a possible link between TAGLN3 and Alzheimer's disease, and some of our ongoing works aim to further elucidate its role in astrocyte function and neurodegeneration. Additionally, our models serve as tools to study dynamic cellular changes contributing to brain aging. For instance, our preliminary findings suggest that hiPSC-derived astrocytes exhibit functional immune memory, aligning with the concept of glial priming, which has been proposed to increase susceptibility to age-related neurodegenerative mechanisms.

In this talk, I will present our hiPSC-based models and discuss how their use can provide novel insights into astrocyte biology, neuroinflammatory responses, and neurodegeneration. By leveraging these innovative platforms, we aim to enhance our understanding of astrocyte contributions to brain disorders, and pave the way for the development of targeted therapeutic strategies.

Acknowledgement

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S7-04

The Alzheimer's Disease risk factor BIN1 is a regulator of glial cell response to neuroinflammation

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In addition to environmental factors, such as ageing and inflammation, Alzheimer's Disease (AD) pathogenesis has a strong genetic component. GWAS have identified Bridging Integrator 1 (BIN1) as the second strongest Risk Factor for developing AD after APOE. BIN1 is an adaptor protein implicated in cell membrane modelling dynamics, highly expressed in neurons and microglia of rodent and human brain. Neuronal BIN1 has been linked to Tau pathology and endocytic pathway defects, however the contribution of microglial BIN1 in AD remains elusive. Using single-nucleus RNA-sequencing of microglia-specific BIN1 conditional knockout (cKO) mouse cortex, we have recently shown that microglial BIN1 is sufficient to alter the expression of key genes regulating microglial reactivity in response to systemic inflammation, with most prominent being the IFN type I pathway. Interestingly, we also found that microglial BIN1 cKO imposes transcriptional changes in the expression of Stat1, complement proteins' and other AD Risk Factors' in astrocytes, implying that microglial BIN1 deletion also exerts a non-cell autonomous impact on astrocytes reactivity. Further analysis of the impact of microglial BIN1 deficiency in the hippocampal brain area, revealed region-specific differences in microglia reactivity in response to inflammation, along with a non-cell autonomous role of microglial BIN1 in regulating adult hippocampal neurogenesis. Taken together our data indicate that deletion of microglial BIN1 promotes the enrichment of proinflammatory microglial subpopulations in response to inflammation, while it elicits a region-specific non-cell autonomous effect on astrocytic and neuronal populations.

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S8-01

INTERACTIONS BETWEEN CANNABINOIDS AND THE KYNURENINE PATHWAY WITH A SPECIAL FOCUS ON KYNURENIC ACID

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Natural and synthetic cannabinoids, can exacerbate psychotic symptoms and potentially increase the risk of developing schizophrenia, particularly in individuals with genetic predispositions or environmental risk factors. In addition, exposure to cannabinoids during sensitive windows of neurodevelopment (i.e. gestation/adolescence) induces learning and memory disruptions, associated with permanent alterations of cortical glutamatergic neurotransmission and cognitive deficits. However, little is known about the mechanisms underlying these detrimental effects. Remarkably, the long-term neurochemical and behavioral consequences of gestational/adolescent cannabinoids are qualitatively very similar to those caused in developing rodents by experimental increases in brain kynurenic acid (KYNA), the major tryptophan metabolite. As KYNA is increasingly viewed as a major player in cognitive dysfunction in individuals with schizophrenia, we hypothesized that KYNA alterations might be involved in long-term cognitive deficits and in the increased risk to develop psychosis associated with early cannabinoid exposure. The first preclinical and clinical evidence supporting the plausibility of this hypothesis will be presented. To date, the possible causal relationship between cannabinoids, KYNA and cognition is under investigation in our laboratory by investigating the impact of natural and synthetic cannabinoids (i.e. delta9-THC and JWH-018, respectively) at different neurodevelopmental windows, as well as the possible efficacy of brain KYNA lowering agents in preventing the long-term detrimental effects associated with neurodevelopmental cannabinoid exposure.

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S8-02

Impacts of maternal cannabis use on long-term psychiatric risk: the promise of interventions targeting the Omega-3 fatty acid signaling network

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With rising rates of maternal cannabis use, there is an urgent need to understand the potential long-term neuropsychiatric risks for offspring, following exposure to cannabinoids. Our research program is exploring how exposure to the major constituents of cannabis, THC and cannabidiol, may adversely impact the developing fetal brain. We use a combination of pre-clinical rodent models of maternal cannabinoid exposure and human cerebral organoid models of early fetal brain development. This presentation will summarize our research into how prenatal cannabinoid exposure can impact the developing neurolipidome and specifically, how prenatal cannabinoid exposure can induce pathological imbalances between the brains Omega-3 and Omega-6 signaling pathways, leading to long-term oxidative stress, neuroinflammation and dysregulation of dopaminergic, GABAergic and GLUTergic neurotransmission in the corticolimbic circuitry. We will describe how prenatal cannabinoid exposure has profoundly different effects on the developing male vs. female brain, and how a dietary intervention with Omega-3 enrichment might prevent some of these long-term mental health risks for offspring.

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S8-03

NEUROBIOLOGICAL SEQUELAE OF THE EXPOSURE TO SYNTHETIC CANNABINOID RECEPTOR AGONISTS DURING ADOLESCENCE

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Synthetic cannabinoids receptor agonists (SCRAs) are the largest group of new psychoactive substances monitored worldwide. Differently from THC, SCRAs can induce more adverse effects and psychiatric consequences, most likely due to their higher potency and affinity at CBRs. Previous studies of our group showed that repeated exposure of JWH-018, the prototypical SCRA, in adult male rats induces changes of mesocorticolimbic dopamine (DA) transmission and its responsiveness to motivational stimuli, abnormalities of emotional state and withdrawal signs after cessation. Despite the growing use of SCRAs, there is a lack of data on the consequences of their repeated exposure during a critical neurodevelopmental period such as adolescence. Considering that vaping SCRAs with electronic cigarette has been gaining popularity among adolescents, in the present study, male and female adolescent rats were passively exposed to JWH-018 vapour once daily for 21 days (Post Natal Day 35-55). Then, pharmacokinetics, behavioural, and neurochemical evaluations were performed at different time points during and after drug inhalation. We found sex differences in JWH-018 plasma pharmacokinetic profiles after single and repeated inhalation. Also, repeated but not acute JWH-018 inhalation induced stronger withdrawal signs in females than males, and impaired locomotion in sex- and dose-dependent manner. Consistently, adolescent JWH-018 inhalation induced sex- and dose-dependent dysregulations of NAc shell and mPFC DA responsiveness to a salient stimulus (i.e. chocolate exposure) at adulthood as estimated by in vivo brain microdialysis. Parallel studies reveled that adolescence male mice that self-administered a newer generation SCRA, namely 5F-MDMB-PICA, showed behavioral and neurochemical abnormalities at adulthood, including a propensity for aggression and reduced social interaction, an anhedonic state, and delayed mPFC DA response to an olfactory stress (i.e. predator odor). In conclusion, by models of administration with high translational value, our studies strongly suggest that adolescent SCRAs use might increase the risk to develop neuropsychiatric disorders later in life.

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S8-04

The effects of synthetic cannabinoids on executive function and related brain activity in fMRI

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The aims of our studies were to investigate the effects of chronic use of synthetic cannabinoids on the brain's structure and function, cognitive and emotional function and schizotypal personality disorder and personality. Synthetic cannabinoid users have exhibited overall smaller grey matter volume than control participants, and in specific regions: insula, the inferior frontal gyrus, the anterior cingulate cortex and the precuneus. These brain regions are rich with cannabinoid CB_1-receptors and are associated with addictive behaviors, cannabis use and abstinence. Secondly, SC users were less accurate and showed longer reaction times on the 2-back and 1-back task than control participants. On the high working memory load, control participants showed additional activation in both the parahippocampal gyrus and the precuneus, areas associated with the default mode network. We have further found impairments in mental flexibility (WCST task), impulsivity (Go No Go task) and response to emotional words (Stroop) in SC users. Furthermore, SC users were more depressed, had higher scores of schizotypal personality disorder and were more introverted, neurotic and less conscientious on the big five questionnaire compared with regular cannabis users and control participants. In conclusion, these findings may have major implications for our understanding of the long-term consequences of synthetic cannabis on cognitive and brain function. We currently run a study using F-DOPA in PET MR to assess dopamine function and neural networks in SC users.

S8-05

Genetic Variations in the Endocannabinoid System and Their Role in Cannabis-Associated Psychosis

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In South London, cannabis use is the most preventable risk factor for psychotic disorders, linked to a distinct symptom profile, i.e., more positive and fewer negative symptoms at both subclinical and first-episode psychosis (FEP) levels, as well as increased risk of violence and aggression. Polygenic scores (PGS), derived from common risk variants, allow individuals to be ranked by genetic liability for schizophrenia. However, the impact of the genetic variation of the endocannabinoid system (EC-PGS) on positive psychotic symptoms and aggression in cannabis-associated psychosis remains unclear.

We analysed data from two incidence studies. In the multinational EU-GEI study (N=617 FEP patients; N=979 controls), we applied bi-factor modelling to estimate transdiagnostic dimensions of psychotic symptoms and experiences, and computed individual EC-PGS across 16 sites in six countries. Linear regression was used to assess the combined effects of EC-PGS and cannabis use on FEP risk and positive symptom dimensions. In the Changes in Psychosis Incidence in South London study (N=3,500 FEP patients), logistic regression was employed to examine the influence of cannabis use and other socio-environmental factors on the likelihood of psychiatric intensive care unit (PICU) admission and seclusion episodes during FEP-related hospitalisation.

In the EU-GEI study, higher EC-PGS was associated with increased FEP risk and greater positive symptom burden in both patients and controls reporting psychotic experiences. Current and daily cannabis use independently predicted elevated positive symptom dimensions across both groups. In the South London study, cannabis use was the strongest predictor of PICU admission at FEP (OR = 2.87; 95% CI: 2.08–3.96) and was also associated with an increased likelihood of seclusion during PICU admission (OR = 2.51; 95% CI: 1.59–4.56).

Cannabis use is associated with more severe psychotic presentations, characterised by more positive symptoms and aggression, jointly and independently with the genetic variation of the endocannabinoid risk. It also increased the likelihood of requiring intensive psychiatric care and seclusion. These findings support the role of cannabis as a modifiable environmental factor influencing the severity and the trajectory of psychotic presentations.

S9-01

Negr1 is a new possible convergent hub for autistic spectrum disorders.

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Autism spectrum disorders (ASDs) are a group of medical conditions with different etiologies that originate during neurodevelopment. Although hundreds of diverse gene variants have been implicated in the pathogenesis of ASD, all ASDs are characterized by common core symptoms (i.e. social impairment and repetitive behaviors). Here, we describe a cleavable cell-adhesion molecule (Negr1) as causative of core ASD behaviors when up-regulated in Wild type (WT) animals, and we indicate Negr1 as a potential convergent molecule dysregulated in ASD. Indeed, up-regulation of Negr1 in the prefrontal cortex (PFC) was sufficient to induce ASD core behaviors in WT mice and brain morphological deficits. By specific overexpression of the soluble Negr1 form in WT animals we demonstrated that the soluble form of Negr1 is sufficient and necessary to cause social deficits in mice. Supporting the relevance of the latter finding, we also found that Negr1 is up-regulated in the PFC of five diverse mouse models of genetic ASD and in postmortem PFC samples of people with ASD and Fragile X.

Altogether, our results on the causal link between Negr1 upregulation and ASD core behaviors in mice together with Negr1 dysregulation in diverse ASD mouse models may explain how a wide variety of ASD genetic variants converge into a unique core group of impaired processes during brain development and of behavioral phenotypes. Our results also suggest regulation of Negr1 pathway and its cleavage as a new target for the design of future therapeutic approaches.

S9-02

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

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

(Supported by MSCA [ITN-#813986] and FCT)

S9-03

FRAGILE X SYNDROME: MOLECULAR AND SYNAPTIC DYSFUNCTIONS AND THERAPEUTIC IMPLICATIONS

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Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and a leading cause of autism spectrum disorder. It is caused by lack of Fragile X Messenger RibonucleoProtein (FMRP), an RNA-binding protein controlling several aspects of RNA metabolism. Abnormal expression of proteins at synapses and altered signaling mediated by metabotropic glutamate (mGlu) receptor subtype mGlu5 have been detected in FXS. FMRP is also implicated in DNA damage response and is a component of stress granules (SGs), cytoplasmatic aggregates that form in response to stress and are protective against apoptosis. Evidence in the *Fmr1* knockout (KO) mouse model of FXS suggests an excess of oxidizing agents in the brain; however, how neurons and glial cells cope with oxidative stress in the absence of FMRP is unknown. Oxidative stress and DNA damage can also trigger cellular senescence. We examined the expression levels of mGlu5 receptors at different ages and detected that mGlu5 is up-regulated in the cortical synaptosomes of young adult, but down regulated in those of aged *Fmr1* KO mice, suggesting an increased sensitivity of synapses to aging in the absence of FMRP. We also examined SGs formation and cell survival in wild-type (WT) and *Fmr1* KO cultured neurons and astrocytes after exposure to oxidative stress and studied markers of senescence in WT and *Fmr1* KO brains. We detected a lower number of SGs in *Fmr1* KO astrocytes, a lower cell survival in *Fmr1* KO neurons and astrocytes compared to WT counterparts upon exposure to H_2O_2; and an increased senescence associated-phenotype in *Fmr1* KO brains compared with WT counterparts. Overall, our data suggest that lack of FMRP sensitizes to oxidative stress-induced damage and accelerates senescence, suggesting new therapeutic options to modify the trajectory of the disease during life.

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S9-04

Novel and complementary pre-clinical approaches to treat the Fragile X Syndrome

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Fragile X syndrome (FXS) is the most common form of inherited intellectual disability and a leading cause of autism spectrum disorder (ASD). It is due to the silencing of the *FMR1* gene coding for an RNA-binding protein, the Fragile X Messenger RibonucleoProtein (FMRP). This protein is part of ribonucleoprotein complexes associated to polyribosomes and is involved in translational regulation. FMRP works mainly as a translational repressor, but in some cases can also enhance translation. By its action, FMRP modulates the expression levels of a large subset of synaptic proteins. Thousands of mRNA targets of FMRP have been identified, modulating dozens of signaling pathways involved in brain development**.** To date no specific treatment is available for this disorder. With this purpose, during the last years, we used different and complementary approaches to identify new effective treatments for FXS**.**

We recently focused our attention on the role of FMRP on the modulation of the expression of several of its target mRNAs that we identified by HITS-CLIP. Among them, we studied the action of FMRP on the metabolism of its target mRNA *Phosphodiesterase 2A (Pde2a),* coding for an enzyme involved in both cAMP and cGMP homeostasis. In FXS neurons the levels of PDE2A are elevated, resulting in reduced abundance of both cAMP and cGMP. We will present data showing that the reduction of the PDE2A activity by pharmacological or genetic tools results into the rescue of socio-cognitive deficits associated with FXS.

In parallel, we established a stable shFmr1 embryonic stem cell (ES) line depleted of FMRP. Differentiation of shFmr1 ES cells into the neuronal lineage results into altered neurogenesis. We used this cell line to screen a library of biomolecules, anticipating that the molecules revealing an ability to actively revert the phenotype of this cell model would likely be candidates for pharmacological treatment of FXS. We found 4 molecules, called SM1-SM4 and we will present their positive impact on *in vitro*, *ex-vivo* and *in-vivo* FXS phenotypes. In particular, chronic treatment of *Fmr1*-KO during their infancy with SM4 definitively modifies the altered brain developmental trajectory of this disorder, resulting into the phenotype rescue in 4 month-old *Fmr1*-KO mice.

We will present the integration of the molecular pathways that are rescued by our approaches and that we defined by multi-omics studies. This will allow to better understand the network of altered pathways underpinning the pathophysiology of FXS.

Phosphodiesterase 2A inhibition corrects the aberrant behavioral traits observed in genetic and environmental preclinical models of Autism Spectrum Disorder; HITS-CLIP in various brain areas reveals new targets and new modalities of RNA binding by fragile X mental retardation protein.; Sumoylation regulates FMRP-mediated dendritic spine elimination and maturation.

S10-01

Mitophagy and mitochondrial biogenesis in ageing and neurodegeneration

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Ageing is driven by the inexorable and stochastic accumulation of damage in biomolecules vital for proper cellular function. Although this process is fundamentally haphazard and uncontrollable, senescent decline and ageing is broadly influenced by genetic and extrinsic factors. Numerous gene mutations and treatments have been shown to extend the lifespan of diverse organisms ranging from the unicellular yeast Saccharomyces cerevisiae to primates. It is becoming increasingly apparent that most such interventions ultimately interface with cellular stress response mechanisms, suggesting that longevity is intimately related to the ability of the organism to effectively cope with both intrinsic and extrinsic stress. Key determinants of this capacity are the molecular mechanisms that link ageing to main stress response pathways. How each pathway contributes to modulate the ageing process is not fully elucidated. Mitochondrial impairment is a major hallmark of several age-related neurodegenerative pathologies, including Alzheimer's and Parkinson's diseases. Accumulation of damaged mitochondria has been observed in post-mortem brains of Alzheimer's disease patients. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to their metabolic state. Little is known about the role of mitophagy in the pathogenesis of Alzheimer's disease. We find that neuronal mitophagy is impaired in animal models of Alzheimer's disease. Indeed, mitophagy stimulation restores learning and memory capacity, in these animals. Moreover, age-dependent decline of mitophagy both inhibits removal of dysfunctional or superfluous mitochondria and impairs mitochondrial biogenesis resulting in progressive mitochondrial accretion and, consequently, deterioration of cell function. Our observations indicate that defective removal of damaged mitochondria is a pivotal event in neurodegeneration. These findings highlight mitophagy as a potential target for the development of innovative, effective therapeutic interventions towards battling human neurodegenerative disorders.

S10-02

FROM ER CALCIUM HANDLING TO MITOPHAGY: PATHWAYS TO NEURONAL HEALTH RESTORATION

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Balanced mitochondrial dynamics—including mitochondrial trafficking, mitochondrial fusion and fission, and mitophagy—are essential for maintaining mitochondrial health and ensuring energy production when and where it is needed. These processes are highly complex, coordinated by multiple molecular players, and their disruption can impair mitochondrial function and compromise cellular energy homeostasis. This is particularly critical in neurons, which have minimal energy reserves and rely on efficient energy distribution due to their extensive compartmentalization. Indeed, disturbances in mitochondrial dynamics are a hallmark of many neurodegenerative diseases.

Mitochondrial function is closely linked to the endoplasmic reticulum (ER), with ER-mitochondria contact sites playing a key role in regulating mitochondrial movement, fusion, fission, and mitophagy. Additionally, calcium transfer from the ER to the mitochondrial matrix through these contact sites is a crucial factor in regulating mitochondrial energy production. This presentation demonstrates how defects in ER calcium handling disrupt mitochondrial dynamics and function, using Wolfram syndrome as a disease model. Wolfram syndrome is a rare genetic disorder caused by mutations in *WFS1* or *CISD2*, which encode ER membrane proteins essential for calcium regulation. Our findings show that reduced ER calcium levels in this model decrease calcium transfer from the ER to mitochondria, leading to severe mitochondrial dysfunction. This includes impaired mitochondrial fusion, disrupted transport, excessive mitophagy, and loss of mitochondrial ATP production. Consequently, this results in a bioenergetic deficit and increased redox stress, particularly evident in presynaptic axonal terminals. Notably, our findings indicate that increasing ER-to-mitochondria calcium flux or mitochondrial matrix calcium levels can restore mitochondrial function and improve axonal health. Wolfram syndrome serves as a valuable disease model for identifying potential therapeutic compounds that target ER-mitochondria calcium signaling. These insights offer promising avenues for treating neurodegenerative and neurodevelopmental diseases linked to impaired ER-mitochondria interactions.

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S10-03

The different roles of mitochondria as modulators of calcium signalling in tauopathies.

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Tauopathies comprise a heterogeneous group of disorders including Alzheimer's disease or frontotemporal dementia, in which misfolded tau protein is accumulated in neurofibrillary lesions. The interplay between mitochondria and calcium signalling imbalance has been long implicated in the pathogenesis of these and other neurodegenerative disorders, where neuronal death is often triggered by a common downstream mechanism: the excessive accumulation of calcium in mitochondria.

We have recently described two different mechanisms by which tau impairs this interplay. We showed that tau directly alters mitochondrial calcium homeostasis by inhibiting its efflux through the sodium/calcium exchanger NCLX both in neurons and astrocytes, thus increasing their vulnerability to calcium overload. In addition, we demonstrated that the role of mitochondria in controlling calcium homeostasis goes beyond their activity as an intracellular store. Mitochondria, through mitochondrial ROS signaling, are able to modulate cytosolic calcium via redox regulation of neuronal glutamate receptors trafficking. In the context of pathology, tau enhances mitochondrial ROS production, thus increasing the surface expression of specific glutamate receptor subunits, leading to calcium overload and neuronal death.

Importantly, mitochondrial antioxidants were able to prevent excitotoxicity and caspase-3 activation triggered by mitochondrial calcium overload in all the models tested, thus pointing at new disease-modifying approaches to target calcium homeostasis.

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S10-04

AXONAL TRANSPORT IMPAIRMENTS IN NEURODEGENERATIVE DISEASES: MECHANISTIC DIVERSITY AND RESULTING THERAPEUTIC STRATEGIES

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The molecular mechanisms causing neuronal death in many neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and Charcot Marie Tooth (CMT) disease, are poorly understood. The key consequence of our incomplete understanding of disease pathogenesis is the lack of effective symptomatic treatments for these widespread global disorders, prompting the necessity for a step-change in treatment strategies to combat these pathologies.

In this view, we are investigating ALS and CMT as disease paradigms to identify new, common targets for pharmacological intervention in these devastating pathologies. We uncovered alterations in axonal transport of several cytoplasmic organelles, such as mitochondria and signalling endosomes, at pre-symptomatic stages of ALS and CMT pathogenesis, suggesting that these impairments may play a causative role in disease onset and progression. Crucially, we have restored axonal transport to physiological levels at early symptomatic stages of disease, thus demonstrating that these pathological changes are fully reversible. At the core of molecular signalling events regulating axonal homeostasis, we identify BDNF as a central node, which activates a variety of transcriptional and phosphorylation programs that foster axonal health.

In light of these promising results, our main goal is identifying novel signalling nodes that modulate axonal transport in healthy and diseased neurons. This will allow us to test the hypothesis that counteracting axonal transport and repair deficits observed in ALS, CMT and other neurodegenerative diseases, represents a novel, effective therapeutic strategy towards treating these pathologies.

S10-05

MOLECULAR DEFECTS IN KIF5A-LINKED NEURODEGENERATIVE AND NEURODEVELOPMENTAL DISEASES

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Mutations targeting the three domains of the neuron-specific kinesin KIF5A cause distinct neurodegenerative or neurodevelopmental diseases, but the molecular bases of this clinical heterogeneity are still poorly understood. To fill this gap, we functionally compared five KIF5A mutants covering the whole spectrum of KIF5A-related disorders: R17Q and R280C KIF5A, linked to spastic paraplegia (SPG); R864* KIF5A, linked to Charcot-Marie-Tooth (CMT) disease; N999Vfs*40 KIF5A, linked to amyotrophic lateral sclerosis (ALS); and C975Vfs*73 KIF5A, linked to neonatal intractable myoclonus (NEIMY).
First, we showed that the CMT-related R864* and ALS-related N999Vfs*40 KIF5A mutants do not undergo autoinhibition and mainly localise at cell periphery. These abnormal behaviours are accompanied by an altered mitochondrial distribution in cells that suggests a disruption of mutant KIF5A transport competence. N999Vfs*40 KIF5A also forms SQSTM1/p62-positive inclusions sequestering wild-type (WT) KIF5A, indicating a gain of toxic function and a dominant-negative behaviour.
We also demonstrated that the newly identified SPG-related R17Q KIF5A mutant and N999Vfs*40 KIF5A both display a faster turnover compared to WT KIF5A and accumulate into detergent-insoluble aggregates when the proteasome is blocked. Moreover, the SPG-related R280C KIF5A mutant and N999Vfs*40-KIF5A both compete for degradation with proteasome-specific substrates.
Finally, we showed that the NEIMY-related C975Vfs*73 KIF5A mutant is characterised by a similar, but more severe aberrant behaviour compared to N999Vfs*40 KIF5A. Notably, the two mutants share an abnormal tail but cause phenotypes on the opposite ends of KIF5A-linked disease spectrum.
Together, our data support the pathogenicity of newly identified KIF5A mutants, highlight previously unknown defects of recurrent variants, and demonstrate that both shared and unique molecular defects underpin KIF5A-linked diseases.

S11-01
Neuro-glia interactions influence cortical morphogenesis across species

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The cerebral cortex is a complex structure responsible for processing sensory and motor information, enabling higher cognitive functions, and influencing personality traits. Its development and functional organization are driven by intricate cellular communication networks, established through diffusible molecular signals and direct physical interactions during development. Disruptions in these interactions can lead to neurodevelopmental disorders. This presentation will focus on the role of cellular interactions in the development of the cerebral cortex, particularly in processes such as neurogenesis, synaptogenesis, and the assembly of cortical circuits. Special emphasis will be placed on cell migration, a critical mechanism for ensuring the proper positioning of neurons within the cortex. The majority of mature cortical neurons originate from distant progenitor regions during embryonic development, making migration essential for their accurate placement. Furthermore, recent findings from our laboratory, as well as other research groups, suggest that migrating cells not only move to specific cortical regions but also transmit instructive signals to neighboring cells and structures, thereby contributing to shape cortical morphogenesis.

S11-02
Unravelling how interneurons gate neocortical plasticity and sensory representation

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Sensory experience and learning are thought to be associated with plasticity of neocortical circuits. Here we used time-lapse, calcium imaging of layer (L) 2/3 neurons in the primary somatosensory cortex (S1) to assess the effects of a bout of rhythmic whisker stimulation (RWS) at a frequency by which rodents sample objects. We found that RWS induced an increase in whisker-evoked L2/3 neuronal activity in most cells and heterogeneously altered the neurons in the active pool. Vasoactive intestinal-peptide-expressing (VIP) interneurons, which promote plasticity through disinhibition of pyramidal neurons, were found to exclusively elevate activity during RWS. When we similarly tested RWS in the Fragile X mouse model, one of the most common monogenically inherited forms of intellectual disability, we found this plasticity to be impaired and this may be due to a lack of inhibitory control on L4 to L2/3 PN synapses. Altogether these findings indicate that cortical neurons' representation of sensory input can be significantly modulated through repetitive sensory stimulation at behaviourally relevant timescales, this maybe gated by activation of disinhibitory circuits, and this function maybe impaired in mouse models of neurodevelopmental disorders.

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S11-03

Prefrontal low gamma oscillations and fear extinction learning rely on early interneuron-oligodendroglia communication

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Emerging evidence links oligodendrocyte lineage cells and myelin to cognition, yet the role of myelination in neuronal networks remains unclear. Our previous studies showed that parvalbumin (PV)-expressing interneurons, the main myelinated GABAergic neurons in the cortex, provide key synaptic input to oligodendrocyte precursor cells (OPCs) during early development. Using a mouse model with disrupted PV interneuron-OPC GABAergic synapses ($\gamma2^{f/f}$ mice), we found that this disruption caused severe myelination defects in PV interneurons of the somatosensory cortex, impairing inhibitory circuits and whisker-mediated texture discrimination. Here we hypothesize that the early postnatal disruption of these synapses also affects adult medial prefrontal cortex (mPFC) function, a region linked to cognition and neurodevelopmental disorders. In a fear conditioning task, $\gamma2^{f/f}$ mice exhibited deficits in tone fear extinction learning and retrieval. Patch-clamp recordings of layers 2/3 pyramidal neurons in the infralimbic (IL) region of the mPFC revealed an excitation-inhibition imbalance caused by a reduced inhibition. These functional impairments were associated with dysmyelination of IL PV axons, despite intact PV interneuron survival, IL myelination, or oligodendrocyte lineage maturation. Attempts to rescue the phenotype—by increasing PV interneuron activity or enhancing myelination—failed to reverse these deficits, indicating the long-term effects of early synaptic OPC disruption. In vivo electrophysiological recordings were used to assess neuronal oscillations in mPFC during the open-field test and the tone extinction retrieval in the fear conditioning test. LFP recordings revealed a specific decrease in the low-gamma power during tone extinction retrieval in $\gamma2^{f/f}$ mice, with minimal effects on high-gamma oscillations or theta phase-gamma amplitude coupling. Our findings reveal the critical role of OPC GABAergic signaling in PV interneuron myelination and mPFC circuit maturation, shaping gamma rhythms crucial for cognitive tasks.

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S11-04

SLEEP CIRCUITS DRIVE INTERICTAL DISCHARGES IN DEMYELINATING NEURODEGENERATIVE DISEASES

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Cognitive processing depends on the cortical computations generated by reciprocally connected pyramidal neurons and interneurons in the deeper layers. Previously, we found that toxin-induced myelin loss causes large aberrant interictal epileptiform discharges (IEDs) on the electroencephalogram (EEG), associated with impaired parvalbumin (PV)-dependent inhibition. Demyelination-induced interictal spikes are reminiscent of interictal spikes in epilepsy and Alzheimer's disease (AD). To better understand the role of myelination and inhibition in the emergence of IEDs, we performed high-density longitudinal EEG recordings in mouse models of AD and MS, determined the cortical myelination patterns and memory functions. IEDs were never observed during wake states, were highly phase-locked to sleep spindles during non-rapid eye movement sleep (NREMs), and emerged during the theta rhythms in REMs. While the EEG oscillation dynamics of spindles and theta oscillations recovered upon remyelination, sleep-associated IEDs continued and were suppressed by a $GABA_A$ receptor agonist. Together with studies in patients I will discuss how the findings suggest that IEDs are selectively generated by the loss of inhibition during corticothalamic sleep circuits and may present a common phenomenon in demyelinating neurodegenerative diseases, emphasizing the need for therapeutic strategies targeting GABAergic inhibition to restore healthy sleep and cognition.

S12-01

GENE-ENVIRONMENT AND BRAIN-BODY INTERACTIONS IN PRECLINICAL MODELS OF NEUROPSYCHIATRIC DISORDERS

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We are interested in how gene-environment interactions mediate and modulate brain function, in health and disease. We have examined the role of various molecular and cellular mediators, and environmental modulators, as they influence healthy cognitive and affective function, as well as cognitive and affective disorders. Our findings have revealed key pathways implicated in the therapeutic impacts of environmental stimuli and identified novel therapeutic targets. We have also discovered altered brain-body interactions, including the first evidence of gut dysbiosis (dysregulated gastrointestinal microbiota) in Huntington's disease (where we model depression, dementia and motor symptoms) and a genetic model of schizophrenia. We have found that environmental interventions which have therapeutic impacts also modulate the gut microbiome. Furthermore, we have shown that a high-fibre diet intervention improved gut function and ameliorated cognitive and affective dysfunction. Ongoing studies are exploring the gut microbiome as a therapeutic target and the possibility that specific environmental factors may modulate brain function via microbiota-gut-brain interactions. Furthermore, we are examining the neuroimmune impacts of the gut microbiome, including area-specific changes in microglial activation states. These approaches to gene-environment interactions may facilitate the development of enviromimetics (including exercise mimetics as a subclass) for various brain disorders.

In order to understand how gene-environment interactions may sculpt brain development and function between generations, we have also been exploring epigenetic inheritance via the paternal lineage. We have discovered transgenerational effects of various paternal environmental exposures, including exercise, stress and immune activation. These lifestyle factors and experiences modulate sperm noncoding RNAs and alter affective behaviors in offspring. We are explore epigenetically mediated changes in the brains of offspring, including including microglial activation states and other forms of neuroimmune modulation. The effects on offspring, including modulation of brain function and behavior, via epigenetic inheritance, have particular relevance to the pathogenesis of depression and anxiety disorders.

S12-02

MICROGLIA-LIPID INTERACTIONS DETERMINE RESILIENCE TO ADVERSE CHILDHOOD EXPERIENCES AND NEURODEGENERATION

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Emerging evidence supports a key role for body-brain interactions in susceptibility to neuropsychiatric disorders, with microglia playing a critical role in detecting and integrating these interactions. This talk will focus on how changes in peripheral lipids affect microglial function, particularly their phagocytic properties, with implications for both the long-term behavioral effects of adverse childhood experiences (ACE) and Alzheimer's disease (AD). We hypothesize that lipid-mediated microglial signaling determines susceptibility vs. resilience to ACE and influences amyloid-beta (Aβ) clearance in AD.

To test this hypothesis, we studied human microglia exposed to serum from ethnically diverse ACE cohorts, including Pakistani children with paternal loss and maternal separation (PLMS) and Bosnian men exposed to genocide. Both cohorts exhibited a consistent reduction in serum high-density lipoproteins (HDLs) in ACE-susceptible individuals. Serum from ACE-susceptible subjects also impaired microglial metabolism and phagocytosis of synaptoneurosomes.

Moreover, the reliance of microglial phagocytosis on lipid metabolism provides a potential strategy for selective Aβ clearance in AD. Using HMC3 human microglia, we demonstrated an enhanced uptake and degradation of Aβ upon lipid starvation. Importantly, knockdown of a lipid-dependent transcription factor abolished the effect of lipid starvation on Aβ phagocytosis without affecting synaptoneurosome phagocytosis, indicating cargo-specificty of these effects.

These findings highlight interactions between peripheral lipids and microglia as a novel cascade that can influences the brain's resilience to insults like ACE and neurodegeneration.

S12-03

Neuroimmune Mechanisms in Metabolic Resilience

Agnes Nadjar

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Over the course of evolution, female mammals have developed exceptional metabolic flexibility, enabling them to sustain reproduction even in times of food scarcity and to optimize energy utilization while supporting the high demands of fetal development. This adaptability provides women with a unique ability to maintain energy balance across varying physiological states, whether processing a calorie-rich meal or enduring fasting.

At the core of these regulatory processes is the arcuate nucleus of the hypothalamus (ARH), which integrates hormonal, neuronal, and nutritional signals. Within the ARH, interactions between distinct neuronal populations and glial cells—including microglia—play a crucial role in fine-tuning whole-body metabolism. However, the precise brain mechanisms underlying the greater metabolic resilience observed in females remain largely unexplored.

In my presentation, I will share novel findings demonstrating how an acute caloric surplus alters neuron-microglia interactions within the ARH and the behavioral consequences in mice. Particular focus will be given to the bioenergetic adaptations of microglia in response to excess calories. Given that a loss of metabolic plasticity in the ARH is a key mechanism in obesity development, this work could pave the way for more effective and personalized treatments for the disease.

S12-04

Therapeutic implications of medium-chain fatty acids in modulating microglia metabolism and neurotransmitter homeostasis in neurodegenerative diseases.

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Microglia, the resident immune cells of the brain, are increasingly implicated in the regulation of brain health and disease. Microglia may impact the progressive pathology of Alzheimer´s disease (AD) bidirectionally. By clearing toxic amyloid-beta (Aβ) aggregates, which are critical in AD pathology, microglia can help in restoring brain homeostasis and halt the progression of AD. However, uncontrolled inflammation by microglia could contribute to neurodegeneration. Microglia functions are closely related to changes in their energy metabolism. Immunometabolism is an emerging concept referring to the ability of immune cells to control their activation by regulating specific intracellular metabolic pathways in response to activating stimuli. This inter-dependency between microglial function and metabolism offers a unique opportunity to modulate microglial activities via nutritional or metabolic interventions. Increasing evidence demonstrates that microglia, their metabolic reprogramming and dysfunction are driving factors in AD pathology, however little is known about how changes in microglia metabolism directly impact neuronal energy metabolism and function. Recently, we found that decanoic acid (C10), a medium-chain fatty acid (MCFAs), improves astrocyte energy metabolism while decreases glia inflammatory markers and Aβ accumulation. Our results also show that C10 may modulate microglia inflammatory responses directly. Therefore, in this talk, it will be discussed how C10 may facilitate immunometabolic regulation in microglia. This research aims at revealing new specific metabolic targets in glial cells involving brain fatty acid signaling and metabolism and may enable the use of metabolic immunomodulators for therapeutic purposes in neurodegenerative diseases.

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S13-01

GETTING A GRIP ON MYELINATION: IN VIVO IMAGING OF AXON-MYELIN ADHESION USING ZEBRAFISH

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Myelination of central axons by oligodendrocytes is essential for CNS function, but how myelin is accurately targeted and grown to appropriate sizes to best support circuit function is unclear. Intercellular adhesion between the axonal and the myelin membranes plays a key role, as manipulating adhesion levels disrupts myelination. Neurofascins are crucial adhesion molecules that organise myelinated axons into their characteristic subdomains, including the paranodal axoglial junctions that anchor myelin to the axon. However, how adhesion is dynamically modulated in individual cells over time, during myelin targeting, formation, and growth, remains unclear. To address this, we took advantage of the zebrafish model organism and of our recently developed gene-editing approach to knock-in fluorescent reporter sequences in frame with the zebrafish neurofascin genes. Using our glial neurofascinb-GFP reporter, the homologue of mammalian NF155, we find that most sheaths contain nfascb at the paranodes immediately upon formation. Fluorescence recovery after photobleaching experimentsreveal that there is slow, but continuous delivery of new glial neurofascin-b protein to paranodal sites during developmental myelination. Our timelapse analysis also shows that the presence of glial neurofascin-b at paranodes does not correlate with the fate of nascent myelin sheaths. We also generated a new zebrafish knock-in line tagging endogenous neuronal neurofascin-a, the zebrafish homologye of NF186. Together, these novel knock-in zebrafish lines enable us, for the first time, to monitor endogenous adhesion molecules and their dynamics, as well as the fate of axonal sundomains, by live-imaging, at subcellular resolution, an intact vertebrate CNS.

S13-02

ILLUMINATING THE INTERPLAY BETWEEN OLIGODENDROCYTES AND AXON FUNCTION

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Oligodendrocytes (OLs) are essential for axonal function, not only by forming myelin but also by regulating axonal metabolism and long-term integrity. Sustaining long myelinated axons is a major challenge for neurons, making them highly vulnerable to metabolic disturbances, aging, and disease. Yet, how OLs contribute to axonal resilience remains incompletely understood. This seminar will explore emerging mechanisms by which OLs regulate axonal function, energy metabolism, and long-term integrity in white matter, with a focus on oxidative stress control and metabolic flexibility. By dissecting the dynamic interplay between OLs and axons, we will discuss novel perspectives on preserving white matter integrity throughout life and in disease.

S13-03

MONOCARBOXYLATES FUEL MYELIN MAINTENANCE AND REPAIR IN THE CENTRAL NERVOUS SYSTEM

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White matter integrity is dependent on correct myelin maintenance and axonal support by oligodendroglia. Myelin maintenance relies on constant turnover of lipids and proteins, which is dependent on the availability of carbon sources for oligodendroglial biosynthetic needs. Moreover, oligodendrocytic provision of energy fuels to the axons adds an additional metabolic demand to the propper functioning of these cells. Monocarboxylates, the family of molecules that comprises lactate, pyruvate, ketone bodies, and some short chain fatty acids are important alternative energy fuels for the cells, but their role in oligodendroglial function during white matter homeostasis and/or myelin regeneration remains underexplored. Here, I will present our data that first demonstrate the expression of monocarboxylate transporter (MCT) 2, the highest affinity MCT previously considered as predominantly neuronal, on mouse and human oligodendrocytes, as well as on mouse oligodendrocyte progenitor cells (OPCs) after demyelination. Notably, we observed downregulation of MCT2 expression on oligodendroglia in progressive multiple sclerosis (MS). We then describe the effects of MCT2 loss of function on the maintenance of myelin and axonal integrity by adult oligodendrocytes, as well as on developmental myelination and myelin regeneration by OPCs.

S13-04

NEURON-MICROGLIA INTERACTION AT THE NODES OF RANVIER IN HEALTH AND DISEASE

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Multiple Sclerosis (MS) is an inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS). While an endogenous repair process exists following demyelination in MS, it is incomplete and varies between individuals. Understanding the mechanisms of remyelination and neuroprotection in this pathology is therefore crucial to promote repair in patients.
In MS, the nodes of Ranvier, which support the fast axonal conduction, are disrupted but can reorganize early during repair, even before remyelination. Furthermore, microglia, the central nervous system resident immune cells, are key players in the disease, as they can engage in pro-inflammatory as well as pro-regenerative processes. Our recent work identified nodal structures as a preferential site for microglia-neuron interaction in both mouse and human. We now demonstrate that neuronal activity promotes microglia-node interaction and the switch towards pro-regenerative microglia. Conversely, adaptive immune cues impair microglia-node interaction in an inflammatory MS model and the extent of these interactions at the onset of remission correlates with recovery outcomes. Taken together, our findings identify factors that influence microglia-neuron crosstalk in disease and suggest that neuronal activity supports remyelination not only by directly regulating oligodendroglia, but also by modulating microglial behavior during repair.

S14-01

INSULIN-REGULATED GLIAL ACTIVATION ALTERS CELLULAR METABOLISM AND UPTAKE OF Aβ IN MOUSE MODELS OF ALZHEIMER'S DISEASE

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Insulin receptors are present on cells throughout the body, including the brain. Dysregulation of insulin signaling in neurons has been implicated in altered mood, cognition, and the pathogenesis of Alzheimer's disease (AD), but little is known on the contribution of impaired insulin signaling in non-neuronal cells, in particular astrocytes and microglia, to the accelerated AD pathogenesis. To define the role of insulin signaling in these cells, we created separate conditional knockout mouse lines, including iGIRKO (inducible astrocyte-specific insulin receptor knockout) and MG-IRKO (inducible microglia-specific insulin receptor knockout). Further, we crossed these mouse lines with the 5xFAD mouse model of AD to generate iGIRKO/5xFAD and MG-IRKO/5xFAD, respectively. Both resultant mice exhibited glial activation and increased levels of Aβ plaque. Specifically, loss of insulin signaling in microglia results in elevated neuroinflammation and metabolic reprograming with an increase in glycolysis, which result in impaired uptake of Aβ. Thus, insulin signaling in astrocyte and microglia plays a key role in cellular metabolism, neuroinflammation and cellular uptake of A such that reduced insulin signaling in these cells alters behaviors and accelerates AD pathogenesis. Together these data not only uncover key roles of insulin action in astrocytes and microglia, but also demonstrate the potential of targeting insulin signaling in these non-neuronal cells in the treatment of AD.

Keywords: insulin, microglia, astrocyte, mitochondria, metabolism, neuroinflammation, mood disorders, Alzheimer's disease

S14-02

CHOLESTEROL DYS-HOMEOSTASIS IN THE BRAIN DURING ALZHEIMER'S DISEASE: IMPLICATIONS FOR AMYLOID PRECURSOR PROTEIN PROCESSING.

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Amyloid pathology in the brains of Alzheimer's disease (AD) patients is primarily driven by the presence of abnormally toxic amyloid-β (Aβ) species. This is especially evident in autosomal dominant forms of AD (ADAD), which result from mutations in the amyloid precursor protein (APP) gene or presenilins that mediate APP processing. Additionally, individuals with Down syndrome, who carry three copies of the APP gene on chromosome 21, face an increased risk of developing AD. Dysregulation of lipids in the brains of AD patients is well-documented, with the *APOE4* allele playing a significant role in driving Aβ production.

Building on a decade of research into the interactions between cholesterol, APOE, and amyloid pathology, I will present a recent project focused on a specific mutation in APP (K28A within the Aβ sequence). This mutation, not yet identified in either AD patients or healthy individuals, produces unusually short Aβ peptides (Aβ33) instead of the toxic Aβ42 and Aβ40 typically associated with the disease. It was discovered serendipitously during an investigation into the interaction site between APP and cholesterol. Our cellular models indicate that APP-K28A not only generates short, non-toxic Aβ peptides but also reverses the production of toxic Aβ42 when combined with APP mutations linked to ADAD. This discovery opens new avenues for potential treatments, including pharmacological and gene-editing approaches targeting the K28 position. Such strategies could effectively prevent the production of toxic Aβ species while preserving the initial steps of APP processing.

S14-03

METABOLIC ALTERATIONS UNDERLYING BRAIN RESILIENCE AND PATHOGENESIS IN ALZHEIMER'S DISEASE

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Biological sex significantly influences Alzheimer's disease (AD) development, particularly regarding brain insulin resistance (bIR) and early energy metabolism defects. Biliverdin reductase-A (BVR-A) plays a crucial role in insulin signaling, with its downregulation promoting bIR. However, sex-related differences in AD neuropathology and underlying mechanisms remain unclear. This study aimed to identify early changes in brain insulin signaling in males and females, shedding light on pathological signs that precede overt bIR and AD neuropathology.
Male and female C57BL/6J mice (wild-type and BVR-A knock-out; n=10 per sex/group) were fed either a standard or a high-fat diet (HFD, 60% kcal from fat) for 1 or 8 weeks. Peripheral metabolic parameters—including fasting glucose, insulin, and IPGTT—were assessed alongside cognitive performance using the NOR and Y-maze tests. Biochemical analyses focused on basal and intranasal insulin-induced signaling, oxidative stress markers, mitochondrial fitness, and AD neuropathology markers in both the cortex and hippocampus. Multivariate analysis was employed to delineate diet- and sex-dependent effects.
After 8 weeks on HFD, male mice developed significant bIR, while female mice primarily exhibited impaired mitochondrial activity. These metabolic alterations were first evident in the cortex and later in the hippocampus. Interestingly, male mice performed better on cognitive tasks than females after 8 weeks of HFD, suggesting that even subtle metabolic disturbances in females may lead to worse cognitive outcomes. Loss of BVR-A significantly correlated with these alterations, as further supported by observations in BVR-A knock-out mice, which exhibited similar metabolic and neuropathological changes after only 1 week on HFD.
Our findings highlight the pivotal role of metabolic alterations—especially the loss of BVR-A—in driving bIR and AD pathogenesis while emphasizing notable sex-dependent differences in brain resilience. These insights offer a valuable framework for developing personalized, early intervention strategies to preserve cognitive function and mitigate AD progression.

S14-04

METABOLIC FACTORS ASSOCIATED WITH COGNITIVE IMPAIRMENT IN ALZHEIMER'S DISEASE

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Metabolic signals have emerged as key players regulating molecular pathways, neural circuits and cognition. Therefore, understanding metabolic defects may result in novel approaches to treat cognitive dysfunction and Alzheimer's disease (AD)-linked dementia. I will discuss experimental findings supporting that reductions in metabolic modulators, such as irisin or lipoxin, impairs memory in rodents. Furthermore, I will present evidence that changes in these metabolic mediators associate with impaired memory in humans affected by dementia. Finally, I will show that exercise-linked increases irisin are neuroprotective in AD models. Overall, our results demonstrate the importance of adequate metabolic signaling to preserve cognition in rodents and humans and support further investigation on these mediators as potential biomarkers for cognitive impairment.

Funding: Alzheimer's Association, Serrapilheira Institute, National Council for Science and Technology (CNPq), Rio de Janeiro State Science Agency (FAPERJ).

S15-01

PROTECTIVE ROLE OF SPHINGOSINE 1-PHOSPHATE SIGNALING AXIS IN NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases (NDD) are heterogeneous neurological disorders affecting millions of people worldwide and entail the progressive loss of neurons. Bioactive sphingolipids have been recently proposed as potent protective agents in NDD such as Alzheimer's (AD) and Parkinson's (PD) diseases. Here, we investigated the involvement of sphingosine 1-phosphate (S1P) signaling pathway in Ca^{2+} homeostasis and oxidative stress in neurons exposed to alpha-synuclein and Aβ42 oligomers (Aβ42 O). S1P is a pleiotropic lipid generated by sphingosine kinase (SK)1 and SK2 isoforms. The majority of S1P effects are mediated by its ligation to GPCR named S1PR1-5 after its export mediated by transporters such as SPNS2. We showed that S1P rescued Ca2+ dyshomeostasis induced by alpha synuclein in human neuroblastoma SH-SY5Y cells and that S1PR1 and S1PR2 mediate the protective action of the sphingolipid. Moreover, we demonstrated that exogenous and endogenous S1P rescued Ca2+ dyshomeostasis induced by toxic Aβ42 O in primary rat cortical neurons and human neuroblastoma SH-SY5Y cells. Further analysis revealed a strong neuroprotective effect of S1PR1 and S1PR4, and to a lower extent of S1PR3 and S1PR5, which result in the endocytic internalization of the extrasynaptic GluN2B-containing N-methyl-D-aspartate receptors. Notably, the S1P beneficial effect can be sustained over time by SK1 overexpression, thus counteracting the down-regulation of the S1P signaling induced by Aβ42 O. Recently, neuroinflammation in glial cells has been demonstrated to be involved in the onset and progression of AD, although the precise roles of glial S1P signaling remain elusive. To elucidate the role of S1P in Aβ neurotoxicity, we investigated the effect of the altered S1P metabolism and signaling in human glial cells on neuronal cell oxidative damage. Interestingly, conditioned media from glial HMC3 cells overexpressing SK1 were protective on Aβ-induced neurotoxicity in SHSY-5Y in comparison to control cells. The pharmacological inhibition of SPNS2 in HMC3 cells reduced the protective effect of their conditioned medium in Aβ-induced neurotoxicity in SHSY-5Y cells, focusing on the key protective role of the sphingolipid in glial/neuronal interplay. Our findings disclose mechanisms underlying the neuronal protective effect of S1P against alpha-synuclein and Aβ42 O, suggesting that S1P signaling axis can be considered promising targets for therapeutic approaches for AD and PD.

S15-02

SPHINGOLIPID HOMEOSTASIS ALONG THE ENDOMEMBRANE SYSTEM AND ITS RELEVANCE IN NEURODEGENERATION

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Sphingolipids are structurally diverse lipid molecules that function as potent signaling and structural lipids. Due to their pleiotropic roles, disruptions in biosynthesis, accumulation, or relative variations in sphingolipid subtypes can be detrimental at the organismal level. Particularly enriched in neuronal tissues, sphingolipids support neural differentiation and function, synaptic transmission, action potential propagation, and the development of the central and peripheral nervous systems.

Sphingolipid synthesis and catabolism are highly compartmentalized processes. Long-chain bases (LCBs), which serve as sphingolipid precursors, are generated in the endoplasmic reticulum by the enzyme serine palmitoyltransferase (SPT). LCBs are subsequently converted into dihydroceramides, which are almost universally converted into ceramides through the formation of a single double bond in the LCB moiety. CERT1 mediates the transfer of ceramides (and dihydroceramides) from the ER to the trans-Golgi for the synthesis of sphingomyelin (and dihydrosphingomyelin). These products form a critical component of nerve cell membranes and myelin. Dihydrosphingolipids, known neurotoxic lipids, accumulate in several neurological disorders and are implicated in severe hypomyelinating leukodystrophy.

We recently identified mutations in the enzymatic subunits of SPT that cause the juvenile form of amyotrophic lateral sclerosis (jALS). SPT-ALS mutations lead to excess sphingolipid production due to the compromised feedback inhibition of the enzyme. Plasma from SPT-ALS patients and mutant cells, in particular, show an accumulation of neurotoxic dihydrosphingolipids.

Additionally, we identified gain-of-function mutations in CERT1 that cause a neurodevelopmental disorder, which we have named **CerTra syndrome**. Interestingly, CerTra syndrome promotes increased LCB synthesis by SPT, leading to sphingolipid and dihydrosphingolipid accumulation. We have now identified a novel sphingolipid homeostatic pathway that links SPT activity with CERT1 function, which acts to restrict dihydroceramide levels in cells. This homeostatic pathway appears impaired in cells expressing SPT-ALS or CERT1 mutants. Importantly, targeting this pathway through CERT inhibition rescued axonal defects and cell death associated with dihydrosphingolipid accumulation in vitro.

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S15-03

GANGLIOSIDES MODULATE POSITIONING AND FUNCTIONS OF PROTEINS INVOLVED IN SYNAPTIC PLASTICITY AND ION HOMEOSTASIS – IMPLICATIONS FOR NEURODEGENERATION

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Gangliosides, sialylated glycosphingolipids, are ubiquitous residents of biological membranes. However, the highest structural diversity, abundance and specific spatio-temporal expression patterns have been firmly evidenced in the mammalian brain. Physiological functions of gangliosides are predominantly linked to nervous system, while deficiencies, accumulation or changed compositional patterns of brain gangliosides are associated with several neuropsychiatric clinical phenotypes including neurodegeneration. Many crucial cellular events mediated by gangliosides are related to their localization in lipid rafts, highly organized membrane domains serving as cellular signaling hubs that process a variety of environmental signals. Structural and functional interactions of gangliosides with other membrane lipid and protein constituents are of pivotal importance for maintaining membrane architecture and dynamics. Based on the results obtained by multi-level methodological approaches, here we describe relationship of specific classes of brain gangliosides with transmembrane proteins participating in synaptic plasticity and ion transport – neuroplastin, plasma membrane calcium ATPases and sodium-potassium ATPases. In addition, we present supportive data that illustrate impact of membrane lipid composition on positioning and functionality of the transmembrane proteins involved in elaborate and regulated molecular events underlying highly complex brain functions such as learning and memory. Finally, we give an overview of the most important findings of our group demonstrating pathophysiological roles of gangliosides in Alzheimer's disease, epilepsy and brain tumors, and discuss on further research strategies clarifying interplay of gangliosides and select proteins in neurodegeneration.

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S15-04

EMOTIONAL BEHAVIOR AND ALCOHOL CONSUMPTION IN EARLY ONSET PARKINSON'S DISEASE: ROLE OF THE SPHINGOLIPID SYSTEM

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Parkinson's disease (PD) is a neurodegenerative disorder, often caused by point mutations in the α-synuclein coding gene. PD is accompanied by other psychiatric conditions, such as anxiety, depression, and drug use disorders. These behavioral phenotypes often precede the development of motor and other symptoms of PD, and could be linked to the point mutations in the α-synuclein coding gene. We investigated whether the familial PD point mutation A53T could be linked to diverse alterations in the emotional state and alcohol consumption behavior of mice at ages not yet characterized by α-synuclein accumulation. The affective and alcohol- drinking phenotypes remained unaltered in female PDGF-hA53T-synuclein-transgenic (A53T) mice during both early and late adulthood. Brain region-specific activation of ceramide-producing enzymes, acid sphingomyelinase (ASM), and neutral sphingomyelinase (NSM), known for their neuroprotective properties, was observed during early adulthood but not in late adulthood. In males, the A53T mutation was linked to a reduction in alcohol consumption in both early and late adulthood. However, male A53T mice displayed increased anxiety- and depression-like behaviors during both early and late adulthood. Enhanced ASM activity in the dorsal mesencephalon and ventral hippocampus may potentially contribute to these adverse behavioral effects of the mutation in males during late adulthood. In summary, the A53T gene mutation was associated with diverse changes in emotional states and alcohol consumption behavior long before the onset of PD, and these effects varied by sex. These alterations in behavior may be linked to changes in brain ceramide metabolism.

S16-01

FROM MOLECULAR PATHOGENESIS TO THERAPEUTIC TARGETS: THE SCA3/MJD CASE

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Ataxin-3 (ATXN3) is a small deubiquitinating (DUB) protein (42kDa) that is expressed ubiquitously throughout different organs, tissues and cells. It contains a structured and well-conserved N-terminal with a catalytic Josephin domain that hosts two binding sites for ubiquitin, and a flexible and divergent C-terminus, with a polyglutamine (polyQ) tract and two or three ubiquitin interaction motifs, depending on the isoform.
Expansion of a CAG repeat in the gene encoding for this protein causes Spinocerebellar ataxia type 3 (SCA3) or Machado-Joseph disease (MJD), a late-onset neurodegenerative disease. A negative correlation between the age of onset and the number of CAG repeats is observed in this disorder, reinforcing the central role of the expanded polyQ tract itself in pathogenesis. The expansion of the polyQ tract within the protein ATXN3 culminates in the formation of toxic protein oligomers and insoluble aggregates, and ultimately leads to neurodegeneration and early death. The gain of toxic function during the ATXN3 aggregation pathway is considered the main contributor to the development of this disorder. Partial loss of ATXN3 function may, however, also contribute for MJD since, as a result of aggregation and/or abnormal interactions, the mutant protein may be unable to fully perform its normal cellular functions, putatively contributing to neurodegeneration. Also, the presence of the expanded ATXN3 in the nucleus has been demonstrated by to be essential for its neurotoxic effect. In this perspective, our work led to the identification of key ATXN3 nuclear interactors - proteins involved in RNA metabolism and nuclear receptors - which show perturbed function in the presence of the expanded version of this protein and functional links to the neurodegenerative process. In some cases, they provide relevant drug targets to be explored for the treatment of SCA3/MJD. An overview of this work will be presented.

S16-02

EXPLORING THE PRODROMAL STAGE IN SPINOCEREBELLAR ATAXIA TYPE 2: GENETIC DAMAGE BIOMARKERS AND MOLECULAR INSIGHTS FOR EARLY DIAGNOSIS AND INNOVATIVE THERAPIES

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Spinocerebellar Ataxia Type 2 (SCA2) is a neurodegenerative disorder with a high prevalence in Cuba, making it a focal point for research and clinical studies.This condition is characterized by progressive cerebellar ataxia, oculomotor abnormalities, and other neurological symptoms. The prodromal stage of SCA2, which occurs before the onset of clinical symptoms, is marked by painful muscle cramps, sensory symptoms, sleep disorders, hyperreflexia, autonomic symptoms, and cognitive impairments. This stage has gained significant attention as a critical window for early intervention. Research in Cuba has identified potential biomarkers of genetic damage, such as expanded CAG repeats in the ATXN2 gene, imaging abnormalities, nerve potential amplitude, maximum saccadic velocity, rapid eye movement (REM) sleep percentage, REM sleep without atonia percentage, corticomuscular coherence, central motor conduction time, and antisaccadic error correction latency.These biomarkers will facilitate the conduction of preclinical trials, early diagnosis, and emerging innovative therapies for SCA2. The distinctive genetic and epidemiological context of SCA2 in Cuba offers a significant opportunity to enhance global understanding of the condition and develop personalized diagnostic and therapeutic solutions.

S16-03

GUT-BRAIN INVOLVEMENT IN SPINOCEREBELLAR ATAXIA TYPE-3

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Spinocerebellar Ataxia Type-3 (SCA3, also known as Machado-Joseph disease, MJD) is a fatal neurodegenerative disease characterised by impaired movement, speech, and swallowing. SCA3 is caused by the inheritance of an expanded trinucleotide repeat region within the ATXN3 gene, leading to an expanded polyglutamine (polyQ) tract within the ataxin-3 protein. PolyQ-expanded ataxin-3 protein is highly aggregation prone, and its presence leads to neurodegeneration within the cerebellum, brain and spinal cord. We have recently identified that alterations to the composition of the gut microbiome occur in the CMVMJD135 mouse model of SCA3, even prior to the onset of motor symptoms. These findings are in line with reports that changes to gut microbiome may occur in a range of different neurodegenerative diseases. Here we report our findings from investigations into the possible causes of this change, in a condition that has a known mono-genetic trigger. We have found that male SCA3 mice have significantly different microbiome communities present as early as 5-weeks-old. We have not identified any changes to gut morphology, enteric neuron number or presence of protein aggregates within enteric neurons. Whilst we have identified that male SCA3 mice develop altered total gut transit times, compared to wild-type control mice, this change is not present until a later age of 9 weeks old. Finally, investigation into the abundance of inflammatory and endocrine factors suggests potential mechanistic changes for further investigation. Together, these findings demonstrate that gut dysfunction and microbiota changes do occur in this mouse model of SCA3, prior to onset of motor impairment, and these changes warrant further investigation for their impact in the disease.

S16-04

GENETIC AND MOLECULAR FACTORS MODIFYING THE PATHOGENESIS OF SPINOCEREBELLAR ATAXIA TYPE 3

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Spinocerebellar ataxia type 3 (SCA3), also known as Machado-Joseph disease (MJD), is the most common autosomal dominant spinocerebellar ataxia worldwide and caused by a CAG repeat expansion in the *ATXN3* gene resulting in a polyglutamine expansion in the encoded Ataxin-3 protein. Statistically, a correlation between the number of CAG repeats and the age at onset of SCA3 patients exists: Patients with more CAG repeats have an earlier onset of symptoms. However, this statistical correlation is not perfect and the number of CAG repeats contributes only about 50% to the age at onset. Therefore, the remaining 50% are influenced by other factors. In order to identify modifiers of the disease progression, we genotyped in a combined European and South American approach more than 500 SCA3/MJD patients for promising polymorphisms in candidate genes.

Candidate genes included *ATXN3* itself, genes coding for known interaction partners of ataxin-3, functional modifiers identified in previous studies as well as genes with known relevance for the pathophysiology of SCA3/MJD including genes involved in the nucleocytoplasmic transport. We selected polymorphisms with a high likelihood of having a functional relevance i.e. polymorphisms in the promoter regions as well as polymorphisms leading to amino acid changes. While controlling for ethnic origin we assessed the contribution of the respective polymorphism to the age at onset in addition to the already known modifying factor, the length of the expanded CAG repeat within *ATXN3*. We indeed identified interesting polymorphisms contributing to the age at onset including certain haplotypes within *ATXN3* itself and could validate their functional impact on pathogenic mechanisms in SCA3/MJD. Our results will improve the prediction of clinical symptoms and contribute to the understanding of pathogenic processes in SCA3/MJD.

S17-01

MODULATION OF PREFRONTAL-HIPPOCAMPAL NEURAL DYNAMICS BY SEROTONIN RECEPTORS IN HEALTH AND PRECLINICAL MODELS OF SCHIZOPHRENIA

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Neural synchrony and functional connectivity are disrupted in schizophrenia. We investigated the abnormal neural synchronization within circuits involving the medial prefrontal cortex (mPFC) and the dorsal hippocampus (CA1) during psychosis-like states and cognitive impairment induced by the NMDAR antagonist phencyclidine (PCP). The acute administration of PCP induces psychosis in both humans and animals, while its subchronic injection to rodents replicates cognitive symptoms observed in schizophrenia patients. The psychotomimetic effects of PCP were linked to prefrontal hypersynchronization, hippocampal desynchronization and disrupted circuit connectivity. Notably, antipsychotic drugs (APDs) risperidone (5-HT2AR and D2R antagonist) and lurasidone (5-HT7R antagonist), but not the classical neuroleptic haloperidol, reduced PCP-induced prefrontal hypersynchrony and hippocampal desynchronization, respectively. These findings suggest that AAPDs, unlike typical APDs, target prefrontal-hippocampal pathways to exert their antipsychotic effects. Furthermore, we investigated the neural correlates of memory and perceptual impairments in mice treated with sPCP and the restoring effects of risperidone and lurasidone. sPCP impaired both short-term and long-term memory, which were once again associated with increased neural synchrony in the mPFC and reduced synchrony in the dHPC, along with disrupted mPFC-dHPC connectivity. Both APDs reversed the memory deficits and mitigated hippocampal desynchronization. We also explored the influence of dopamine D2R and serotonin receptors (5-HT1AR, 5-HT2AR, 5-HT4R, 5-HT7R) on healthy and pathological prefrontal-hippocampal pathways. Our studies suggest that during NMDAR hypofunction, the connection between the PFC and dHPC becomes compromised, potentially contributing to psychosis and cognitive impairment in schizophrenia. AAPDs suppress excessive cortical synchrony to exert their antipsychotic effects, while simultaneously restoring hippocampal synchronization to alleviate cognitive symptoms in patients.

S17-02

OSCILLATIONS, SYNCHRONY AND WAVES: THE LINGUA FRANCA OF CORTICAL COMPUTATIONS.

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The dynamics of neuronal systems are characterized by hallmark features such as oscillations and synchrony. However, it remains unclear whether these characteristics are mere epiphenomena or are utilized for computation. Due to the difficulty of specifically interfering with oscillatory network dynamics in neuronal systems, we simulated recurrent networks of damped harmonic oscillators, where oscillatory activity is enforced in each node, a choice well supported by experimental findings. When trained on standard pattern recognition tasks, these harmonic oscillator networks (HORNs) outperformed non-oscillatory architectures in terms of learning speed, noise tolerance, and parameter efficiency. HORNs also reproduced many characteristic features of neuronal systems, such as those found in the cerebral cortex. In trained HORNs, stimulus-induced interference patterns holistically represent the results of comparing sensory evidence with priors stored in recurrent connection weights. The learning-induced weight changes align with Hebbian principles. Implementing additional features characteristic of natural networks, such as heterogeneous oscillation frequencies, inhomogeneous conduction delays, and network modularity, further enhanced HORNs' performance without requiring additional parameters. Taken together, our model offers plausible a posteriori explanations for features of natural networks whose computational roles have remained elusive. We conclude that neuronal systems likely exploit the unique dynamics of recurrent oscillator networks, whose computational superiority critically depends on the oscillatory patterning of their nodal dynamics. Implementing the proposed computational principles in analog hardware is expected to facilitate the design of highly energy-efficient devices that could ideally complement existing digital technologies.

S17-03

THE TRANSITION FROM ENDOGENOUS NETWORK ACTIVITY TO EPILEPTIFORM DISCHARGES: A COMPUTATIONAL APPROACH

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Epilepsy is a chronic condition characterized by abnormal, synchronized electrical discharges in the brain. It affects individuals across all age groups and remains one of the most prevalent neurological disorders, with an estimated global incidence of approximately 50 million cases. Despite advances in pharmacological and surgical interventions, nearly 30% of individuals with epilepsy are classified as refractory, highlighting the pressing need for a deeper understanding of the mechanisms underlying seizure generation and propagation.

In this study we used ex vivo brain slices to investigate the transition between the endogenous physiological cortical activity, in the form of recurring Up and Down states, and epileptiform discharges. Characterizing and predicting such transitions is crucial for understanding the dynamics of ictogenesis and for developing therapeutic tools.

Network activity was assessed with local field potential recordings and epileptiform activity was induced by three pharmacological protocols (low Mg^{++} aCSF, 4AP, or gabazine) that targeted different aspects of the excitation-inhibition balance. We then used computational methods in order to (a) describe the complex network metrics as the cortex makes the transition to seizure-like events and (b) identify possible critical points that predict a change in network dynamics. Our results reveal the capacity to discriminate between spontaneous, pre-ictal and seizure-like states, for several metrics and independently of the activity level of the signal. Collectively, the observed differences indicate that as the system is heading towards epilepsy, its behaviour gradually becomes less complex, and suggest that the relevant entropic changes could be used as a means of predicting seizure-like states.

S17-04

CORTICAL CIRCUIT CORRELATES OF PERCEPTION: LESSONS FROM A MOUSE MODEL OF PERCEPTUAL BI-STABILITY

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Understanding how cortical dynamics give rise to percepts—and how these percepts spontaneously transition—remains a central question in systems neuroscience. To address this, we adapted a bistable visual motion paradigm developed in primates for use in the mouse, enabling the study of perceptual dynamics with modern optical imaging techniques. We employed "type I plaids," stimuli formed by the superposition of two gratings moving 120° apart, which support two distinct percepts: component motion and pattern motion. Mice viewing these stimuli exhibit optokinetic responses (OKRs) that alternate between tracking component and pattern motion, providing a reliable behavioral readout of their perceptual state. Using this paradigm in conjunction with mesoscopic two-photon calcium imaging, we mapped cortical activity associated with perceptual content and transitions. Lesions of primary visual cortex (V1) altered the ratio of pattern:component motion OKRs, confirming that mouse V1 contributes to shaping the visual motion percept and that the bistable OKR cannot be attributed solely to subcortical or non-V1 circuitry. Contrary to classical models suggesting that V1 primarily encodes local motion features, we found a surprisingly high fraction of pattern-motion selective neurons and a lower-than-expected fraction of component-selective neurons in mouse V1, suggesting a greater than previously appreciated contribution of V1 to complex visual motion in mice. Perceptual transitions were accompanied by shifts in relative activity across these subpopulations. In addition, we identified a distinct population of neurons with transient, ramping activity, which preceded perceptual reversals and diminished after the new percept stabilized. These *reversal-locked* neurons, a ~10-25% of the imaged population- were activated specifically around perceptual reversals, but not during OKR epochs within stable percepts. As over 95% of layer 1 ChAT+ interneurons fell in this category, chemogenetic modulation, using DREADDs, significantly altered the rate of perceptual transitions, suggesting they play a causal role in regulating perceptual switching dynamics.

Given the hypothesized role of impaired perceptual flexibility in autism spectrum disorder (ASD), we applied this paradigm in the MECP2-duplication mouse model of autism. We observed a significant reduction in the perceptual switching rate, along with a markedly increased dominance of the component (local) visual motion percepts at the expense of the pattern (global) motion percept. These findings parallel observations in human patients with ASD, including reduced rates of visual rivalry reversals and altered global motion perception. Together, our results establish this mouse model of bistable perception as a powerful platform for dissecting the cortical mechanisms of perceptual (cognitive) flexibility, while promises a translational bridge between circuit-level mechanisms and the cognitive inflexibility observed in neuro-developmental disorders such as autism.

S18-01

SYNAPTIC PLASTICITY RELATED NOVEL TARGETS IN ANIMAL MODELS AND CLINICAL COHORTS OF DEPRESSION

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Major depression is the most frequent psychiatric disorder, affecting an estimated 350 million people globally. Antidepressant (AD) drugs designed to target monoamine-dopamine, norepinephrine, serotonin- neurotransmission are widely used. However, for one third of the patients these ADs have little effect in the long-run. Even for those that respond, full AD effect is generally observed only after several weeks. Beyond monoamines, the newer ketamine/esketamine, which target glutamate neurotransmission and synaptic plasticity, are reserved for "difficult-to-treat-depression" and given exclusively in a hospital setting because of side effects and safety constraints. Similar limitations are expected for psilocybin and other hallucinogens, now in development as future ADs. Therefore, innovating with faster and better acting AD compounds is crucial, in particular for the 30% of patients who don't respond to current treatments.

Elk1 is a transcription factor that acts downstream of the ERK and SGK cascades to integrate signals relevant to stress and to modulate synaptic plasticity. Better understanding the regulation of this pathway paves the road for AD interventions with an unprecedented mode of action. We will present recent data from the ANTARES project and clinical cohort on Elk1 and its regulation in depression. We will also discuss proof-of-concept evidence from animal models. We show selective "add-on" strategies that encompass the Elk1 pathway and target neuroplasticity to confer faster onset as well as better antidepressant efficacy.

S18-02

PSILOCYBIN AND OPTOGENETIC REGULATION OF NEUROGENIC CIRCUITRY IN STRESS-INDUCED DEPRESSIVE PATHOLOGY

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Studies in rodents have shown that Major Depressive Disorder (MDD) impairs the formation of new brain cells in the hippocampal dentate gyrus, affecting both cognitive and emotional functions. With current treatments being ineffective for many patients, understanding the underlying mechanisms of depression is crucial.

Using optogenetic techniques, we investigated how controlling newly formed brain cells affects depression in a rat model. We specifically examined whether these cells influence the therapeutic effects of psilocybin, a promising compound for treatment-resistant depression.

Our results demonstrate that manipulating new brain cell formation influences depression-like symptoms, including emotional and cognitive deficits. Notably, psilocybin reversed these symptoms through a mechanism dependent on new hippocampal cells. These findings suggest new therapeutic approaches for treating MDD.

S18-03

EXOSOMES AND CANNABIDIOL TREATMENT IN STRESS AND ALZHEIMER'S DISEASE BRAIN PATHOLOGY

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In the new era of Precision Medicine, the diagnosis, prognosis and treatment of complex and multifactorial brain diseases such as Alzheimer's disease (AD) and depression are increasingly based on the evaluation of the individual's lifestyle, risk factors and multilevel biological analyses that aim to clarify the degree of brain pathology as well as the effectiveness of new therapeutic schemes in each, individual/patient. Dr Ioannis Sotiropoulos' talk will focus on the impact of chronic, psychological stress, a risk factor for AD, on different disease pathomechanisms (e.g. Aβ, Tau, neuroinflammation) and their relationship to brain exosomes; the later are small extracellular vesicles (EVs) secreted by cells that carry different biological material (e.g. proteins, RNA and DNA) from the cell of origin, and they are suggested to contribute to both spreading of brain pathology as well as its diagnosis in periphery (e.g blood). He will also present data related to the therapeutic potential of cannabidiol against Stress and AD brain pathologies. Given that the modern lifestyle increasingly exposes individuals to high stress loads, it is clear that understanding the mechanistic link(s) between chronic stress and AD pathogenesis may facilitate the treatment of AD and other stress-related disorders.

S18-04

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-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.

S19-01

UNVEILING THE ROLE OF MIR-802 IN THE ONSET AND PROGRESSION OF BRAIN INSULIN RESISTANCE IN TRISOMIC ASTROCYTES

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Down syndrome (DS) is characterized by a variety a marked predisposition to cognitive decline and cognitive decline in DS. Metabolic dysfunctions, particularly insulin resistance (IR), contribute to neurodegeneration in DS. MicroRNAs (miRs), small non-coding RNAs, are key post-transcriptional regulators and have gained attention for their role in modulating metabolic pathways. miR-802 is triplicated in DS and has been shown to have a role in the development of IR in obesity and diabetes. Remarkably, we found that miR-802, is upregulated in DS brain and likely play a role in the onset of brain IR, by impairing the transcriptional regulation of insulin signaling (IS) targets, GSK-3β and PTEN. Here **we aim to investigate the effects of miR-802 overexpression in trisomic astrocytes,** being these glial cells tightly involved in brain metabolism and neuroprotection.
Cortical primary astrocytes were isolated at PND0 from euploid (Eu) and Ts2Cje (Ts) mice. First, Ts astrocytes were characterized for their insulin response following stimulation with 100 nM insulin (15 min) and a second stimulus (10 nM) at 15, 30, and 45 minutes to induce IR. We then quantified miR-802 expression and assessed its impact on target genes PTEN and GSK-3β, previously identified via bioinformatics.

Our results show that Ts astrocytes exhibited reduced insulin sensitivity, markers by decreased activation of $pIRS1^{Y216}$ and $pAKT^{S473}$. This impaired response correlated with a significant upregulation of miR-802 and reduced GSK-3β and PTEN expression, supporting its role in IR onset in Ts astrocytes, potentially disrupting their metabolic functions. Given the central role of astrocytes in neuronal homeostasis, miR-802-mediated IS dysfunction could have broader implications for brain metabolism and neurodegeneration in DS. Elucidating the role of miR-802 in an IR-associated model provides novel insights into the molecular mechanisms underlying IS dysfunctions in DS.

S19-02

PATHOGENIC ROLES OF MICROGLIAL EXTRACELLULAR VESICLES IN NEURODEGENERATION

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Synaptic dysfunction is an early mechanism in Alzheimer's disease (AD) which involves progressively larger areas of the brain over time. However, how it starts and propagates is unknown. Our recent findings demonstrate that large extracellular vesicles (EVs) released by microglia play a crucial role in these early events.

We isolated large EVs from primary microglia exposed or not to AD-related misfolded proteins ($A\beta_{42}$ or tau protein; Aβ-EVs, tau-EVs). We proved the involvement of EVs in the onset and propagation of synaptic dysfunction in the brain by stereotaxically injecting Aβ-EVs or tau-EVs into naïve mice entorhinal cortex (EC), a vulnerable region in AD, and evaluating: i) long-term potentiation (LTP) in EC and its main target region, the dentate gyrus of the hippocampus (DG) in brain slices; ii) possible alterations in cortico-hippocampal network activity by chronic EEG recordings; iii) progressive memory impairment in behavioural tasks. We then studied the mechanisms mediating this phenomenon. Optical manipulation was combined with time lapse imaging to study EV-neuron interaction dynamics, revealing the ability of EVs to efficiently move at the neuronal surface, a feature that was enhanced for Aβ-EVs (carrying Aβ) compared to tau-EVs and EVs from untreated microglia (ctrl-EVs). Taking advantage of high-resolution accurate-mass spectrometry SWATH™-MS, we investigated the molecular differences between Aβ-EVs and ctrl-EVs, providing a mechanistic understanding of the enhanced Aβ-EV motion at the neuronal surface.

Our data provide evidence of the involvement of microglial EVs in early synaptic dysfunction in AD, unveiling a new mechanism controlling the diffusion of large EVs and related pathogenic signals in the brain parenchyma (i.e. extracellular motion at the neuronal surface), paving the way for novel therapeutic strategies.

S19-03

MITOCHONDRIAL FATTY ACID B-OXIDATION IN ASTROCYTES IS IMPORTANT FOR BRAIN LIPID HOMEOSTASIS

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The synthesis, degradation, storage and recycling of lipids within cells directly affect energy production, cell membrane turnover, signaling pathways and lipotoxicity. Therefore, lipid homeostasis plays a crucial role in optimal cellular function across diverse tissues. In the brain, dysregulated lipid metabolism is linked to several neurodegenerative disorders, highlighting the significance of lipid homeostasis for normal brain function. Recent studies have emphasized the role of astrocytes in neutralizing toxic lipids from stressed neurons through lipid transfer and mitochondrial fatty acid beta-oxidation (FAO)[1,2,3]. However, the contribution of astrocytic FAO to lipid homeostasis in normal brain function remains poorly understood. To investigate this, we characterized the expression pattern of one of the key enzymes of FAO, carnitine palmitoyl-transferase 1a (Cpt1a), and evaluated the functional role of FAO in astrocytes by selectively deleting Cpt1a at both early and late stages of postnatal mouse brain development. Our findings demonstrate that Cpt1a is predominantly expressed in glial progenitors and astrocytes throughout postnatal development, adulthood, and during the progression of Alzheimer's disease (AD). Single-nucleus RNA sequencing, lipidomics, behavioral tests, and imaging revealed that the deletion of Cpt1a in astrocytes disrupts lipid homeostasis, which adversely affects the postnatal and adult brain.

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S19-04

THE EFFECT OF ASTROCYTIC TAU DEPOSITION ON SYNAPTIC PLASTICITY

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Soluble tau species are associated with synaptic loss and memory decline in Alzheimer's disease (AD). In addition to the intraneuronal accumulation of tau in AD, it was shown recently that extracellularly applied oligomeric tau (oTau) could enter astrocytes *in vitro* where it impacts negatively the physiology of both neurons and astrocytes. Thereafter we investigated the impact of pathological Tau expression onto astrocytes. Expression of pathological tau into astrocytes, was achieved by intrahippocampal injection of an AAV9 vector containing the green fluorescent protein (GFP) and the human 4R0N tau isoform with the P301L mutation flanked by lox sites (AAV-P301L-GFP) in 2-month-old GFAP-Cre mice that express Cre recombinase in astrocytes. Two weeks post-injection hippocampal slices were collected for electrophysiological recordings, assessing short- and long-term synaptic plasticity. Short-term synaptic plasticity recordings showed that expression of pathological Tau in astrocytes leads to derangement of presynaptic mechanisms depicted by impaired postsynaptic potentiation, synaptic fatigue and replenishment of the readily releasable pool of neurotransmitters. The latter was consistent with a reduction in vesicular docking markers Synaptotagmin I and Synaptogyrin I. Additionally, assessment of long-term synaptic plasticity showed that expression of pathological Tau in hippocampal astrocytes leads to impairment of long-term potentiation (LTP), a synaptic surrogate of memory, that is associated to impairment of associative memory tested with the contextual fear condition paradigm. Importantly, application of the Hsp90β inhibitor Tanespimycin (17-AAG) ameliorated LTP impairments due to tau deposition in the astrocytes of the hippocampus. Altogether these results extend our understanding regarding plasticity impairments induced by astrocytic deposition of pathological Tau and suggest a possible approach for rescuing these deficits.

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S20-01

MITOCHONDRIA DYNAMICS AT THE CROSSROADS OF ANXIETY AND BEHAVIOR

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Understanding how stress and lifestyle affect highly anxious populations will facilitate identification of intervention-specific biosignatures, accurate diagnosis and selection of effective treatment strategies for pertinent pathologies. Mitochondria quality control lies at the epicenter of the bioenergetic regulation in the brain in health and disease. Here, we discuss how acute stress, early life interventions and dietary regimes shape molecular brain responses in high anxiety through mediating mitochondria pathways and mitochondria dynamics processes. To address this question, we use mouse models, behavioral biology, in vivo pharmacology, mitochondrial biochemistry and mass spectrometry-based proteomics/metabolomics. Our data shed light on the regulatory role of mitochondria dynamics in mammalian responses to external stimuli and provide novel targetable pathways for pharmacological treatments in high anxiety.

Funding

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S20-02

MITOCHONDRIA-NUCLEUS P53 SIGNALING IN ALZHEIMER'S DISEASE AND STROKE

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The p53 tumor suppressor protein, a key regulator of cell apoptosis, accumulates in affected brain areas from stroke and AD (Alzheimer´s disease) patients. However, whether p53 plays any role in the etiopathology of these neurological disorders remains unknown. We found that both oligomerized amyloid β (Aβ) peptide and experimental ischemia triggered Cdk5-induced p53 phosphorylation and stabilization in neurons, leading to mitochondrial dysfunction and neuronal apoptosis. Moreover, subcellular localization of p53 is an important regulator of neurodegeneration. The human single nucleotide polymorphism (SNP) of p53 at codon 72, which yields an arginine to proline aminoacidic substitution (Arg72Pro), modulates protein nuclear-mitochondrial localization and controls neuronal susceptibility to apoptosis induced by both Aβ and ischemia. While both polymorphic variants accumulate within the nuclei, the Arg72p53 variant, but not the Pro72p53 one, interacts directly with mitochondrial Bcl-xL, which triggers mitochondrial dysfunction and activates the intrinsic apoptotic pathway in neurons. Furthermore, the Arg72Pro SNP also dictates functional outcome of stroke and AD patients Our results reveal that p53 mitochondrial-nuclear crosstalk is essential to determine neuronal apoptosis and, consequently, brain damage, which finally controls functional prognosis of AD and stroke patients.

S20-03

MITOCHONDRIA STRESS SIGNALLING DETERMINES THE CELLULAR FAITH IN PARKINSON'S DISEASE

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Mitochondria under stress trigger a plethora of signalling mechanisms involving cellular transformations that are designed to restore mitochondrial function and include changes in gene expression alongside protein synthesis and metabolic reprograming. With the significant increase in our understanding of mitochondrial stress signalling and quality control mechanisms, we are now able to distinguish that mitochondrial dysfunction may lead to mitochondrial unfolded protein response (mtUPR), integrated stress response (ISR), mitochondrial import stress responses and mitophagy. Importantly, mitochondrial stress signalling may act in a cell nonautonomous and transgenerational manner.

Mitochondrial dysfunction and impairment of quality control are well documented in Parkinson's disease (PD). Alongside mitochondrial dysfunction, evidence for mitochondrial stress signalling is present in PD. The question remains how are the signalling mechanisms generated by mitochondrial stress influencing the general cellular homeostasis, and how are they influencing the cellular decision to survive or die? Here we will discuss evidence that moderate, transient stress signalling has a positive effect and supports the cell to resolve a temporary imbalance while, sustained stress signalling is detrimental and leads to cell death, particularly during the ISR activation via ATF4/CHOP signalling. Moreover, we will show that impairment in mitochondria stress signalling ability and dysfunctional quality control lower the threshold for activation of innate immune responses. Finally, we will discuss how mitochondria nucleus crosstalk operates cellular signalling in conditions of combined mitochondrial dysfunction and nuclear DNA damage accumulation, two key hallmarks of age-related neurodegenerative diseases.

S20-04

REGULATION OF BEHAVIOR BY MITOCHONDRIAL CB1 RECEPTOR SIGNALING

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The brain is the most complex and interconnected organ of the body. My laboratory uses the study of type-1 cannabinoid receptors (CB1) as a tool to try better understanding brain complexity. For instance, the impact of the balance between neuronal excitation and inhibition, of astrocyte activity, of sensory perception and, more recently, of mitochondrial and bioenergetic processes on brain functions and behavior were investigate through the CB1 receptors' lens. In this talk, I will particularly focus on the recent data about CB1 receptor-dependent control of brain metabolism and its impact on behavior.

S21-01

OLIGODENDROCYTE PROGENITOR CELLS: ROLE AND FUNCTION IN THE HEALTHY AND INJURED BRAIN

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The idea that NG2-glia and myelin can also influence diseases mainly associated with neurons has emerged in the last years. Here, I am going to introduce NG2-glia and their role in health, injury and disease and show an example of a neuronal disease with a synaptic phenotype where glia and myelination are also affected and can even cause at least part of the pathology.

Phelan-Mc-Dermid syndrome (PMDS) is a subtype of Autism Spectrum Disorders (ASDs), which is caused by mutations or deletion of the postsynaptic scaffold protein SHANK3, leading to synaptic deficits. PMDS-patients show alterations in the white matter tracts and we could recently identify various myelin defects in SHANK3 deficient mice. As synaptic connections also occur between neurons and NG2-glia, we aimed to study whether these myelin abnormalities could be due to a disruption in this synaptic communication. Indeed, deletion of SHANK3 specifically in NG2-glia affects their proliferation and differentiation and leads to motor and behavioural abnormalities. Using this novel mouse model, we reveal new insights into the role of NG2-glia in ASDs as well as into the physiological function of neuron-NG2-glia synapses in the adult CNS.

These results show an early and strong involvement of oligodendrocytes and myelin in neurological/-psychiatric diseases that were thought to be fully neuronal and open new avenues for the development of therapeutic strategies this time targeting glia.

S21-02

FUNCTION AND REGULATION OF PLPPR3 MEMBRANE PROTEINS IN NEURONS

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Filopodia are slender, finger-like plasma membrane protrusions. In neuronal cells, filopodia mature into specialized compartments including neurites, axon branches and synapses. Consequently, filopodia are important for the formation of neuronal circuits during brain development.
Primarily composed of parallel bundles of actin filaments, filopodia formation, stabilization and dynamics are regulated by various proteins that associate with both actin filaments and the plasma membrane. Thus, a precise actin-membrane communication underlies the process of filopodia formation during neuronal growth and morphogenesis.
Among the membrane proteins implicated in filopodia formation, Phospholipid Phosphatase-Related Protein 3 (PLPPR3) has emerged as important regulator of this process in neurons. PLPPR3 is composed of six transmembrane domains and a long disordered intracellular domain (ICD).
Here, we show that the intracellular domain of PLPPR3 forms liquid condensates both in cells and in vitro, and induces strong co-partitioning of actin monomers into condensates, promoting actin nucleation and polymerization. Our findings suggest a biophysical mechanism by which PLPPR3 regulates actin cytoskeleton remodeling at plasma membranes, providing insights into how phase separation can drive neuronal morphogenesis and growth.

S21-03

BIOACTIVE LIPID-DEPENDENT REGULATION OF AXONAL GROWTH DURING DEVELOPMENT

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Axonal growth occurs in a highly polarized and localized fashion and is manifested as axon elongation and axon branching. Axon growth is intimately associated with attraction or repulsion of growth cones, a process dictated by extracellular guidance cues that ensure innervation of the correct anatomical targets. Despite extensive knowledge of protein-based guidance and branching cues, much less is known about transient, locally acting signals such as extracellular bioactive lipids. Here, we revisit lysophosphatidic acid (LPA), which has long been recognized for its potent yet variable effects on neuronal morphology and growth—ranging from cell rounding, axonal growth cone collapse and neurite retraction, to the formation of axonal filopodia and branches. LPA utilizes an expanded set of bona-fide LPA-specific GPCRs to alter cytosolic signaling machinery and the dynamics of the F-actin and microtubule cytoskeleton during neuron responses. We will discuss and highlight additional plasma membrane proteins that appear to constitute a second-tier of LPA responders and may serve to expand and fine-tune LPA morphological and other responses during development.

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S21-04

GENE REGULATION NETWORKS IN NERVOUS SYSTEM DEVELOPMENT AND CANCER PROGRESSION

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The generation of cellular diversity in the developing central nervous system (CNS) relies on finely tuned gene regulation networks that control the balance between neural stem cell proliferation and differentiation. Dysregulation of these networks is increasingly recognized as a key contributor to the onset and progression of CNS tumors, including glioblastomas and neuroblastomas. We have identified a core regulatory network comprising the orphan nuclear receptor NR5A2 (LRH-1), the homeobox transcription factor Prox1, and the long non-coding RNA Ariel, which exerts critical functions in both neural development and tumorigenesis. NR5A2 promotes neurogenesis by inducing Prox1 and facilitating cell cycle exit, while Ariel counteracts this effect by repressing NR5A2 and Prox1 to favor astrogenesis. Importantly, we find that this network is dysregulated in glioblastomas, where NR5A2 expression correlates with improved patient prognosis as well as inhibition of tumor cell growth *in vitro* and *in vivo*. Furthermore, pharmacological activation of NR5A2 using the agonists DLPC and DUPC inhibits proliferation of both NSCs and glioblastoma cells in vitro and reduces tumor growth in vivo in heterotopic and orthotopic xenograft mouse models. These findings uncover a developmental gene regulatory circuit that also plays important roles during tumorigenesis and highlight NR5A2 as a promising therapeutic target in nervous system malignancies. Together, our study underscores the translational potential of targeting developmental pathways to uncover new therapeutic targets for aggressive CNS tumors.

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S22-01

INTERPLAY OF GLIA, EXTRACELLULAR MATRIX, AND NEURONS AT TETRAPARTITE SYNAPSES

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The extracellular matrix (ECM) in the brain, composed of lectican-family proteoglycans, hyaluronic acid, tenascins, and link proteins, forms a scaffold that regulates cellular interactions and synaptic plasticity. During brain development, activity-dependent formation of perineuronal nets (PNNs) — dense, lattice-like ECM structures around parvalbumin-expressing interneurons in the cortex and hippocampus — marks the closure of critical plasticity windows. PNN development limits juvenile plasticity, enhances perisomatic GABAergic inhibition, reduces memory redundancy, and sharpens memory precision (1). Additionally, glial release of CS56+ ECM proteoglycans in an activity-dependent manner modulates spine plasticity (2). Experiments depleting microglia in young and aged mice highlight their role in organizing PNNs and the perisynaptic ECM of excitatory synapses, as well as regulating spine density and expression of vesicular glutamate (VGLUT1) and GABA (VGAT) transporters (3). Recent findings show that reducing neural ECM alters microglial states, preventing complement-mediated tagging of excitatory synapses. This leads to shorter microglia-synapse interactions and lower spine elimination rates, as observed via live two-photon microscopy of microglia-spine dynamics (4). These effects contrast with those seen in inflammatory states, such as those induced by lipopolysaccharide or tau-rich Alzheimer's brain fractions (5). Together, these insights reinforce the tetrapartite synapse model (6), where neurons and glia collaboratively shape the ECM, which in turn governs diverse glial and synaptic functions.

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S22-02

HYALURONAN-BASED NEURAL ECM IN PATHOPHYSIOLOGICAL PLASTICITY OF THE BRAIN

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The brain's extracellular matrix (ECM) is uniquely composed of proteoglycans and polymeric carbohydrates like hyaluronan, and it controls and maintains neuronal excitability, synaptic functions and blood-brain barrier (BBB) functionality. The proteomic and glycomic composition of the neural ECM is affected by neural activity states, development, aging and disease. Pathophysiological remodeling of the ECM and its structural specialization, the perineuronal nets (PNN), occurs at the level of biosynthesis, glycosylation, secretion and proteolytic cleavage. Increasing evidence points to a role for ECM components in the pathogenesis of neurological disorders. In murine models of epilepsy levels of core components of the hyaluronan-based ECM like Brevican, Neurocan, Aggrecan and link proteins HAPLN1 and HAPLN4 levels reliably predicted seizure properties across models, suggesting a link between ECM state and epileptic phenotype.
In independent samples of post-mortem brains and RNAseq data (from http://aging.brain-map.org/) of AD patients we observed a complex picture of ECM reorganization which reveals spatially segregated molecular rearrangements at transcript or protein levels in different subcellular fractions from cortical areas and the hippocampus, which may contribute to the pathogenic process.
The neural sheddome and ECM secretome are detectable in human plasma and cerebrospinal fluid (CSF), opening a window into their composition under pathophysiological conditions and allowing the detection of candidate molecules. In the CSF of Alzheimer's patients, levels of Brevican and Neurocan positively correlated with age, total tau, p-Tau, neurofilament-L and A beta 1-40, and in ALS patients' CSF samples higher levels of a cleaved Neurocan fragment were detected, indicating disorder-specific neural ECM rearrangements. On the other hand, Brevican serum levels in healthy young and elderly humans decrease with age and correlate with measures of cognitive flexibility.

S22-03

ASTROCYTES AND PERINEURONAL NETS IN EXTRASYNAPTIC TRANSMISSION AND NEUROPLASTICITY

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Extracellular space (ECS) volume, deposits of macromolecular substances and altered extracellular matrix (ECM) during pathological states and aging can limit plasticity due to compromised extrasynaptic volume transmission, diffusion of neurotransmitters, neuroactive molecules, growth factors, drugs and clearance of waste products from the brain. Extracellular matrix (ECM) is a network of macromolecules which has two forms - perineuronal nets (PNNs) and a diffuse ECM - both influence brain development, aging, synapse formation, neuroplasticity, progression of neurodegenerative diseases and CNS injury (for rev see Syková and Nicholson, 2008). Inhibition of hyaluronan synthesis induced by oral treatment with 4-methylumebelliferone disrupts perineuronal nets (PNNs), diffuse ECM, reduces the astrocytic network and staining for microglia. The treatment increases extracellular space (ECS) volume by about 35% and changes ECS geometry (Syková et al, 2025). The changes of diffusion barriers significantly affect diffusion parameters in the adult brain and spinal cord. Our findings suggest that disruption of ECM allows for more efficient transport of ions, neurotransmitters and neuroactive substances in the ECS and thus ensures broader inter-neuronal communication by extrasynaptic transmission. Disruption of PNNs and an increase in ECS volume can result in enhanced crosstalk between synapses, spill-over of transmitters, formation of new synaptic contacts and thus increased synaptic plasticity. Manipulation of PNNs, diffuse ECM and changes in ECS volume and geometry might be beneficial during treatment of brain diseases by opening plasticity, facilitating cell migration, growth of axons and formation of new synaptic connections in adulthood.

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S22-04

MODULATION OF CHONDROITIN SULFATES IN THE PERINEURONAL NETS MEDIATE DIFFERENTIAL GLIAL RESPONSES AFTER CNS INJURY

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Astrocytes play an essential role in regulating homeostasis and physiological functions within the central nervous system (CNS). One critical mechanism involves the homeostasis of extracellular matrix formation and degradation. Following traumatic brain or spinal cord injury, astrocytes upregulate the expression of chondroitin sulphate proteoglycans (CSPGs), which facilitate the formation of glial scars, and inhibit axonal regeneration and neurite outgrowth. Previous studies have demonstrated that the enzymatic removal of CS glycosaminoglycans (GAGs) on CSPGs using chondroitinase ABC enhances axonal regeneration and facilitates functional recovery. However, the specific role of CS-GAGs in astrocytes remains to be elucidated. In this seminar, I should illustrate how the upregulated CS-GAGs contribute to the maintenance of reactive astrogliosis post-injury in the CNS.

S23-01

DEVELOPING NEUROMARKERS OF DISEASE PROGRESSION AND COGNITIVE FUNCTION IN HUMAN PATIENTS WITH CTNS GENE MUTATIONS (CYSTINOSIS)

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Cystinosis, caused by bi-allelic mutations in the 17p13.2-located CTNS gene, is an autosomal recessive disorder with an incidence of ~1:100,000-200,000 live births. CTNS mutations often result in end-stage renal disease, hypothyroidism, and retinopathy. With the emergence of renal replacement therapy and the use of cysteamine that slows renal failure progression, researcher focus has shifted on the neurological, cognitive, and behavioral aspects of the disease.

One set of skills likely to explain some of the cognitive and academic difficulties seen in cystinosis falls under "executive functions", namely, cognitive processes that: 1. guide action and behaviors essential to aspects of learning and everyday human performance; 2. contribute to the monitoring or regulation of performance; and 3. relate in addition to socioemotional and behavioral domains. We will report on findings from a series of studies designed to probe sensory processing and executive function in individuals with cystinosis compared to age-matched controls, with the use of behavioral tests and high-density electrophysiological recordings of underlying brain activity. Cognitive testing reveals lower scores on verbal IQ and perceptual reasoning in both children and adults. Electrophysiological data show that the neural responses to basic tone stimuli are highly typical in these patients, yet in children we see a weakness in generating the MMN brain response to the deviant tones under conditions designed to tax the auditory sensory memory system in children. Furthermore, individuals with cystinosis showed a surprising increase in the amplitude of their early visual sensory responses, which may indicate some hypersensitivity to visual inputs. In the cognitive domain, poorer performance on tasks that required inhibition is accompanied by clear differences in response-inhibition and conflict monitoring related neurophysiological responses, and a reduction in neural responses associated with awareness of making an error.

Overall, electrophysiological assessments of sensory and cognitive functioning in cystinosis across the age span reveals mild-to-moderate deficits in this population. These electrophysiological techniques provide objective measures of neural functioning that may have excellent utility as biomarkers against which to test the efficacy of both pharmacological and other therapeutic interventions.

S23-02

SEX-RELATED EFFICACY IN RARE DISEASES: DEVELOPING DAVUNETIDE TO TREAT INDIVIDUALS WITH MUTATIONS OF THE ACTIVITY-DEPENDENT NEUROPROTECTIVE PROTEIN GENE (ADNP), A RARE MONOGENIC VARIANT OF AUTISM

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We revealed activity-dependent neuroprotective protein 25 years ago, protecting neurons against electrical blockage. Taking a reductionist approach to avail brain penetration, we discovered davunetide (NAP=NAPVSIPQ). To assess ADNP activity in vivo, we developed the first knockout model, presenting neural tube closure defects and inability to form a brain. Heterozygous ADNP mice live, exhibiting developmental delays, cognitive, social, and motor deficits predicting a developmental syndrome, the ADNP syndrome. A major pathology associated with ADNP deficiency in mice and children is tauopathy, pathological Tau depositions characterizing Alzheimer's disease (AD) and related tauopathies. Thus, davunetide's main interacting site are microtubule (MT) end binding proteins (EB1/EB3), dramatically increasing Tau-MT interaction, protecting against MT breakdown and oxidative stress, underlying neurodegenerative processes. Cell cultures and animal models presenting ADNP deficiency or ADNP mutations are protected by davunetide implicating efficacy in children suffering from the ADNP syndrome and in neurodegenerative tauopathies. Our recent results show that ADNP is essential for sex-dependent hippocampal neurogenesis, through male unfolded protein response and female mitochondrial gene regulation, implicating a dramatic sexual dichotomy, but including davunetide protection in both sexes (Mol Psychiatry. 2024 Dec 23). As such, ExoNavis Therapeutics (IG VP Drug Development) is planning a clinical trial in ADNP syndrome children. Davunetide safety profile has been demonstrated in 11 clinical trials involving 564 adult subjects (healthy, pure tauopathy, progressive supranuclear palsy, PSP, schizophrenia and prodromal AD). Efficacy was shown in women suffering from PSP (Transl Psychiatry, 2023, ExoNavis, in development) as well as sex-dependent memory boosting in patients presenting mild cognitive impairment, with somatic mutations in ADNP associated with increased tauopathy and protected by davunetide treatment.

S23-03

EXPLORATIONS OF THE MOLECULAR AND CELLULAR PATHWAYS ASSOCIATED WITH MUTATIONS OF THE *SLC9A6* GENE IN CHRISTIANSON SYNDROME

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Christianson syndrome (CS) is a monogenic condition involving X-linked intellectual disability, autism, neurodegeneration pathology, and epilepsy. CS arises from mutations in the *SLC9A6* gene located on the X-chromosome which encodes the endosomal pH regulator (Na^+, K^+)/H^+ exchanger isoform 6 (NHE6). NHE6 maintains accurate endosomal pH in recycling and early endosomes for cargo trafficking to synapse, affecting synapse development and plasticity. Loss-of-function of NHE6 lead to over-acidification of recycling endosomes which disrupts the trafficking of cargo, potentially contributing to an excitation/inhibition (E/I) imbalance in the brain. NHE6 is highly expressed in the brain, particularly in the hippocampus, which is important for learning/memory mechanisms but is vulnerable in epilepsy. Using a CS mouse model, we aim to understand how NHE6 dysfunction contributes to learning and memory deficits and hyperexcitability due to an imbalance in E/I neurotransmission. In other neurological disorders similar to CS, the BDNF/TrkB signaling pathway, essential for neuronal growth and synaptic refinement, is disrupted. Disruptions in this pathway are linked to deficits in neurotransmission. Additionally, the the K^+/Cl^- cotransporter KCC2 (SLC12A5) maintains low intracellular chloride levels and is essential for GABAergic inhibition. KCC2 is found in recycling endosomes and defects in its activity may contribute to seizures. In CS mice, we observed a significant reduction in KCC2 levels in the hippocampus, suggesting either delayed maturation or mistrafficking. We found KCC2 colocalized more in lysosomes in CS mice compared to early/recycling endosomes, suggesting a mistrafficking in the absence of NHE6. Future experiments will explore whether these changes affect GABAergic transmission and if modulating KCC2 can restore hippocampal function in CS models. These findings offer insights into KCC2 trafficking mechanisms and potential therapeutic targets for CS.

S23-04

DEVELOPING A COMMON NEUROPHYSIOLOGICAL ENDOPHENOTYPE (NEUROMARKER) IN BOTH HUMAN PATIENTS AND A MOUSE MODEL WITH CLN3 GENE MUTATIONS (BATTEN DISEASE)

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CLN3 disease is a prevalent form of Neuronal Ceroid Lipofuscinosis (NCL) caused by inherited mutations in the CLN3 gene, with symptoms such as vision loss, language impairment, and cognitive decline. The early onset of visual deficits complicates neurological assessment of brain pathophysiology underlying cognitive decline, while the small number of CLN3 mutation cases in humans hinders the study of sex differences. Building on our recent progress in assessing auditory neurophysiological changes in CLN3 patients, we developed a parallel approach using electroencephalography arrays in Cln3 knockout (Cln3-/-) mice to investigate the longitudinal progression of auditory processing deficits in both sexes. We employed a duration mismatch negativity (MMN) paradigm, similar to that used in our CLN3 patient studies, to assess the automatic detection of pattern changes in a sequence of stimuli. Wild-type mice of both sexes showed robust duration MMN responses when assessed longitudinally in the same subjects from 3 to 9 months of age. In contrast, female Cln3-/- mice developed consistent MMN deficits throughout this age range, while male Cln3-/- mice exhibited MMN deficits at younger ages that were mitigated at older ages. Analyses of auditory brainstem responses indicate that MMN abnormalities in Cln3-/- mice are not due to peripheral hearing loss. Instead, these deficits originate centrally from sex-specific and age-related changes in auditory evoked potentials elicited by standard and deviant stimuli. Our findings reveal a sex-specific progression of central auditory processing deficits in Cln3-/- mice, supporting auditory duration MMN as a translational neurophysiological biomarker for mechanistic studies and therapeutic development.

S24-01

ENGINEERING NEUROTROPHIN RECEPTORS AT THE SURFACE OF EXTRACELLULAR VESICLES CARRYING SMALL NON-CODING RNA THERAPEUTICS

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RNA therapeutics are emerging as groundbreaking treatment approaches for addressing a range of diseases of the nervous system and advancing precision medicine. Yet, their widespread use in clinics is hindered by several challenges, including instability and inefficient cellular delivery. Extracellular vesicles (EVs) appear as promising delivery vehicles for small non-coding RNA (sncRNA) due to their nanosized scale, lipid bilayer coating, high stability and low immunogenicity. However, significant challenges remain: how can sncRNA be loaded into EVs? Are these methods robust enough to evolve into a new pharmaceutical technology? How can EVs be decorated to yield specific cell targeting and avoid sncRNA off-target effects? In this talk, I will show the results recently obtained towards the development of a methodology to load EVs with sncRNA and to check the quality of the obtained nanocarrier. Furthermore, the possibility that two neurotrophic receptors, namely the TrkA and p75NTR receptors, and their shared ligand nerve growth factor (NGF), can mediate a targeted interaction between EVs and recipient cells was investigated. TrkA and p75NTR can separately bind NGF, and their synergistic effect in transducing NGF signal has suggested for decades the possibility that a ternary p75NTR-NGF-TrkA complex can be formed. Interestingly, co-crystal structures have demonstrated that NGF binds to TrkA and p75NTR extracellular domains in an antiparallel manner. This raises the hypothesis, that we are currently addressing, that a ternary complex can be formed in trans, between one receptor present on the EV surface and the other one on recipient cells. To verify this hypothesis, we are producing EVs carrying either receptor, and performing cellular internalization and functional assays, to verify the advantage of co-delivering these EVs with NGF. The possibility that such interaction can serve as a way to potentiate NGF signalling will be discussed.

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S24-02

LOSS OF NEUROPROTECTION IN ALZHEIMER'S DISEASE: SHEDDING LIGHT ON BDNF RECEPTOR CLEAVAGE AND ITS MIRRORING IN EXTRACELLULAR VESICLES

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The neuroprotective BDNF/TrkB-FL system is compromised in Alzheimer's disease (AD). Amyloid-beta triggers calpain-mediated TrkB-FL receptor cleavage, leading to TrkB-ICD formation, a novel intracellular fragment. Biological fluids are used to pinpoint potential disease biomarkers and extracellular vesicles (EVs) are cell-specific carriers of promising pathological biomarkers. Thus, this work aimed to 1) investigate TrkB-ICD effects and 2) its presence in human AD samples and EVs. To that endeavour, neurons were transduced with either LV-CamKII-GFP or LV-CamKII-TrkB-ICD-IRES-ZsGreen and used for patch clamp, dendritic spine and transcriptomic analysis. For plasma samples, patients fulfilled the criteria for Mild Cognitive Impairment due to AD (MCI_{AD}), whereas controls ($MCI_{CONTROL}$) reported cognitive complaints but had no evidence of Aβ pathology or neuronal injury. MCI_{AD} plasma-derived EVs (pdEVs) were isolated using the ExoQuick reagent and characterized. EVs from 48-hour conditioned medium of control, GFP- and TrkB-ICD-V5-transduced differentiated SH-SY5Y cells and primary neuronal cultures were also isolated through differential ultracentrifugation and characterized to understand TrkB-ICD EV incorporation. Neurons overexpressing TrkB-ICD were enriched in phosphotyrosine proteins and possessed fewer dendritic spines. TrkB-ICD neurons are also more hyperexcitable given by mEPSC increase and concomitant decrease in resting membrane potential. Transcriptomic data revealed that TrkB-ICD upregulates 23 GO terms, encompassing genes implicated in for synaptic structure/function. PDEVs of MCI_{AD} patients contained higher levels of TrkB-ICD. TrkB-ICD and TrkB-ICD-V5 were detected in both SH-SY5Y EV subpopulations. Altogether, these data demonstrate TrkB-ICD extracellular secretion, alluding for its potential toxicity dissemination.

S24-03

TRANS-SYNAPTIC SIGNALING VIA EV AND MICRORNA CARGO MEDIATES BDNF-DEPENDENT NEURONAL CIRCUIT FORMATION

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Extracellular vesicles (EV) are secretory vesicles that have emerged as important regulators of inter-cellular communication, in part by delivering their cargo, including proteins, lipids and RNA. Despite collective evidence implicating EV in several neurodegenerative diseases, their physiological significance in inter-neuronal communication is still largely unclear. We recently showed that neuron-derived EV regulate neurotrophin signaling via the inter-neuronal transfer of specific microRNA, small non-coding RNA that are important regulators of local protein production. Specifically, brain-derived neurotrophic factor (BDNF) signaling led to the secretion of specific microRNA in EV, which was important for the clustering of excitatory synapses, as well as excitatory synaptic transmission and the synchronization of neuronal network firing. Ongoing work further suggests the trans-synaptic transmission of neuronal small EV, which could thereby modulate neuronal circuit connectivity in health and disease. Overall, this work provides evidence for a novel constituent of trans-synaptic signaling that may be highly relevant to several neurological diseases characterized by aberrant BDNF signaling.

This project was funded via the BONFOR program of the University of Bonn Medical Faculty and the German research foundation (DFG: project number 502254462).

S24-04

NEURON-DERIVED EXTRACELLULAR VESICLES: FROM SYNAPTIC MODULATION TO TRANSNEURONAL TOXICITY

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Extracellular vesicles (EVs) play a crucial role in neuron-to-neuron communication by transporting signaling molecules that influence synaptic plasticity and neuronal survival. Neuron-derived EVs are taken up by recipient neurons, where they enhance spine density and activate key signaling pathways, promoting resilience against nutrient deprivation. However, EVs can also transfer toxic proteins like RTP801, which is linked to neurodegeneration by inducing apoptosis and reducing neuronal complexity. Elevated RTP801 levels in EVs impair protective signaling pathways, while silencing RTP801 in EVs restores neuronal arborization and maintains survival mechanisms. These findings suggest that EVs are double-edged swords in neurodegenerative diseases—supporting neural health in some contexts while spreading toxicity in others—making them potential targets for therapeutic intervention.

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S25-01

PHYSIOLOGICAL DETERMINANTS OF ALZHEIMER'S DISEASE IN THE NLGF MOUSE MODEL – PATCH CLAMP AND VOLTAGE IMAGING STUDY

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Alzheimer's disease becomes irreversible after the accumulation of neurotoxic protein plaques in the brain. Preventing this progression requires early detection of subtle neuronal dysfunctions that precede plaque formation. Emerging evidence indicates that disruptions in neuronal communication—specifically in electrical signaling—occur before these harmful aggregates appear. Evidence suggests that neuronal hyperactivity precedes significant amyloid-beta (Aβ) deposition, highlighting early physiological changes as critical insights into AD progression. This study investigates early physiological and molecular alterations in AD model mice, focusing on (i) neuronal membrane excitability; (ii) synaptic voltage dynamics, and (iii) protein expression levels. Using electrical recordings and optical voltage imaging of the neuronal physiological responses, combined with proteomics (UPLC-MS/MS), we monitor these parameters in cortical brain slices. By studying both younger mice (prior to plaque formation) and older mice (with plaques), we aim to identify early disruptions in network physiology and cortical protein expression. Our objective is to develop sensitive imaging tools for assessing cortical network health. By integrating (correlating) four key measures, never combined before—Aβ load, membrane excitability, synaptic population voltages, and proteomic changes—we aim to establish an assay capable of identifying neuronal network dysfunction prior to the irreversible phase of AD (significant amyloid plaque accumulation). This comprehensive approach, which synergizes molecular profiling with physiological testing, holds promise for advancing Alzheimer's translational research, potentially enabling earlier and more effective clinical interventions before irreversible damage ensues. Furthermore, a straightforward and sensitive assay to detect early network dysfunction—prior to amyloid plaque formation or behavioral, learning, and memory deficits—could transform how AD is studied in laboraory and treated in clinics, advancing efforts to mitigate disease progression at its earliest stages (prodromal phase). This approach could accelerate research into the earliest cellular changes in AD models (e.g. animal model of AD), and potentially foster more effective clinical interventions.

S25-01

THE FUNCTION OF THE VOLTAGE-GATED SODIUM CHANNEL $Na_v1.2$ IN PHYSIOLOGY AND PATHOLOGY

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The voltage-gated sodium channel (VGNC) $Na_v1.2$ is the principal sodium channel expressed in the axon initial segment (AIS) of pyramidal neurons, responsible for the generation, propagation, and back-propagation of the action potential (AP). This critical role highlights the significance of this ion channel for neuronal excitability and its clinical relevance linked to various neurological disorders often arising from spontaneous mutations in the *SCN2A* gene. To address the precise physiological mechanisms underlying $Na_v1.2$ function, we use ultrafast optical imaging techniques, including sodium (Na^+), calcium (Ca^{2+}), and membrane potential (V_m) imaging, paired with patch-clamp recordings, to resolve the submillisecond dynamics of AP generation at the AIS of layer-5 neocortical pyramidal neurons. These techniques enable a high-resolution analysis (50-100 μs scale) of the ionic currents and voltage in physiological conditions and disease models. In our ongoing project, we performed ultrafast recordings of Na^+, Ca^{2+}, and V_m from a heterozygote $Na_v1.2$ knock-out mouse model (*Scn2a*$^{+/-}$) associated with a loss-of-function (LOF) of the channel, inducing features of autistic-like behavior, and compared them to those obtained in wild-type (*Scn2a*$^{+/+}$) mice. We observed a significant reduction in Na^+ influx at the AIS in the *Scn2a*$^{+/-}$ model, consistent with an expected decreased number of functional $Na_v1.2$ channels. To further probe $Na_v1.2$ interactions, we applied pharmacological interventions targeting specific ion channels by locally delivering toxin-derived agents such as Dendrotoxin and Iberiotoxin. This analysis revealed distinct modulatory effects taking place in the AIS in SCN2A-related channelopathies. Moreover, using the same imaging methods, we assessed the effects of a potential $Na_v1.2$ modulator, the scorpion venom peptide AaH-II. We showed that it increased the slow component of Na^+ influx, and produced a spurious slow Ca^{2+} influx through Ca^{2+}-permeable VGNCs. In summary, our findings shed light on physiological changes observed in the mouse model of $Na_v1.2$ LOF to correlate behavioral dysfunction with the cellular mechanism of AP generation. By integrating our techniques with targeted pharmacology, we provide a robust platform for targeted therapeutic assessments in the channelopathy research field.

S25-02

APPLICATION OF HUMAN BRAIN ORGANOIDS IN NEURODEGENERATION RESEARCH

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Brain diseases represent a significant burden for modern society, leading to substantial healthcare costs, loss of productivity, and emotional strain on patients and families. The growing prevalence of neurovascular and neurodegenerative disorders underscores the urgent need for effective treatments. Here we present research strategies arising from emerging technologies, including 3D cultures of human brain tissue in the form of brain organoids. By applying protocols significantly upgraded by our own group we developed models which include cells of the nervous tissue (neurons, astrocytes) in various stages of their maturity. Moreover, by long-term growing (up to 120 days) of brain organoids during which markers of all 6 layers of neocortex develops, we are able to observe events present both in normal, but as well in disturbed brain cortical structures. Here we present an overview of application of advanced models of the nervous tissue with the goal to detect and influence phenomena present after hypoxic/ischemic incident, including neuroinflammation and cell death. Moreover, we use brain organoid models to decipher cellular and molecular phenomena present in the Down`s Syndrome and in the Alzheimer`s disease. This already allowed us to detect genes involved in both detrimental processes in the cortical tissue (e.g. DYRK1A, involved in cellular aging) or genes which bring cell-protective effects (e.g. BACE2, anti-amyloidogenic action).

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S25-04

SWEET DISRUPTIONS: N-GLYCOSYLATION ALTERATIONS IN NEURONAL DIFFERENTIATION AND TRISOMY 21

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N-glycans are complex carbohydrate structures attached to proteins, essential for cellular communication, adhesion, and immune regulation. In the nervous system, N-glycosylation is critical for neurodevelopment, synaptic function, and myelination. It regulates key processes such as neuronal differentiation, axon guidance, and receptor signaling, influencing both normal physiology and disease pathology. Our recent findings demonstrate that N-glycan profiles differ significantly between trisomy 21 and disomic stem cells, indicating that N-glycosylation changes occur at the earliest stages of neural development. These differences are in line with the previously observed premature aging phenotype at both the cellular and organismal level, and as such may contribute to altered cellular behavior, potentially affecting brain structure and function later in life. Given its widespread influence, N-glycosylation represents a key but often overlooked factor in neurological research. Recognizing the impact of N-glycans in the nervous system could transform our understanding of neurodevelopmental and neurodegenerative diseases. By integrating N-glycomics into neuroscience, we may uncover novel biomarkers and therapeutic strategies, opening new avenues for diagnosis and intervention.

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YMS I-1

JUVENILE mGluR2/3 Agonist LY379268 TREATMENT ENHANCES PREFRONTAL GABAERGIC TRANSMISSION AND IMPROVES BEHAVIORAL AND SYNAPTIC DEFICITS IN BOTH THE MAM MOUSE AND MAM RAT NEURODEVELOPMENTAL MODELS OF SCHIZOPHRENIA

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Schizophrenia is a severe, neurodevelopmental psychiatric disorder emerging in late adolescence/early adulthood lacking effective treatment.

We aim to understand the synaptic properties of layer 5 (L5) pyramidal neurons (PN) of medial prefrontal cortex (mPFC) in adolescence and dissect the effect of a novel mGluR2/3 agonist, LY379268 (LY37), on rescuing cognitive and negative symptoms (e.g. social withdrawal) in the methylazoxymehtanol acetate (MAM) model of schizophrenia, both in mice and rats.

We performed voltage-clamp recordings in mPFC brain slices from adolescent (P40-P45) saline and MAM-treated C57BL/6 male and female mice to investigate spontaneous inhibitory and excitatory postsynaptic currents (sI/E-PSC) in L5 PN. We studied cognitive function through the temporal order object recognition task and social interaction in a homecage (SIH) in adolescence and adulthood. For the rescue experiments, saline- and MAM-treated mice received LY37 (1mg/kg) or saline i.p. during peri-juvenile development (P25-P31), followed by voltage-clamp recordings, TOR and SIH tests in adolescence, but also we evaluated the effects of peri-juvenile LY37 treatment on behaviour in adulthood.

Adolescent MAM mice exhibit a significant decrease in sIPSC frequency accompanied by reduced number of parvalbumin (PV) interneurons, but not change in sEPSC frequency, compared to controls, shifting the excitation/inhibition (E/I) ratio towards excitation for MAM animals. Peri-juvenile LY37 treatment rescued the number of PV+ INs, shifted E/I ratio to control levels and improved TOR and SIH behaviors in adolescent and adult MAM animals.

Overall, juvenile LY37 intervention successfully prevented E/I imbalance, but also TOR and SIH performance observed in MAM mice during adolescence and adulthood.

YMS I-2

DELETION OF THY-1 INDUCES A DISTINCT PARTIALLY ACTIVATED ASTROCYTE PHENOTYPE IN MICE

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Thy-1 (CD90) is a highly conserved glycosylphosphatidylinositol-anchored cell surface protein with a peculiar expression pattern. In the brain, Thy-1 is expressed exclusively on the surface of mature neurons. Although the Thy-1 promoter is widely used as a neuron-specific promoter for transgenic expression, the exact role of the endogenous Thy-1 protein remains largely unknown. Thy-1 receptors, ITGB1, ITGB5 and Syndecan 4, are expressed on astrocytes suggesting a potential interaction of both cell types. Since the interplay of neurons and astrocytes is crucial for maintaining normal CNS health and function, we investigated the role of Thy-1 in neuron-astrocyte communication using a complete as well as a neuron-specific Thy1-KO mouse model. In both mouse lines, astrocytes exhibited increased expression of a distinct set of activation-associated genes, such as Gfap, Vim, and Tnc. These changes were more prominent in aged mice, indicating a delayed onset of the astrocytes' phenotype. Further, interaction of cultured astrocytes with recombinant Thy-1 in vitro confirmed this phenotype. Functional assays demonstrated that Thy-1 significantly restricts astrocytes growth and inhibits proliferation. Whole genome expression analysis showed that Thy-1 regulates the expression of neurotransmitter receptors and potassium channels, highlighting its role in synaptic clearance. Taken together, our data demonstrate that Thy-1 controls the activation of astrocytes, resulting in a distinct astrocyte phenotype characterized by reduced expression of a subset of activation-associated genes and reduced proliferation. These findings provide valuable insights into the molecular mechanisms underlying astrocyte activation and suggest potential therapeutic targets for modulating astrocyte function in CNS diseases.

YMS I-3

ROLE OF DEVELOPMENTAL REGULATORS OF AXONAL LOCAL TRANSLATION IN ADULT AXON REGENERATION

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Local translation (LT) is a key mechanism for development, maturation and regeneration of axons, as it provides them with the ability to modulate their proteome *in situ* and independently of the soma. Indeed, LT is one of the first processes activated after injury, and high capacity of axons for LT is correlated with their intrinsic ability for regeneration. However, the mechanisms underlying regulation of LT in adult axons remain elusive. Previous work from our lab has identified a dual function of the protein Mena in developing axons; Mena orchestrates cytoskeletal dynamics, and regulates LT of specific mRNAs by forming a ribonucleoprotein complex (Mena-RNP). Here, we explore the role of Mena in the adult nervous system, and its potential contribution to axon regeneration after injury. We show that the axonal interactome of Mena significantly changes pre- and post-injury, from a translation regulation- to a cytoskeletal regulation-mode respectively, in good agreement with a dual Mena function. Moreover, we observe that genetic ablation of Mena in mice leads to a significantly decreased LT capacity of PNS axons in response to injury, resulting in their impaired regeneration *in vivo*. This is mediated by impaired LT of key molecules that orchestrate regenerative responses in axons. More specifically, we show that Mena is crucial for LT of PI3K and the activation of the PI3K/Akt/mTOR pathway post injury. Overall, our study sheds light on adult axon biology, by introducing Mena as a novel key molecular player in the acute axon response to injury and subsequent regeneration.

YMS I-4

ADVANCED IN VITRO MODELS OF BLOOD-BRAIN BARRIER LEAKAGE POST-TRAUMATIC BRAIN INJURY: INSIGHTS INTO ASTROCYTE AND NEURONAL INTERACTIONS

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Traumatic brain injuries (TBI) are a debilitating condition that results in the progressive death of brain cells, leading to motor disorders, cognitive decline, and disability. Current therapies for TBI primarily address symptoms rather than the underlying pathological changes, making the development of effective treatments a significant challenge for healthcare providers and research scientists alike.

The brain depends on a stable microenvironment for proper function. Following TBI, disruptions in cellular interactions lead to widespread damage, including increased permeability of protective barriers like the blood-brain barrier. This "leakiness" exacerbates injury by enabling inflammatory mediators to infiltrate brain tissue. Astrocytes and neurons are central to this process, as astrocytes regulate neurovascular signalling and support neuronal health, while neurons influence astrocytic responses. Despite their importance, the mechanisms underlying these interactions post-TBI remain poorly understood.

Traditional cell models often fail to capture the complexity of these cellular interactions, highlighting the need for advanced research platforms. To address this, we have developed a physiologically relevant in vitro model using primary human brain cells. These cells are cultured in a modular system that mimics TBI by applying mechanical injury to replicate trauma. This model enables the study of how astrocyte-neuron interactions influence injury progression and facilitates the discovery of therapeutic targets.

By focusing on the dynamic interplay between astrocytes and neurons, this research offers new insights into the pathophysiology of TBI. It also provides a powerful platform for identifying novel drug targets and signalling pathways, ultimately advancing strategies to improve brain health and recovery following TBI.

YMS I-5

THE ROLE OF TUMOR-ASSOCIATED MACROPHAGES IN THE BRAIN METASTASIS MICROENVIRONMENT

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Background: Brain metastases (BrM) occur in 10%-25% of cancer patients, making up over half of adult brain tumors. Despite advances, median survival remains under a year. Microglia (Mg) and monocyte-derived macrophages (MDM) respond to BrM, but their specific roles in tumor progression are unclear. Whether tumor-associated microglia (TA-Mg) and tumor-associated MDM (TA-MDM) (collectively termed TAMs) impact BrM similarly to gliomas and how metastatic cell origin and brain location influence these responses, particularly their spatial distribution, requires further investigation.
Methods: To distinguish between TA-Mg and TA-MDM, we employed genetic tracing mouse models that enable us to trace these cells specifically. Experimental BrM of melanoma (RET), lung carcinoma (D122), and breast cancer (E0771) were established in these mice by intracardiac or intracranial injection.
Results: Our results show that Mg are the first myeloid cells that respond to the occurrence of early-stage parenchymal metastatic lesions of lung and breast BrM. They proliferate, contact the tumor cells, are activated, and accumulate at the surface of the tumors. While some MDMs are present in small masses, their density is lower than that of Mg. In larger tumors, Mg density decreases, and MDMs outnumber them inside the tumor. However, in melanoma RET models, neither Mg nor MDM accumulate, suggesting a limited functional role.
Conclusions: The results also show that the activation, distribution, and ratio of TA-Mg to TA-MDM vary across BrM of different sizes and origins and in different brain regions, indicating that tumor cell genetics influence TAM behavior in a brain-region-specific manner.

YMS I-6

ASTROCYTE-ENRICHED 3D CONSTRUCTS ENHANCE TRAUMATIC BRAIN INJURY REPAIR

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The use of regenerative medicine as a potential treatment for brain injuries is gaining momentum, though challenges remain in ensuring neuronal survival and maturation. Recent advancements show promise, particularly through the implantation of human cells into rodent brains, which exhibit remarkable plasticity similar to young human progenitor cells. These cells have the ability to integrate into neural circuits and help reduce functional deficits. However, challenges such as insufficient support cells, poor integration, and lack of vascularisation continue to hinder progress. Astrocytes are crucial for neuronal recovery, as they release growth factors, support synaptogenesis, and assist in blood vessel formation. In this study, we compared neuronal progenitor cells (NPCs) cultured alone with NPCs co-cultured with astrocytes in microfluidic-based three-dimensional constructs that resemble the human cerebral cortex. Our results indicate that the co-cultures enhance neurodevelopmental marker expression and neuronal maturation while increasing viability and density of cell constructs. Upon implantation into mouse brains, we observed a significant reduction in lesion surface area and an increase in astrocytic size and axonal processes extending into target areas. Moreover, astrocyte co-cultures stimulated blood vessel formation and elevated aquaporin-4 expression, suggesting improved implant functionality. This study underscores the potential of astrocyte co-cultures to improve the integration and efficacy of cell implants for brain injuries, with significant implications for regenerative medicine. Our findings could guide future approaches to enhance recovery and integration of implanted neuronal tissues for better therapeutic outcomes.

YMS I-7

ATP DEPLETION AND RESTORATION IN CULTURED PRIMARY ASTROCYTES

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The cellular adenosine triphosphate (ATP) content in brain declines in traumatic events like ischemic strokes or mechanical impacts. To test for the mechanisms involved in impaired ATP regeneration and efficient ATP restoration we used primary astrocyte cultures as model system. A rapid and extensive decline in cellular ATP levels was only observed, if both cytosolic glycolysis and mitochondrial oxidative phosphorylation had been compromised, demonstrating that each of those ATP regenerating pathways can at least in part compensate for an impairment of the other one. The observed ATP loss was not matched by a corresponding increase in the levels of ADP and AMP, but rather severe declines in the total content of all adenosine phosphates and in the adenylate energy charge were observed. Cellular levels of creatine phosphates were depleted even faster than those of ATP, supporting the function of creatine phosphate as buffer for rapid ATP regeneration in astrocytes. ATP restoration was studied in glucose-deprived astrocytes that had been depleted by 70% of their ATP due to a 60 min exposure to the mitochondrial uncoupler BAM15. Removal of the uncoupler and application of glucose allowed only a slow ATP restoration (up to 80% of the initial ATP content in 6 h), while co-application of glucose plus adenosine strongly accelerated ATP restoration and the initial cellular ATP content was found to be fully restored already within 1 h. This demonstrates that in addition to the energy substrate glucose an adenine source is required to foster rapid ATP restoration in ATP-depleted astrocytes.

YMS I-8

CONTACTIN 2 IS IMPORTANT IN THE REGULATION OF MYELINATION OF SST+ INTERNEURONS

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The axons of hippocampal interneurons are covered by myelin sheaths, which are properly organized with the contribution of cell adhesion molecules and ion channels. Contactin 2 (CNTN2 or TAG-1), an immunoglobulin superfamily adhesion molecule, organizes the juxtaparanodal region of myelinated fibers and clusters voltage-gated potassium channels there. CNTN2 is a dynamic molecule that plays a pivotal role during development and is implicated in neuropathology (multiple sclerosis, AD).

Our data support the idea that not only is CNTN2 expressed in hippocampal interneurons [Parvalbumin (PV^+) and Somatostatin (Sst^+)] but also that it selectively affects the myelination of Sst^+-expressing hippocampal interneurons while PV^+ interneuron myelination remains unaffected. We have demonstrated that in the CNTN2-deficient interneuron populations *in vivo,* the clustering of Kv1.2 channels is decreased. Here, we present our electrophysiology analysis in Sst+ and PV+ cells, which shows reduced excitability of the former and no alteration in the intrinsic properties of the latter. Those observations prompted us to examine the Inhibitory Postsynaptic Currents (IPSCs) that pyramidal neurons receive and we observed an increase in the spontaneous IPCS they receive.

We are currently examining why and how the absence of CNTN2 selectively affects the Sst+ interneuron myelination. What is its impact on the regulation of hippocampal circuits and, thus, on behavior? To answer these questions, we have generated a conditional knock-out mouse model that will be crucial for understanding the contribution of CNTN2 to hippocampal organization and function. Overall, in this work, we report on the novel analysis of Sst+ hippocampal interneuron myelination.

YMS I-9

MOUSE MODEL OF GRIN2D-DEVELOPMENTAL AND EPILEPTIC

ENCEPHALOPATHY RECAPITULATE THE HUMAN DISEASE

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Pathogenic variants in *GRIN2D*, encoding one of the subunits of the NMDA receptor (NMDAR), are associated with developmental and epileptic encephalopathies (DEEs). Unusual for de novo mutations, the recurrent, de-novo, gain of function, missense mutation c.1999G>A (p.Val667Ile) was discovered in multiple patients.

We characterized a mouse model carrying the orthologous *Grin2d* mutation, using behavioral paradigms, electrophysiological recording in acute brain slices focusing on the activity of Purkinje neurons (PNs) in the cerebellum, and electrocorticography (ECoG) recordings monitoring brain activity and the response to several drugs.

Grin2d mutant mice exhibit a range of phenotypes that closely mirror the human disease, including premature mortality, spontaneous seizures, and early onset of motor deficits followed by cognitive impairment. In addition, we observed complex developmental changes in PNs with reduced spontaneous firing in immature mice and augmented synaptic response to NMDA application in older mice. ECoG recordings demonstrated a profound and continuous abnormal brain activity, with altered spectral properties and a prominent narrowband activity in the theta, alpha, and beta bands, paralleling the patterns seen in a patient with the same *GRIN2D* pathogenic variant. The acute administration of ketamine at a low dose (0.5 mg/kg) had a limited effect on the spectral properties, and higher dosages (4 or 10 mg/kg) caused seizures. Conversely, memantine (10 mg/kg) and phenytoin (10 mg/kg) demonstrated a small corrective effect on ECoG properties.

Together, *Grin2d* mutant mice recapitulate key phenotypes of patients with pathogenic *GRIN2D* variants, including unique abnormal brain oscillations, which may serve as a biomarker for quantifying drug responses and guiding future research efforts.

YMS I-10

EXPLORING EPENDYMAL CELL REPROGRAMMING AS A THERAPEUTIC INTERVENTION FOR HYDROCEPHALUS

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Hydrocephalus is a frequently occurring neurological disorder, affecting approximately 1 in 1000 births, defined by abnormal accumulation of cerebrospinal fluid in the brain's ventricles. A major factor in the pathophysiology of hydrocephalus is the dysfunction of multiciliated brain ependymal cells. These cells are essential for regulating cerebrospinal fluid (CSF) flow and composition while also sustaining neural stem cells within the ventricular/ subventricular neurogenic niche. Moreover, disturbances in the niche's structural organization play a significant and lasting role in hydrocephalus progression. The primary treatment for hydrocephalus typically involves CSF's neurosurgical diversion, a procedure associated with high rates of morbidity and failure. This underscores the urgent need to develop innovative therapeutic strategies for hydrocephalus.

Our study aims to evaluate the reprogramming potential of GemC1 and McIdas, critical regulators of multiciliated ependymal cell fate determination, and investigate whether reprogramming cells to ependyma could be beneficial for hydrocephalus. **Our study demonstrates that GemC1 and McIdas induce direct cellular reprogramming towards ependyma.** We provide evidence that ectopic expression of GemC1 and McIdas reprograms cortical astrocytes and programs mouse embryonic stem cells into ependyma. McIdas is sufficient to induce functional activity in reprogrammed astrocytes. Additionally, we demonstrate that McIdas expression supports ependymal cell regeneration in two distinct postnatal hydrocephalus mouse models—one involving intracranial hemorrhage and the other a genetic form of hydrocephalus—while also improving the structural integrity of the neurogenic niche. **Our study presents evidence supporting the restoration of ependymal cells in animal models of hydrocephalus, offering potential pathways for developing future therapeutic interventions.** This study was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "2nd Call for H.F.R.I. Research Projects to support Faculty Members & Researchers" (Project Number: 2735) and the Hydrocephalus Association USA.

YMS II-1

INTERPLAY OF SERUM LIPIDS AND MICROGLIA IN THE SUSCEPTIBILITY TO THE LONG-TERM BEHAVIORAL EFFECTS OF ADVERSE CHILDHOOD EXPERIENCES

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Adverse childhood experiences (ACE) constitute a significant risk factor for adult-onset neuropsychiatric disorders. However, individual susceptibility to the long-term effects of ACE varies widely and the biological mechanisms of susceptibility vs. resilience to ACE remain largely unknown. Emerging evidence supports the role of peripheral metabolic factors, in particular, serum lipids and their associated non-coding RNAs in the effects of ACE. We hypothesize that microglia centrally integrate lipid-mediated signals and alter their phagocytic and inflammatory outputs to determine the susceptibility vs. resilience to ACE.

Our study synergizes investigations of in vitro human microglia with serum samples collected from ethnically diverse human ACE cohorts. These include a cohort of Pakistani children who experienced ACE in the form of paternal loss and maternal separation (PLMS), as well as adult Bosnian men with exposure to genocide during their childhood. Analyses from both the children and adult men confirm consistent decrease in serum high-density lipoproteins (HDLs) in the ACE-susceptible subjects. Furthermore, treating HMC3 human microglia like cells with serum from ACE-susceptible vs. ACE-resilient subjects impairs microglial metabolism and synaptoneurosomes phagocytosis.

Our ongoing research focuses on ascertaining a role for miR-142-3p, an-HDL associated miRNA in microglial dysregulation induced by ACE-induced serum. Furthermore, we are developing an iPSC-derived model of human microglia for pre-clinical validation of our results with an eventual goal of elucidating microglia-based interventions to mitigate the long-term effect of ACE.

YMS II-2

ASPIRIN AS A MODIFIER OF EPIGENETIC RESPONSES: DNA METHYLATION CHANGES IN A SOCIAL INSTABILITY MODEL OF DEPRESSION IN FEMALE WISTAR RATS

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Abstract

DNA methylation, an epigenetic modification, has been implicated in increased susceptibility to depression. Aspirin has been reported to have possible antidepressant effect. This study aimed to determine the epigenetic mechanism of antidepressant effect of aspirin in a social instability stress (SIS) model of depression. Eighty adult female Wistar rats weighing between 180 g and 220 g were exposed to SIS to induce depression-like behaviours. Before and after induction, rats were subjected to behavioural tests to determine resilient and susceptible rats. Rats were divided into nine groups (n=7), and treated as follows: (i) control+distilled water (DW) (1 ml/kg) (ii) resilient+DW (1 ml/kg) (iii) susceptible+DW (1 ml/kg) (iv) susceptible+escitalopram (ESC) (10 mg/kg) (v) susceptible+RG108 (0.4 mg/kg) (vi) susceptible+ASA (10 mg/kg); (vii) susceptible+ASA (10 mg/kg)+ESC (10 mg/kg); (viii) susceptible+ASA (100 mg/kg); and (ix) susceptible+ASA (100 mg/kg)+ESC (10 mg/kg). ASA (oral), ESC (oral), and RG108 (intraperitoneal) were administered once daily for twenty-one days. Rat brain samples were collected for gene expression and whole genome methylation studies. The result showed: (i) reversal of depression-like behaviours (ii) DNMT3L gene expression upregulation in susceptible+ASA (100 mg/kg) and downregulation in susceptible+ESC groups compared to the control (iii) knockout of methylated depression susceptibility genes in the susceptible+ASA (100 mg/kg) and susceptible+RG108 (0.4 mg/kg) groups. In conclusion, aspirin had DNA methylation inhibitory properties comparable to the standard DNA methyltransferase inhibitor - RG108, and exerted its antidepressant effect through this epigenetic mechanism. This study recommends that aspirin should be used as an epigenetic-targeted (adjunct) antidepressant.

YMS II-3

THE RETINA AS A WINDOW TO THE BRAIN: DYSREGULATED ENDOCANNABINOID SIGNALLING AS A BIOMOLECULAR MARKER OF EARLY ALZHEIMER'S DISEASE

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The concept of the retina as a "window to the brain" in Alzheimer's disease (AD) is increasingly emerging because of its extra-cerebral manifestations in the eye. Here, we explored the possible alterations of the endocannabinoid system (ECS), the onset of gliosis and oxidative stress in the retina of AD-like mice at an early stage of AD phenotype. 12-month-old Tg2576 (TG) mice over-expressing the amyloid precursor protein (APP) were used and the major enzymes, receptors and lipids belonging to the ECS were quantified in the retina. Through western blotting and immunofluorescence, we found that TG retinas displayed an up-regulation of cannabinoid receptor 2 (CB_2) and of monoacylglycerol lipase (MAGL), the enzyme responsible for the degradation of 2-AG. Consistently, UPLC–MS/MS demonstrated a significant reduction of 2-AG in TG retinas, while a trend toward increase was found for the other eCB AEA. No statistically significant differences were found for the other enzymes/ receptors of the ECS under study. ECS alterations were not associated with hippocampal or retinal β-amyloid plaques, retinal degeneration or excitotoxicity; instead, oxidative stress burden and gliosis (particularly microglia reactivity) was observed. Moreover, linear regression analysis for individual animals showed a significant correlation between amyloid precursor protein (APP) and CB_2 /MAGL. Overall, our data indicates that ECS dysregulation, neuroinflammatory events and oxidative stress burden in the retina of AD-like mice may be considered early retinal biomarkers of AD; yet our study further supports the retina as a suitable tool to investigate the molecular underpinnings of brain diseases.

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YMS II-4

MONITORING THE IMPACT OF PROLONGED USE OF CANNABIDIOL IN THE HEALTHY BRAIN: A MULTISCALE ANALYSIS

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Despite the approved use of pharmaceutical cannabinoids in humans for pathological conditions and their promising results in neurological/neuropsychiatric disorders, Cannabidiol (CBD) has become increasingly popular as a food supplement for self-reported stress relief. CBD-based supplements are driving growth in the global cannabidiol market, valued at USD 5 billion in 2021. However, the effects of prolonged CBD use on the healthy brain remain largely unknown. We exposed 5-6 month-old wild-type mice and C.elegans to CBD treatment (mice 30 mg/kg; i.p for 7 weeks; C.elegans 50μM, from egg until day 5) with behavioral, molecular, biochemical, cellular, neurostructural, and proteomic analysis. Surprisingly, prolonged CBD administration in wild-type mice induced anxiety and mild cognitive deficits accompanied by spine loss, but not dendritic atrophy, of hippocampal neurons. Furthermore, molecular and proteomic analyses of the hippocampus showed that CBD increased autophagy pathways and decreased synaptic modulation, alongside mitochondrial dysregulation. In line with our mice findings, C. elegans exposure to CBD dysregulated mitochondrial and lysosome activity damaging synaptic activity and neuronal function, leading to reduced motility and behavioral deficits. Our findings suggest a detrimental effect of prolonged CBD use on the healthy brain, highlighting the urgent need for further investigation into the uncontrolled use of cannabis-derived products. The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 83145/114219/β6.ε).

YMS II-5

α-SYNUCLEIN IS A NOVEL FUNCTIONAL INTERACTOR OF THE EXOCYST COMPLEX

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The study aims to investigate the potential interaction between α-synuclein and the exocyst (EXOC1-8), a multi-protein assembly critical for vesicle tethering to the plasma membrane. Exploring the role of α-synuclein in cellular trafficking and its physiological interactions may support the understanding of pathways disrupted in neurodegenerative diseases.

Mass spectrometry analysis in SHSY5Y cells inducibly expressing α-synuclein, highlighted vesicular trafficking pathways, implicating the exocyst. Immunostaining showed that α-synuclein colocalizes with EXOC2, ECOC3 and EXOC7. Co-immunoprecipitation experiments in crosslinked and non-crosslinked cellular and protein liquid chromatography fractions did not indicate direct interaction. However, immuno-electron microscopy demonstrated recurrent proximity between EXOC3 and α-synuclein in the cytoplasm, nucleus and tethered vesicles in SHSY5Y cells and primary cortical neurons. EXOC3 depletion of CRISPR-Cas9-mediated EXOC3 knockout cells led to significant downregulation of α-synuclein mRNA and protein levels, indicating EXOC3's role in the regulation of α-synuclein levels. Furthermore, α -synuclein-KO adult mice exhibited significant and striatum-specific downregulation of the exocyst complex compared to wild type mice, further supporting a reciprocal regulation. In the striatum of wild type mice, EXOC3 was found in GABAergic and cholinergic neurons, whereas in α-synuclein KO mice, EXOC3 was found expressed in astrocytes and less in neurons.

These findings suggest a functional interplay between α-synuclein and the exocyst, which likely exist as part of a large hyper-complex. Our findings shed light on a yet unidentified physiological function of α-synuclein and highlight the exocyst complex as a novel regulator of intracellular α-synuclein levels.

YMS II-6

SPATIOTEMPORAL DYNAMICS OF SYNAPTIC DYSFUNCTION IN p.A53T-αSyn MODELS: INVESTIGATING EARLY PATHOLOGY AS A THERAPEUTIC TARGET

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Alpha-synuclein (αSyn) is a highly expressed & conserved presynaptic protein, considered a critical factor in Parkinson's disease (PD) development. Synapse dysfunction is thought to be an early & progressive feature of PD pathology, with its initiating mechanisms yet to be determined. This study focuses on the p.A53T-αSyn mutation, which has been found to affect synapse formation and function, even from the neural progenitor cell stage and aims to investigate the mechanisms underlying early synaptic dysfunction in PD. Herein, a transgenic mouse model expressing the human p.A53T-αSyn in brain neurons under the control of the PrP promoter, alongside with human-derived neurons bearing the p.A53T mutation are employed. Proteomic analysis of synaptosomes from 6-m.o. mutant mice reveals dysregulation of presynaptic proteins particularly involved in synaptic vesicle trafficking. The investigation of p.A53T synapse ultrastructure at 3-m.o. further corroborates these findings, showing diminished synaptic vesicle numbers & impaired PSD formation. These structural abnormalities are accompanied by imbalance in glutamatergic & GABAergic systems across brain regions, long before dopaminergic neuron loss. Additionally, *in vitro* analysis reveals early synaptogenesis defects & compromised excitatory & inhibitory contacts, while longitudinal electrophysiological studies in developing hippocampal neurons indicate aberrant network activity and synaptic dysfunction. Notably, the administration of dual-allosteric NMDAR antagonists, Memantine & Nitrosynapsin, potentially reverses the observed synaptic dysfunction. Altogether, these findings highlight the spatiotemporal emergence of synaptic dysfunction as a key feature of p.A53T-αSyn pathology. Early intervention with neuromodulatory agents offers a promising strategy to mitigate PD progression. Funded by H.F.R.I. (PN: 1019);GSRI (PN: TAEDR-0535850-BrainPrecision [EU (NextGenEU); Greece 2.0];HPI Excellence Scholarship-Nostos Fdn.

YMS II-7

AN INNOVATIVE DECOY PEPTIDE STRATEGY TO MITIGATE A-SYNUCLEIN PATHOLOGY IN PARKINSON'S DISEASE MODELS

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Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by motor and non-motor symptoms. Motor symptoms such as tremors and rigidity, result from the degeneration of dopaminergic neurons in the substantia nigra. Non-motor symptoms such as constipation and dementia arise from the dysfunction of additional neurons. Both Motor and non-motor symptoms are caused by accumulation and aggregation of α-synuclein (α-syn). Notably, α-syn also aggregates in other neurodegenerative diseases, including Dementia with Lewy bodies (DLB) and Multiple System Atrophy (MSA). Currently, there is no therapeutic approach available to prevent α-syn pathology. In PD, treatment is primarily symptomatic, and relies on the replenishment of dopamine in the brain to alleviate the motor symptoms.

We have previously shown that α-syn is monoubiquitinated, leading to the proteasomal degradation of α-syn. We also found that an additional post-translational modification, SUMOylation, compete with and decrease α-syn monoubiquitination, leading to reduced proteasomal degradation and α-syn accumulation. Therefore, we sought to develop a technology to decrease α-syn SUMOylation. In this framework, we developed a cell-penetrating peptide that works as a decoy to reduce α-syn SUMOylation. We found that this decoy peptide facilitates the degradation of α-syn and decreases the levels of endogenous and pathological α-syn in neurons. We also found that the decoy peptide is specific towards α-syn as it does not decrease the levels of other proteins related to neurodegenerative diseases such as tau and general protein SUMOylation. The decoy peptide crosses the blood-brain barrier and decreases the levels of endogenous α-syn in the brain.

Most importantly, we found that the decoy peptide reduces α-syn pathology when administered intraperitoneally in the PD mouse model of α-syn pre-formed fibrils (α-SynPFF). We propose that this decoy peptide represents a promising strategy for targeting α-syn aggregation and may hold potential as a disease-modifying treatment for PD and possibly other α-synucleinopathies.

YMS II-8

SNCA-TARGETING ANTISENSE OLIGONUCLEOTIDES AS A THERAPEUTIC APPROACH FOR ALPHA-SYNUCLEINOPATHIES

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According to clinical, genetic, and experimental evidence, alpha-synuclein (SNCA) accumulation is implicated in synucleinopathies like Parkinson's disease, multiple system atrophy, and dementia with Lewy bodies. In the absence of disease-modifying treatments, we explored the therapeutic potential of antisense oligonucleotides (ASOs) targeting SNCA to suppress its expression in physiologically relevant models.

We tested modified SNCA-targeting ASOs in human neuroblastoma cell cultures and conducted in vivo studies using two-month-old bacterial artificial chromosome (BAC) transgenic rats expressing the human wild-type SNCA locus. ASOs were delivered intracerebroventricularly, and outcomes were evaluated after forty-five days using molecular, cellular, and behavioral analyses.

SNCA-ASOs efficiently reduced SNCA mRNA and protein levels both in vitro and in vivo across multiple brain regions. Transcriptomic analysis revealed diminished synaptic activity and neuronal gene expression, coupled with region-specific alterations in glial gene expression. Protein assays demonstrated intricate changes in neuroimmune mediators and significant AKT1S1/PRAS40 phosphorylation alterations in the olfactory bulb, with an overall trend of reduced AKT pathway activation in most brain regions. Behaviorally, treated rats exhibited increased mobility, enhanced exploration, and improved olfactory function.

These findings provide molecular, cellular, and behavioral evidence supporting the efficacy of SNCA-targeting ASOs in reducing SNCA levels and improving behavioral outcomes, underscoring the potential of intrathecal ASO delivery as a promising disease-modifying therapy for alpha-synucleinopathies.

YMS II-9

CHOLESTEROL ESTERIFICATION IS HAMPERED IN ALZHEIMER'S DISEASE

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Alterations in brain lipids are a central feature of AD, nevertheless therapeutic strategies targeting brain lipid metabolism are still lacking. Our lab recently reported that a pharmacological inhibitor of the fatty acid enzyme, stearoyl-CoA desaturase (SCD), led to recovery of hippocampal synapses with associated improvements in learning and memory in 3xTg-AD mouse model. Here, we use the 5xFAD model to further delve into lipid metabolism disruptions in AD, and into the effect of the SCD inhibitor (SCDi) on fatty acids (FAs) alterations and synapse loss.

Hippocampi from 5xFAD and NC mice were collected at 5 and 8 months of age for FAs profile, analysed by gas chromatography–flame ion detection (GC–FID), and IHC for β-amyloid, GFAP and Iba-1. SCDi was infused in 5xFAD and NC mice 5 MO, through an intracerebral ventricular osmotic pump for 28 days. Hippocampi were processed for GC-FID and Golgi staining for dendritic spines quantification.

FAs alterations, appeared in female hippocampus at 5 months (together with plaque pathology and gliosis) and worsened by the age at 8MO, while males began to show alterations at 8MO. The desaturation index of the SCD enzyme associated with the conversion of palmitic to palmitoleic acid showed a significant increase in 5xFAD mice, notably at 8 months of age, but starting at 5MO in females. Treating 5xFAD females' mice 5MO for 1 month with a SCDi improved dendritic spine density and normalized fatty acid levels.

Taken together, data demonstrate that at symptomatic stages the central SCD inhibition has beneficial effects in a second mouse model of AD, 5xFAD characterized by a more aggressive and rapid progression of disease. These findings identified SCD as a novel promising therapeutic target for AD.

YMS II-10

NOVEL ANTISENSE OLIGONUCLEOTIDES AGAINST TAU BRAIN PATHOLOGY

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Emerging evidence suggests the mediating role of Tau protein and its malfunction in neuronal deficits found in brain pathologies with diverse etiology, including Alzheimer's disease and other Tauopathies, epilepsy, traumatic brain injury and chronic pain. Thus, as targeting Tau protein is a promising therapeutic approach, the present study designed and tested 50 novel Antisense Oligonucleotides (ASOs) –a novel therapeutic tool that modifies gene expression- against total Tau or selectively 4R-Tau. ASOs efficiency in reducing pathological Tau and downstream damage of neuronal structure, as well as cognitive deficits, was tested in primary neurons and mice expressing mutated human Tau.

After initial screening in cell lines, primary neurons from wild-type (WT) or P301L-Tau transgenic mice were incubated with selected ASOs before being treated with amyloid beta ($A\beta_{1-42}$) oligomers or vehicle. Additionally, THY-Tau22 transgenic male and female adult mice and their WT littermates were intracerebroventricularly injected with ASOs or a non-targeting control followed by multidimensional behavioral assessment.

In primary neurons, ASOs blocked Aβ-induced neurotoxicity and reversed neuronal atrophy, as measured by the number of MAP2 positive neuronal cells and neurostructural analysis. Moreover, ASOs blocked the Aβ-induced increase in Tau accumulation. In THY-Tau22 transgenic mice, ASOs ameliorated Novel Object Recognition performance and increased freezing time in contextual fear conditioning, displaying an attenuation of Tau-related memory deficits.

Altogether, these data provide *in vitro* and *in vivo* evidence of the beneficial use of ASOs against Tau-related neuronal malfunction, supporting ASOs as an innovative therapeutic approach in Tau-related neurodegenerative pathologies.

M001-01

White Matter Myelination and Axonal Function in a Rat Model of Fragile X Syndrome

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Fragile X Syndrome (FXS) is a neurodevelopmental disorder resulting from the lack of the protein FMRP due to the transcriptional silencing of the FMR1 gene. FXS is one of the leading single gene causes of autism spectrum disorder (ASD), affecting approximately 1 in 4000 males and 1 in 8000 females. Individuals present with intellectual disability, ASD, sensory hypersensitivities and epilepsy amongst other comorbidities.

Whilst neuronal deficits have been extensively described in rodent models of FXS, the contribution of glial cells, specifically oligodendrocytes, to FXS pathophysiology is largely understudied, despite FMRP being expressed in these cells. Oligodendrocytes are the myelin producing cells in the central nervous system. Normal circuit function relies on appropriate myelination, which ensures the fast transmission of action potentials through the formation of the nodes of Ranvier.

MRI imaging studies in FXS individuals as well as *Fmr1* KO mice have revealed white matter abnormalities consistent with impaired myelination across a number of white matter tracts, including the *corpus callosum*. Recently published data from our lab suggests that cell autonomous defects in oligodendrocyte maturation and morphology observed *in vitro* contribute to altered cortical myelination *in vivo* in a *Fmr1* KO rat model of FXS. However, we do not know how myelination defects translate to functional deficits in FXS.

Given the evidence for white matter defects in FXS humans and rodents and the role of myelin in regulating action potential propagation, the current project aims to assess axonal function and white matter myelination in the *Fmr1* KO rat model of FXS. We are using electrophysiology to assess action potential conduction velocity in the *corpus callosum* of *Fmr1* KO rats. Additionally, we're using immunohistochemistry to assess oligodendrocyte maturation and nodal architecture, and electron microscopy to assess myelin ultrastructure. Preliminary data indicates nodal architecture is altered in juvenile *Fmr1* KO rats, potentially contributing to altered axonal function.

MO01-2

REACTIVE ASTROCYTES AND ASTROCYTE TNF SIGNALING CONTRIBUTION IN DEMYELINATION AND REMYELINATION

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Astrocytes are the most abundant glial cells in the CNS, critical for brain homeostasis but also involved in the pathogenesis of neurodegenerative disorders, like multiple sclerosis. Recently, we showed that selective pharmacological inhibition of soluble TNF by XPro1595 led to faster repair of demyelinated lesions in a cuprizone demyelination model[1]. We previously identified a cell autonomous role for microglia in enhancing remyelination in the absence of TNF receptor 1[1]. Here we explored the contribution of astrocytes and the role of TNFR1 signaling to cuprizone demyelination and remyelination. Neuropathological analysis and 3D-reconstructed cell morphometrics revealed strong reactivity of grey matter astrocytes in control and XPro1595-treated animals at demyelination onset, and this reactivity was significantly enhanced by soluble TNF inhibition. Transcriptomic profiling of astrocytes and enrichment analysis of single-nuclei RNA-Seq data[1] in demyelination revealed that significantly regulated pathways by soluble TNF are involved in cholesterol metabolism and steroid biosynthesis, as well as neuronal activity. To interrogate the direct role of TNF signaling in astrocytes, we used astrocyte-specific TNFR1 knockout mice during cuprizone demyelination and remyelination. Myelin quantification revealed that astrocyte-specific TNFR1-depletion resulted in greater myelin loss at peak demyelination and impaired remyelination compared to controls, indicating that TNFR1-deficiency in astrocytes severely impairs their contribution to brain repair following demyelination. Our results identify astrocytes as significant components of brain demyelination and remyelination in a multiple sclerosis model and highlight the importance of intact TNFR1 pathways to the homeostatic and repair functions of cortical astrocytes.

1. Boutou A et al. Microglia regulate cortical remyelination via TNFR1-dependent phenotypic polarization. Cell Rep. 2024; doi:10.1016/j.celrep.2024.114894

MO01-03
ROLE OF SPHINGOLIPIDS IN FRACTALKINE MEDIATED MYELIN REPAIR

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Remyelination is a complex event that requires the orchestrated intervention of various cell types present in the lesion niche. Recruitment of oligodendrocyte precursor cells (OPC) to the injured site, while crucial for remyelination, sometimes fails, thus facilitating this process and stimulating OPC maturation into myelinating oligodendrocytes (OL) could prevent disease progression. Fractalkine (FKN) is able promote remyelination acting both on OPC and microglia, enhancing the proliferation and the differentiation of the former.

Analysis of the sphingolipid content of OPC, differentiating OL and BV2 microglial cells treated with FKN revealed that while the treatment does not affect the phospholipid content, sphingolipid composition is altered. In particular, total levels of ceramide, a sphingolipid that represents not only an important pro-apoptotic signal, but also a signal for the re-arrangement of sphingolipid- and cholesterol-rich signaling platforms, is significantly reduced in all cells treated with FKN. Moreover, treated BV2 cells also show reduced cholesterol levels, however cholesterol content in OPC and OL remains unchanged.

Protein levels of integrin receptors and kinases of the Src family in OPC and BV2 cells treated with FKN have also been analysed. In BV2 cells, the treatment leads to a decrease in the levels of integrin receptor subunits αV and β3, while no difference is observed for Src nor Lyn. On the other hand, FKN in OPC induces an increase in integrin αV and β3. Moreover, while total levels of Lyn and Src kinases are reduced, there is an increase in their activation.

Altogether, these results support the notion that FKN signaling might be mediated by alterations of lipid-dependent membrane organization and/or signalling in different glial cell present in the lesion niche.

MO01-4

Beyond Multiple Sclerosis: Exploring the Spectrum of Autoimmune-Mediated Demyelination

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Multiple sclerosis (MS) is a central nervous system (CNS) autoimmune demyelinating disease characterised by neuroinflammation, myelin loss, and axonal damage. Diagnosing MS can be challenging due to similarities with other conditions, including systemic autoimmune diseases (SADs) which can present with similar symptoms. Without specific biomarkers, MS diagnosis is based on the McDonald criteria, which emphasise ruling out other conditions. This diagnostic uncertainty leads to a significant misdiagnosis rate, with around 20% of patients being misdiagnosed.

Our study included 250 patients referred to Aeginition Hospital in Athens under the initial suspicion of MS. Only 66.3% were diagnosed with MS spectrum disorders, while 24.9% were classified under the CNS autoimmune group, which included 6.7% of patients diagnosed with SAD and CNS involvement and 18.1% belonged to the "demyelinating disease with autoimmune features" (DAF) group, showing signs of CNS demyelination and autoimmunity without fitting MS or SAD criteria. Transcriptomic analysis on RNA from whole peripheral blood of treatment-naïve patients showed that MS patients overexpressed genes regarding blood coagulation and platelet aggregation, SAD patients overexpressed genes of innate immunity while DAF patients overexpressed genes related to cell structure and organisation. MS, SAD, and DAF groups had unique gene expression profiles, indicating diverse underlying pathogenetic mechanisms.

This study so far underscores the need for biomarkers to differentiate these conditions, aiding accurate diagnosis and personalized treatment. Improved diagnostic precision will help avoid misdiagnosis, reducing unnecessary disability and enhancing patient outcomes.

M001-5

MICROGLIA PROMOTE CNS REMYELINATION IN THE CORTICAL GREY MATTER UPON POLARIZATION TO AN INFLAMMATORY REGENERATIVE PHENOTYPE

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Microglia are implicated in neurodegenerative demyelinating diseases with increasing evidence for roles in CNS regeneration. Here we combine pharmacological and conditional gene-targeting tools, together with transcriptomics and 3D brain imaging, in in vivo and in vitro experimental models of multiple sclerosis, to investigate mechanisms of microglia-mediated CNS remyelination. Pharmacological inhibition of soluble TNF (solTNF) and microglia-specific genetic deletion of TNF receptor 1 (TNFR1) revealed an altered microglia polarization characterized by beneficial Arginase-1 expression, highly activated morphology and enhanced phagocytic clearance of myelin debris, that accelerate cortical remyelination and motor recovery. Longitudinal brain RNA-Seq revealed distinct transcriptome signatures of demyelination and remyelination, with earlier recovery upon microglia TNFR1 deletion. Single cell transcriptomics at disease onset revealed that activated disease-associated microglia maintain an IL-10-reparative inflammatory phenotype in the absence of solTNF, instead of switching to a default IL-1-related damaging profile. The functional relevance of microglia inflammatory polarization is validated in vivo, by administering an IL-1 receptor antagonist (IL-1Ra, Anakinra) in cuprizone experimental demyelination. Furthermore, disease-state microglia producing IL-1/IL-18/CASP1 targets are validated in human demyelinating lesions. Overall, our results show that highly activated inflammatory microglia mediate cortical remyelination, and demonstrates a critical role of microglia solTNF-TNFR1-IL-1 axis in controlling the switch between reparative and damaging microglia, with potential therapeutic relevance for CNS demyelinating diseases.

Reference: Boutou et al. “Microglia regulate cortical remyelination via TNFR1-dependent phenotypic polarization.” Cell Reports (2024)
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MO01-6

Concurrent COVID-19 Infection and Epstein-Barr Virus Reactivation at the First Clinical Episode in a Greek Cohort of Multiple Sclerosis Patients

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Background: Multiple sclerosis (MS) is a chronic autoimmune disorder strongly linked to Epstein-Barr virus (EBV) infection. Double-stranded RNA (dsRNA) is a key marker of viral genomes, replication, and transcription, produced by DNA viruses like EBV and positive-strand RNA viruses like SARS-CoV-2. Here we investigate the interplay between viral infections and autoimmunity, particularly whether COVID-19 is associated with EBV reactivation and influences the first MS clinical episode.

Methods: Plasma and cerebrospinal fluid (CSF) from 78 MS patients were analyzed for: 1) dsRNA using an in-house sandwich-ELISA (J2/K2 antibodies, Nordic-MUbio), 2) antiviral cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12p70, IFN-α2, IFN-β, IFN-λ1, IFN-λ2/3, IFN-γ, TNF-α, CXCL10, GM-CSF, Biolegend), 3) EBV-specific antibodies (VIDIA), and 4) SARS-CoV-2-specific antibodies (anti-Spike/Nucleocapsid IgG and IgM), by in-house ELISAs.

Results: Plasma dsRNA levels correlated significantly with all 13 antiviral cytokines in the plasma of MS patients. Recent COVID-19 infection (anti-Nucleocapsid IgM+, n=8) and EBV reactivation (anti-EBNA-1 IgG+/IgM+, n=9) were associated with elevated dsRNA and cytokine levels. A strong correlation was observed between anti-Nucleocapsid IgM and both anti-VCA and anti-EBNA-1 EBV IgM, indicating concurrent COVID-19 infection and EBV reactivation. Anti-Spike IgG was detected in 71% (35/49) of CSF samples, with higher antibody levels associated with an increased number of SARS-CoV-2 vaccine doses.

Conclusions: This study provides the first evidence that COVID-19 infection is associated with EBV reactivation, possibly triggering the first MS clinical episode in some patients. These findings underscore the importance of monitoring EBV reactivation in the context of MS pathogenesis, particularly in the aftermath of COVID-19 infection.

MO01-7

ASSOCIATION OF B-CELL ACTIVATING FACTOR GENE VARIANTS WITH SERUM ANTI-JCV ANTIBODY POSITIVITY IN MALE PATIENTS WITH MULTIPLE SCLEROSIS UNDER NATALIZUMAB TREATMENT: IMPLICATIONS FOR PROGRESSIVE MULTIFOCAL LEUKOENCEPHALOPATHY RISK STRATIFICATION

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Introduction: Progressive multifocal leukoencephalopathy (PML) is a life-threatening complication among Multiple Sclerosis (MS) patients under natalizumab treatment, with serum anti-JCV antibody titters being used for stratification risk. Given the critical role of interferon (IFN)/B-cell activating factor (BAFF) axis in humoral immune anti-viral responses, we explored whether it is involved in the generation of serum anti-JCV antibodies among these patients.
Methods: 162 patients with RRMS under natalizumab treatment were included. Serum anti-JCV antibodies were measured at baseline, 12 and 24 months after treatment initiation. Type I and II IFN-inducible genes and BAFF expression were quantitated in peripheral blood by qRT-PCR. BAFF rs9514828, rs1041569, and rs9514827 gene variants were assessed by RFLP-PCR.
Results: Anti-JCV serum titter were significantl correlated with BAF gene expression (r=0.35, p=0.01). The prevalence of BAFF TTT haplotype was found to be significantly higher in anti-JCV (+) male MS patients at baseline (23.9% vs 5.7%, OR [95% CI]: 5.0 [1.4–18.2], p = 0.008) and 12 months post-treatment period (22.5% vs 6.3%, OR [95% CI]: 4.3 [1.8–15.3], p = 0.02). A higher, but marginally not significant, prevalence of BAFF TTT haplotype was observed at 24 months post-treatment (19.9% vs 6.9%, OR [95% CI]: 3.30 [0.90–12.40], p = 0.07).
Conclusions: Our study suggests an implication of BAFF axis in the production of serum anti-JCV antibodies. Moreover, the BAFF TTT haplotype derived from the rs9514828, rs1041569, and rs9514827 variants may represent a novel risk factor for anti-JCV seropositivity and indirectly for PML among male MS patients treated with natalizumab.

MO01-8

LIPIDOMICS OF RESPONSE TO TREATMENT IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic disease, where the immune system attacks myelin, leading to pain, spasticity and, ultimately, death. As there is no cure for MS, treatment focuses on reducing relapses, slowing disease progression and, overall, managing MS symptoms.

In this study, we investigated the lipidome of MS patients that were treated with two different disease-modifying therapies - dimethyl fumarate (DMF, a small molecule Nrf2 activator) and ocrelizumab (OCZ, an anti-CD20 monoclonal antibody), with the aim of discovering potential biomarkers relevant to disease progression and drug response.

Plasma samples from 71 patients were grouped into DMF and OCZ according to the response to treatment, remission of symptoms and/or relapses (R, Responders; NR, Non-Responders). Patients treated with DMF were 23 (6 NR *vs* 17 R), while 48 patients were treated with OCZ (11 NR *vs* 37 R).

Differential abundance of specific lipids was assessed using univariate statistical t-est. After data validation we identified 608 lipid species in all four groups. In both treatments, PLS-DA showed a modification in circulating lipidome between NR *vs* R. Among DMF discriminant lipids, 9 species had a p value<0.05. Particularly, SM44:6 and PC O-38:8 were higher in DMF/R, while NAGly 24:4 was lower. In OCZ, 21 species had a p value<0.05, with CL86:11, NAE16:4 and some triacylglycerol species augmented in the R group.

In short, some lipid species may have a role in MS response to treatment, depending on the type of treatment patients undergo. Even if the results of this study seem promising, further analyses are needed to better understand the role of lipids in MS response to treatment and pathogenesis.

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MO01-9

CIRCULATING LEVELS OF FREE OLEIC ACID POSITIVELY CORRELATES WITH NEUROLOGICAL IMPAIRMENT IN MULTIPLE SCLEROSIS' PATIENTS

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Multiple sclerosis (MS) is a chronic inflammatory and neurodegenerative disease of the central nervous system, and it is one of the leading neurological causes of disability in young adults. The Expanded Disability Status Scale (EDSS) is used to quantify the level of neurological impairment, assessing it across various functional systems, such as motor skills, sensory functions, visual acuity and cognitive abilities. Changes in the circulating lipid profiles can be associated with the disease progression, and specific lipid alterations may correlate with the level of disability.
69 patients (30 males and 39 females, mean age 42.5±10.7) were clustered in three groups according to their EDSS score: without disability (score between 0.0-1.5) (19 pz), minimal disability (score between 2.0-3.5) (25 pz) and severe disability (score ≥4) (25 pz). Their circulating lipidome was assessed through HPLC/HR-MS, identifying, after data validation, 608 lipid species. The association between the EDSS groups and the circulating lipids was assessed using Spearman's rank correlation.
Results. 42 lipid species significantly correlated with the aggravation of the disability score. In particular, several PC O (PC O-38:6, PC O-32:, both $p<0.0001$, and PC O-36:5, PC O-32:0, PC O-38:5, PC O-34:3, PC O-32:2, PC O-36:4, PC O-40:8, PC O-38:7, PC O-44:3, all $p<0.01$), SM 40:6;3O ($p<0.01$), CE 22:1 ($p<0.01$), and FA 36:9 ($p<0.01$) negatively correlated with EDSS severity, while FA 18:1 ($p<0.01$) positively correlated with EDSS severity.
Conclusion. MS patients with severe neurological impairment presented a characteristic pattern of circulating lipids with lower level of several plasmalogen phosphatidylcholines and higher levels of oleic acid.

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MO02-1

ALTERED HIPPOCAMPAL SHARP WAVE RIPPLES PLAYS A ROLE IN IMPAIRED MEMORY CONSOLIDATION IN CHRISTIANSON SYNDROME MOUSE MODEL

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In many neurological disorders, sleep disturbances and intellectual disability are strongly associated. Therefore, by studying these types of disorders, we can attempt to elucidate molecular and cellular mechanisms with broad relevance to brain function and disease. Christianson syndrome (CS) is an example of a monogenic condition involving X-linked intellectual disability and sleep disturbances. The lack of treatment options for these children is directly related to our poor understanding of the mechanisms underlying the disease. CS arises from mutations in the *SLC9A6* gene which encodes the endosomal pH regulator (Na^+, K^+)/H^+ exchanger isoform 6 (NHE6). NHE6 regulates the pH of intracellular membrane vesicles to facilitate cargo trafficking needed for proper function and development of neurons. However, the relation between loss of NHE6, sleep disturbance and cognitive deficits is currently unclear. In the brain, the hippocampus is the region identified in long-term memory formation. It contains specific neurons, "place cells," that code for the animal's position in its environment. During sleep, the hippocampus replays previously formed patterns of place cell activity **associated with population bursts orchestrated by "sharp wave-ripples" (SWRs),** which are necessary for the **consolidation of memories.** In a CS murine model, we found impaired spatial cognition while hippocampal place cells are mostly unaffected. However, SWRs are more frequent but of lower oscillatory frequency and power in CS. Our results provide novel insights into understanding impaired cognition during sleep in CS to contribute to a further understanding of the disease and contribute to future therapeutic interventions.

MO02-2

CIRCUIT MECHANISMS UNDERLYING THE SEXUAL DIMORPHIC EFFECTS OF RESTRAINT STRESS ON PREFRONTAL CORTICAL NEURONAL PROPERTIES

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Stress is a critical factor in various psychiatric disorders exhibiting sex-specific differences, involving cognitive dysfunctions associated with the prefrontal cortex (PFC).

This study aims to assess the sexually dimorphic effects of acute stress on PFC functions and neuronal circuits that regulate the stress response. Specifically, PFC synaptic properties, as well as the intrinsic and synaptic characteristics of PFC-BLA and PFC-PFC projection neurons, were studied.

Two groups of adult male and female mice were used, one to study the effects of acute restraint stress (RS) and a second cohort to investigate the effects of a crucial mediator of stress response, CORT on PFC physiology *in vitro*. PFC brain slices were isolated for field or voltage-clamp recordings to study the induction of long-term potentiation (LTP) and the synaptic properties of PFC, respectively. RS and CORT impaired the induction of LTP in male mice exclusively. Conversely, PFC functions in female mice appeared unaffected by RS, but exhibited reduced inhibition following CORT incubation and RS exposure.

To further investigate these sex-specific differences, mice were stereotaxically injected with cholera toxin B into the PFC or BLA, to label and analyze cortico-cortical and cortico-amygdala neuronal properties following RS. Voltage-clamp recordings from fluorescently labeled neurons in PFC slices revealed no significant sex-based differences in the synaptic properties of PFC->PFC neurons for both RS and controls. The examination of RS effects on the PFC->BLA circuit is ongoing.

These findings highlight distinct sex-specific responses to acute stress, suggesting a potential protective mechanism in the female PFC against stress-related effects.

MO02-3

B-ADRENERGIC RECEPTOR-INDUCED E-S POTENTIATION IN THE DORSAL AND VENTRAL HIPPOCAMPUS

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β-adrenergic receptors (β-ARs) play a critical role in modulating learning, memory, emotionality, and long-term synaptic plasticity. Recent studies indicate that β-ARs are necessary for long-term potentiation (LTP) induction in the ventral hippocampus under moderate synaptic activation conditions that do not typically induce LTP. To explore potential dorsoventral differences in β-AR-mediated effects, we applied the β-AR agonist isoproterenol (10 μM, 30 min) to dorsal and ventral hippocampal slices, recording field excitatory postsynaptic potentials (fEPSPs) and population spikes (PSs) from the CA1 region. Isoproterenol induced robust, long-lasting PS increases, with effects three times greater in the dorsal compared to the ventral hippocampus. Isoproterenol did not significantly affect fEPSP in either segment of the hippocampus, leading to strong excitatory-to-spike (E-S) potentiation—twice as large as that in the ventral hippocampus. E-S potentiation was not associated with significant paired-pulse inhibition changes in either hippocampal segment. These differences do not appear to result from β1-AR expression levels, as they are comparable across dorsal and ventral hippocampal regions. Overall, the findings suggest that β-AR activation enhances the dorsal hippocampus's role during stress, facilitating heightened alertness, rapid spatial information processing, and effective navigation necessary for "fight-or-flight" responses. This research work has been supported by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant no. 83343 (AM).

MO02-4

SPIKE TIMING-DEPENDENT LONG-TERM DEPRESSION REQUIRES ASTROCYTE D-SERINE AT L2/3-L2/3 SYNAPSES OF THE MOUSE SOMATOSENSORY CORTEX

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Spike timing-dependent plasticity (STDP) is a learning rule important for synaptic refinement and for learning and memory during development. While different forms of presynaptic t-LTD have been deeply investigated, little is known about the mechanisms of somatosensory cortex postsynaptic t-LTD. In the present work, we investigated the requirements and mechanisms for induction of developmental spike timing-dependent long-term depression (t-LTD) at L2/3-L2/3 synapses in the juvenile mouse somatosensory cortex. We found that postnatal day (P) 13–21 mice of either sex show t-LTD at L2/3-L2/3 synapses induced by pairing single presynaptic activity with single postsynaptic action potentials at low stimulation frequency (0.2 Hz) that is expressed postsynaptically and requires the activation of ionotropic postsynaptic NMDA-type glutamate receptors containing GluN2B subunits. In addition, it requires postsynaptic Ca^{2+}, Ca^{2+} release from internal stores, calcineurin, postsynaptic endocannabinoid synthesis, activation of CB_1 receptors, and astrocytic signaling to release the gliotransmitter d-serine to activate postsynaptic NMDARs to induce t-LTD. These results show direct evidence of the mechanism involved in developmental postsynaptic t-LTD at L2/3-L2/3 synapses, revealing a central role of astrocytes and their release of D-serine in its induction.

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MO02-5

DESCENDING CONTROL OF PAIN BY THE INSULAR CORTEX

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Emergent studies emphasize the role of the insular cortex in a plethora of functions including basic survival, complex emotional functions, as well as body- and self-awareness. One function with which the insular cortex has been strongly associated is the sensation pain. Indeed, the insula is activated during the experience of pain, while stimulation of the insula is capable of evoking pain sensation. Furthermore, increased insular activity has been observed in cases of chronic pain, hinting at a role of the insula in pathological pain conditions. Here we suggest that the involvement of the insula with pain is mediated by the Descending Pain Modulatory System (DPMS), a brainstem network that bidirectionally modulates pain. We use stereotactic injections of dyes and viruses, chemogenetic approaches and behavioral assays to study the role of the insula in pain. We verified the anatomical insula-DPMS projection and use the Descending Control of Nociception (DCN) behavioral paradigm to study the functional insula-DPMS connection. Activation of the insula with the hM3Dq or its inhibition with the hM4Di Designer Receptor Exclusively Activated by Designer Drugs (DREADD) indicate a role of the posterior insula in inhibiting DCN. In addition, we also study the effect of chemogenetic insula inhibition in the Spared Nerve Injury neuropathic pain condition. Our results accentuate the role of the insula-DPMS pathway in pain perception.

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MO02-6

TARGETING MEMORY SYSTEMS TO COMBAT DRUG ADDICTION: SEX-SPECIFIC APPROACHES TO DISRUPTING REWARD-CONTEXT MEMORIES

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Environmental contexts associated with previous drug trigger relapse through persistent "reward-context memories." These memories endure long after drug cessation, demonstrating addiction's profound impact on neural circuitry. Our research studies the dentate gyrus (DG), a brain region with unique properties that make it particularly amenable to engram research in addiction neurobiology. Using a 10-day conditioned place preference (CPP) paradigm with fentanyl (0 [saline], 0.5, 1, 2, or 4 mg/kg, IP) in C57BL/6J mice, we investigated sex differences in drug-context associations. Mice underwent pre-testing, 8 days of alternating conditioning (contexts paired with either saline or fentanyl), and post-testing. Movement and location were analyzed using SLEAP machine learning software for precise behavioral quantification. Our preliminary findings reveal significant sex-specific differences in fentanyl place preference, with female mice exhibiting stronger CPP than males. We are currently assessing whether these behavioral differences correlate with sex-specific patterns of immediate early gene (c-fos) expression in the DG, which would suggest differential engram formation or reactivation between sexes. The next phase of our research employs TRAP-Fos virus for inducible, activity-dependent labeling of DG granule cells, combined with chemogenetics to manipulate these putative reward-context engrams. This approach will address our central question: Does targeted inhibition of DG engrams disrupt context-reward memory recall? Our findings could reveal novel mechanisms for weakening maladaptive reward-context associations that drive relapse, potentially leading to sex-specific therapeutic interventions for addiction — an urgent need given fentanyl's devastating public health impact.

MO02-7

CO-CLINICAL EVALUATION OF COGNITIVE IMPAIRMENTS INDUCED BY CHEMOTHERAPEUTIC AGENTS IN MICE AND HUMANS: A RESEARCH PROTOCOL

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The co-clinical approach holds substantial clinical, biological, and pharmacological significance, as it enables the simultaneous investigation of treatment effects in both animal models and humans. This parallel analysis provides critical insights into mechanisms of action and potential therapeutic interventions. Among these treatments, chemotherapy remains a cornerstone of cancer therapy—effectively targeting malignant cells but frequently leading to adverse side effects. One such consequence is cognitive impairment, commonly referred to as "chemobrain," which may be transient or persist for years after treatment. This condition affects attention, memory, visuospatial abilities, and executive functions.

Co-clinical studies involving chemotherapeutic agents offer the potential to predict cognitive effects and to identify the precise onset of impairment, ultimately guiding strategies for prevention and management. In this study, clinical chemotherapy protocols will be adapted for mice, with dosages adjusted based on body surface area (mg/m^2) to enhance translational accuracy. Age equivalence between species will be established using stage-specific equations, ensuring valid cross-species comparisons.

Cognitive impairment will be assessed using touchscreen-based tasks analogous to CANTAB tests employed in human research, with validation through gold-standard behavioral assays. In addition, the inflammatory profile of the animals will be evaluated via plasma cytokine quantification, paralleling human analyses, and by detecting astrocyte and microglia activation through immunofluorescence techniques.

The findings of this study aim to elucidate the temporal dynamics of chemotherapy-induced cognitive dysfunction, contributing to the identification of potential therapeutic targets for the prevention or mitigation of such adverse effects, thereby promoting the preservation of cognitive function in cancer patients.

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MO02-8

DEVELOPMENTAL AND SEX-SPECIFIC EFFECTS OF A5-NICOTINIC ACETYLCHOLINE RECEPTOR DELETION ON COGNITIVE BEHAVIOUR AND ULTRASONIC VOCALIZATIONS IN MICE

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The α5 subunit of nicotinic acetylcholine receptors (nAChRs) contributes to neural circuit modulation, influencing cognitive behaviours. Mice lacking the α5-subunit of nAChRs (ACNA5 mice) exhibit decreased pyramidal activity in the prefrontal cortex due to increased SST activation, leading to altered PFC and hippocampus-related behaviours. This study examines the developmental and sex-specific effects of the α5 subunit deletion on behaviour and ultrasonic vocalizations in mice.

Adolescent (PND 35) and adult (PND 60) WT and ACNA5 C57BL/6J mice of both sexes were examined in the Open Field Test (OFT), Object Location Memory Task (OLT) and Elevated Plus Maze (EPM). Ultrasonic Vocalizations (USVs) were recorded in juvenile (PND 21), adolescent and adult WT and ACNA5 males during social interaction in the OF Arena.

ACNA5 mice lacked the typical developmental increase in locomotion seen in WT mice, indicating α5 subunit deletion affects maturation. Nevertheless, all ACNA5 groups -except for adult males- exhibited increased locomotion in the OFT. In addition, ACNA5 mice manifested reduced location memory in the OLT, compared to WT; while adolescent female and adult ACNA5 mice of both sexes failed to distinguish the novel object location, suggesting spatial memory impairments. In the EPM, female adult ACNA5 mice exhibited increased anxiety compared to both female adult WTs and adolescent ACNA5, suggesting developmental and sex-dependent influences of α5 subunit deletion on anxiety-like behavior. Finally, USV analysis revealed significant alterations in vocalization patterns, indicating potential communication deficits.

These findings highlight the significance of α5-nAChRs in shaping proper neurodevelopmental trajectories. Distinct developmental and sex-specific behavioral patterns observed in ACNA5 mice, reveal cholinergic signaling's importance for functional network maturation, with disruptions potentially causing neurodevelopmental disorders.

MO02-9

ANTICONVULSANT POTENTIAL OF *ARTEMISIA VULGARIS* ROOT EXTRACT IN A PTZ-KINDLING MOUSE MODEL

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Epilepsy is a chronic neurological disorder characterized by recurrent seizures. Drug-resistant forms remain a serious clinical problem. Since inflammation and oxidative stress play an important role in the pathogenesis of epilepsy, compounds with anti-inflammatory and antioxidant properties are of particular interest. The medicinal plant *Artemisia vulgaris* (common wormwood, AV) is considered as a potential source of such compounds.

The aim of this study was to evaluate the anticonvulsant effect of the ethanol extract of AV roots in a pentylenetetrazol (PTZ)-induced kindling model in mice. The experiment involved 72 CD-1 mice (2–2.5 months old; 33 females, 39 males) divided into 7 groups: control (10% DMSO + saline), PTZ (10% DMSO + PTZ 35 mg/kg), AV-50, AV-250, AV-500 (AV extract at doses of 50/250/500 mg/kg + PTZ 35 mg/kg), naringin (80 mg/kg + PTZ 35 mg/kg) and levetiracetam (50 mg/kg + PTZ 35 mg/kg). All drugs were administered intraperitoneally for 45 days: 36 days of induction (18 PTZ injections every other day), then 9 days of behavioral testing. The severity of seizures was assessed using the Racine scale. $P < 0.05$.

AV at a dose of 50 mg/kg significantly reduced the proportion of animals that reached stage Racine 6 or repeated seizures of stage 5, compared with the PTZ group. The protective effect was evident from the 15th injection, similar to naringin. Levetiracetam showed an earlier effect – from the 9th injection. In behavioral tests (open field, elevated O-maze, Barnes maze), no significant differences were found, probably due to the death of the most sensitive to PTZ animals before testing or the cessation of PTZ injections in early kindled mice, which provided them with time to recover.

Molecular studies are currently underway to identify systemic and brain-specific markers of inflammation, oxidative stress and neuronal damage.

Despite the encouraging results in the AV-50 group, the high interindividual variability of the low-dose PTZ kindling model limits its applicability for screening. In the future, it is planned to use the status epilepticus model for a more accurate assessment of the neuroprotective and anti-inflammatory properties of the extract.

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MO03-1

Voltage-gated calcium channels as a possible new approach for Alzheimer´s disease

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In AD the accumulation of amyloid-beta drives excessive Ca^{+2} influx via NMDA receptors, which in turn leads to calpain-mediated BDNF receptor cleavage to generate a novel intracellular fragment, TrkB-ICD, with known nefarious effects. Nevertheless, a component responsible for at least 20% of Ca^{+2} entry induced by oligomers remains unidentified. L-type voltage-gated calcium channels (VGCC) are found in the membrane of excitable cells, mediating Ca^{+2} influx responses to membrane depolarization. VGCC inhibitors are mostly used for hypertension, however, if VGCC contribute to BDNF receptor cleavage is yet to be studied.

This work aimed to investigate novel sources of Ca^{+2} influx as potential triggers of TrkB-FL cleavage. Firstly, DIV7 neocortical neurons were incubated with KCl, to induce membrane depolarization, and/or EGTA, a calcium chelator, to assess the contribution of extracellular Ca^{+2} in TrkB-FL cleavage. In parallel, the contribution of VGCC to calpain-mediated TrkB-FL cleavage was assessed by co-incubating KCl with either Nifedipine (L-type antagonist), ω-Agatoxine (P/Q-type antagonist), ω-conotoxin (N-type antagonist), SNX-482 (R-type antagonist) or Ethosuximide (T-type antagonist). Neuron homogenates were collected after 24h and their TrkB-FL, TrkB-ICD and ratio protein levels measured by Western-blot. A time chase assay of KCl+Nifedipine was also conducted, assessing TrkB-FL and TrkB-ICD at 8, 16 and 24h.

KCl-depolarized neurons increased levels of TrkB-ICD as compared to controls ($p<0.05$ n=4-8), with no changes in TrkB-FL immunoreactivity, which was rescued when co-incubated with EGTA ($p<0.05$). Experiments performed with VGCC showed that only Nifedipine rescued TrkB-ICD formation ($p<0.05$, n=3-5) and TrkB-ICD/TrkB-FL levels ($p<0.05$, n=3-5) at 24h post treatment. Results from the time chase assay were similar, as co-treatment with Nifedipine decreased TrkB-ICD immunoreactivity at 16h ($p<0.05$, n=4) and 24h ($p<0.05$, n=4).

Altogether, these results showcase that TrkB-FL cleavage may be by both NMDA- Ca^{+2} entry and by other Ca^{+2} channels, suggesting a potential new strategy for addressing AD by utilizing approved VGCC blockers. **Funding:** AstraZeneca Young Doctors Research Grant, EU Horizon 2020, grant No. 952455, Scientific, Technological and Innovation Research Support Office; Lisbon Holy House of Mercy (MB37-2017, MB35-2021), HORIZON-WIDERA-2023-ACCESS-04-01, grant No. 101160180 (PANERIS)

MO03-2

NEURONAL RTP801 AFFECTS ADULT HIPPOCAMPAL NEUROGENESIS IN VIVO IN HEALTH AND ALZHEIMER'S DISEASE

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Neurogenesis is the process of new neuron formation in the nervous system and it is maintained throughout life in specific neurogenic niches. Among them, the dentate gyrus (DG) of the hippocampus is gaining attention given the role that adult hippocampal neurogenesis (AHN) has in cognition and memory. RTP801/REDD1 is a stress-induced protein that inhibits mTOR signaling pathway. RTP801 has been linked to embryonic neurogenesis. Selective silencing of RTP801 in radial glia, impairs migration and promotes premature differentiation of neural progenitors during cortex development. Moreover, neuronal RTP801 regulates neuroinflammation in a murine model of Alzheimer's disease (AD). Neuroinflammation is known to be a key regulator of AHN which dramatically halts the neurogenic process.

Here, we wanted to study the role of neuronal RTP801 in AHN in physiological conditions and in the 5xFAD mouse model of AD. 6-month-old male WT and 5xFAD mice were subjected to bilateral injections of shCt or shRTP801 AAV at CA1 and DG. After 4 weeks animals were euthanized, and samples were processed for immunohistochemical analysis.

Immunofluorescence analyses of mice hippocampi revealed that RTP801 knockdown in neurons increases the number of Sox2+ cells in the subgranular zone (SGZ) of the DG. In this line, the number of mature NeuN+ neurons increases, independently of the genotype. Altogether results suggest that neuronal silencing of RTP801 increases the differentiation of neural stem cells (NSCs) of the SGZ to mature neurons.

This new putative role of RTP801 paves the way for further studies aimed to unravel the significance of such process but already suggests an important role in migration and differentiation of NSCs in AHN.

MO03-3

BREAKING THE FLOW: HOW AMYLOID-β DISRUPTS AXONAL AUTOPHAGY IN EARLY ALZHEIMER'S DISEASE

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The pathological accumulation of proteins observed in the brain of Alzheimer's disease (AD) patients suggests a role for autophagy dysregulation in the disease's pathogenesis. Autophagy is essential for the degradation and recycling of cellular components in a physiological manner, and it is vital for maintaining synaptic function. The autophagic process is tightly regulated by several proteins, including WD-repeat protein interacting with phosphoinositides (WIPIs), which control the formation and maturation of autophagosomes. During aging, autophagosomes undergo modifications and their production declines, but the mechanisms driving this process remain unclear. In this study, we demonstrate that amyloid-β oligomers (AβO) impair autophagosome biogenesis through a WIPI2-mediated mechanism. We hypothesize that this disruption occurs early in AD development, exacerbating age-related changes and contributing to axonal dysfunction. Using 3xTg-AD mice, we found increased levels of phosphorylated WIPI2 (p-WIPI2) in the hippocampus of young female mice. Additionally, using primary cultures of rat hippocampal neurons, we observed early alterations in the axonal levels of p-WIPI2, WIPI2, ATG16L1, and LC3 (an autophagy marker). These alterations were also detected at the synaptic level. Given the critical role of WIPI2 phosphorylation in autophagosome formation, we further investigated the molecular mechanism underlying these changes. Our findings indicate that the AβO-induced autophagy disruption involves NMDA receptor activation and CaMKII signaling, which likely modulate WIPI2 phosphorylation. Together, these findings highlight WIPI2 phosphorylation dynamics and ATG16L1 regulation as promising therapeutic targets for early intervention in AD.

MO03-4

Aβ ISOMERIZATION ACCELERATES PATHOLOGICAL PROCESS IN AB-INDUCED ALZHEIMER'S DISEASE MODEL

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Alzheimer's disease (AD) is a common neurodegenerative disorder marked by the accumulation of phosphorylated tau and β-amyloid (Aβ) isoforms. A prevalent modification of Aβ is the isomerization of the aspartic acid residue Asp7 (isoAβ), which was detected in up to 50% of Aβ in AD brain tissue. IsoAβ is significantly more toxic to neuronal cells than Aβ and triggers extensive amyloidosis in AD models. Yet, its impact on early, before plaque formation AD progression was not studied. Our work aimed to compare the effects of Aβ and isoAβ on behavioural, biochemical, and molecular changes in an Aβ-induced AD model *in vivo*, focusing on inflammation, oxidative stress, and the cholinergic system.

To create an acute model of AD, BALB/c mice were neurosurgically operated by injecting Aβ or isoAβ into the brain ventricle. Animal behaviour was assessed in Open Field and Social Interaction tests on days 14 and 21 post-injection. Brain homogenates were analysed for biochemical and molecular markers using TBARS assay, Elman's method, ROS production measurement, cholinesterase activity assays, Western blotting, RT-PCR, and ELISA one month after injection.

IsoAβ notably, ahead of Aβ increased mice aggression level that is typical for early AD stages. IsoAβ-induced behavioural changes were associated with pathological microglia and astrocyte activation, leading to heightened production of pro-inflammatory IL-1β and NF-κB. While Aβ also elevated cytokine levels, it did not enhance GFAP and Iba1 level. Only isoAβ induced tau pathology, reducing total tau level and increasing phosphorylated tau. Both peptides engendered oxidative stress and pathological changes in the cholinergic system. However, isoAβ lowered reduced glutathione level and increased the level of the α4 subunit of nicotinic acetylcholine receptor to a higher degree than Aβ. These findings underscored that Aβ isomerization sharpens debut of AD pathology, that makes it a worthwhile therapeutic target.

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MO03-5

Brain Insulin Resistance and Oxidative Stress: Unraveling the Role of BVR-A in Neurodegeneration

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Background: Biliverdin reductase-A (BVR-A) is a pleiotropic enzyme involved in multiple intracellular processes and cellular redox homeostasis. BVR-A has been identified as a fundamental regulator of insulin signaling (IS), and studies from our lab have demonstrated that its loss induces insulin resistance in both peripheral tissues and the brain. Recent evidences suggest that BVR-A dysfunction is involved in brain insulin resistance, a crucial factor in the progression of Alzheimer's disease (AD). Here, we aimed to explore whether a reduction in BVR-A protein levels represents an early molecular event linking brain insulin resistance, oxidative stress (OS) imbalance, and AD-markers related neurodegeneration.

Methos: Male and female C57Bl/6J wild-type (WT) and BVR-A knock-out (BVR-A$^{-/-}$) mice were fed either a standard diet (SD) or a high-fat diet (HFD, 60% kcal from fat) for 1 or 8 weeks. A subgroup of mice received intranasal insulin (INI, 2 UI total) to assess brain insulin sensitivity. Markers of OS (protein carbonyls, 3-nitrotyrosine, 4-hydroxynonenal) and AD (APP cleavage) were analyzed.

Results: INI significantly increased AKT activation after 8 weeks on SD, an effect lost under HFD, indicating impaired IS. In BVR-A$^{-/-}$ mice, INI induced aberrant signaling activation and mTOR hyperactivation, regardless of diet. While a HFD induced early IS alterations in WT mice after 8 weeks, it was not sufficient to trigger an increase in OS. However, BVR-A$^{-/-}$ mice exhibited elevated OS, suggesting that the absence of BVR-A is enough to induce these changes. APP cleavage analysis showed higher C99 fragment levels in HFD-fed male WT mice, similar to those in BVR-A$^{-/-}$ mice, suggesting that the loss of BVR-A activates the amyloidogenic pathway. This response is absent in female.

Conclusions: Our results suggest that a HFD induces early alterations in IS in WT mice without increasing OS. However, in BVR-A$^{-/-}$ mice, the absence of BVR-A is sufficient to drive a rapid increase in OS, highlighting its crtical role in cellular stress regulation and the aberrant activation of IS. These findings support the hypothesis that disruptions in IS are among the earliest events involved in neurodegeneration, preceding the increase in oxidative damage, supporting BVR-A as a potential early marker of brain insulin resistance.

MO03-6

STRUCTURAL AND THERMODYNAMIC ANALYSIS OF ALZHEIMER'S DISEASE-PROTECTIVE APOLIPOPROTEIN E VARIANTS

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Apolipoprotein E (apoE), an important protein of the lipid transport system in peripheral tissues and the brain, has three common isoforms, APOE2, APOE3 and APOE4. APOE4 is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). New rare APOE variants recently identified in patients with AD appear to exert protective roles. These variants include the APOE3-Christchurch(R136S), APOE3-Jacksonville(V236E) and APOE4(R251G). The molecular basis behind the protective effects and reduction in AD risk by these variants has not been elucidated. APOE is characterized by structural plasticity and thermodynamic instability and can undergo significant structural rearrangements as part of its biological function. To examine whether there is a structural basis behind the properties of the protective APOE variants, we analyzed their structural and thermodynamic integrity both in APOE3 and APOE4 allelic background and in comparison, to their wild-type counterparts. Circular dichroism spectroscopy revealed that only the V236E variation significantly alters the secondary structure of APOE3 and APOE4 in lipid-free form. This variant was also found to be less prone to oligomerization as compared to the other APOE forms. Chemical denaturation analysis showed changes in the chemical unfolding profile of V236E and R251G APOE variants in lipid-free form. Finally, thermal unfolding analysis revealed small thermodynamic alterations in each variant compared to their wild-type APOE counterparts, both in lipid-free form and associated to lipoprotein particles. Overall, our findings suggest that the specific single amino acid changes that are found in AD-protective APOE variants can induce distinct changes in the molecule's stability and conformation and may underlie functional consequences.
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MO03-7

LONG-TERM IMPACTS OF CHRONIC STRESS: THE ROLE OF NEUROINFLAMMATION AND TRKB IN STRESS-ACCELERATED ALZHEIMER'S PATHOLOGY IN MICE

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While most Alzheimer's (AD) studies focus on the cognitive aspects of the disease, less focus is given to affective symptoms. In this study, we investigated the long-term consequences of exposure to chronic stress. 5xFAD AD model mice were exposed to Unpredictable chronic mild stress, and cognitive and emotional aspects were examined at 3-time points (up to 4 months after exposure to stress). We found that exposure to chronic stress exacerbates neuropathology in the 5xFAD mouse model in adulthood, accompanied by changes in the neurotrophic system. In-vitro, we show that corticosterone impairs the ability of microglia to uptake Aβ and reduces microglial activation. To conclude, our study shed light on mechanisms through which chronic stress might contribute to the onset and advancement of Alzheimer's disease symptoms.

MO03-8

EXPRESSION AND PHARMACOLOGICAL MODULATION OF 18kDa TRANSLOCATOR PROTEIN IN DIFFERENT CELLULAR MODELS OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a form of dementia caused by widespread degeneration of neuronal cells in the brain, leading to memory deficits and cognitive decline. The main molecular determinants include extracellular β-amyloid peptide (Aβ) brain plaques and neurofibrillary tangles of hyperphosphorylated Tau protein.The 18 kDa translocator protein (TSPO), a transmembrane protein mainly located in the outer mitochondrial membrane, has been recently proposed as a promising therapeutic target for AD treatment[1]. Indeed, TSPO mediates the transport of cholesterol to the inner mitochondrial membrane to initiate the synthesis of neurosteroids, potent neuroprotective modulators. Little is known about TSPO activation's ability to rescue neurons from Aβ neurotoxicity. Here, we evaluated the potential ability of two highly steroidogenic TSPO ligands, PIGA1138 and XBD-173 [2], to rescue the main AD cytological hallmarks reproduced by challenging with fibrillated Aβ differentiated SHSY5Y cells, primary mouse cortical neurons and human neurons derived from the differentiation of induced pluripotent stem cells (iPSCs). Results showed an increase in TSPO expression promoted by Aβ administration. Furthermore, both TSPO ligands were able to decrease Aβ-induced neuronal death, neuritic damage and synaptic loss. Interestingly, the two ligands were able to regulate the compromised autophagic flux, though to a different extent. All together, these data unravel a role of TSPO as direct modulator of AD neurotoxicity, strengthening the rationale of its therapeutic targeting in AD.

References

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[2] https://doi.org/10.3390/ijms17071028

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MO03-9

Disruption of synaptic balance in primary neuronal models of Alzheimer's disease and tauopathy

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Despite being the most common form of dementia, the pathways underlying the pathogenesis of Alzheimer's disease (AD) remain poorly understood. Aggregation of tau protein in neurons is a pathological hallmark of AD, while synaptic loss and dysfunction are early phenotypes of the disease. We have developed rat primary neuronal culture models of tauopathy using lentiviral expression of human wildtype and mutant aggregation-prone P301L tau. Using these tools, we investigated early-stage synaptic changes in excitatory and inhibitory synapses in cells expressing wild type P301L-mutant tau. We used biochemical, cell biological and fixed and live cell imaging to interrogate tau protein's effects in these cells. Our data show enhanced association of P301L tau with the presynaptic protein bassoon as well as a significant loss of bassoon in cells overexpressing tau. Utilizing SynBot, an automated and machine learning-assisted analysis tool to assess synaptic puncta, we observed significant losses of excitatory and inhibitory synaptic puncta in cells overexpressing both wildtype or P301L tau. Calculation of Excitatory/Inhibitory (E/I) ratio of synaptic puncta on single neurons showed a significant increase in the E/I ratio in cells overexpressing the aggregate-prone P301L tau compared to wildtype tau. These data indicate aggregation-prone tau causes synaptic loss and perturbs the E/I ratios. Consistent with these results, our current live cell imaging with the calcium reporter GCaMP to visualize postsynaptic activity indicates disrupted synaptic activity in tau overexpressing neurons. Taken together these results begin to elucidate mechanistically how tau impacts synaptic number and function. We anticipate that further work will continue to identify and characterise novel pathological mechanisms and could provide targets for therapeutic intervention in in early-stage AD.

MO03-10

ANALYSIS OF DIFFERENTIAL EXPRESSION OF GENES IN CELLULAR MODELS FOR THE STUDY OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disorder, with both familial and sporadic incidence. Mutations in the genes encoding for the Amyloid Precursor Protein (APP), and Presenilin 1 (PSEN1), which is part of the γ-secretase complex that cleaves APP and releases the toxic Aβ peptide, cause early onset AD. The physiological role of these proteins is however not completely elucidated.

In order to determine the functions and processes of these proteins, we established cells lines, knockout (KO) for Amyloid Precursor Protein (APPKO) and Presenilin 1 (PSEN1KO), and alpha-synuclein (SNCAKO) as a specificity control for AD related changes. The KOs were obtained using the CRISPR Cas9 system with 2 single-guide RNAs, and validated by PCR and Western Blot.

Based on our hypothesis that mutations in the APP and PSEN1 genes lead to a disturbance in neurotrophic processes and in the response to neurotoxic insults, we examined the response of these cells to H_2O_2 (oxidative stress) and $CoCl_2$ (chemical hypoxia) using the MTT assay. Our results show that our cells were resistant to these treatments.

KEGG and GO analysis after RNA sequencing showed that the genes that have changed their expression in the APPKO and PSEN1KO cell lines but not in the SNCAKO cell line are involved mainly in brain development processes. We therefore hypothesise that early on in development the mutations may trigger the disease processes and we propose that some of the differentially expressed genes may be potential biomarkers.

MO03-11

KLK6 MODULATES AB PLAQUE ACCUMULATION IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative process that gradually causes cognitive decline. AD is characterized by excessive amyloid beta (Aβ) accumulation and deposition in the form of plaques throughout the brain which affect the neuronal function. Neuroinflammation appears to have a significant part in the pathophysiology of AD. Here, we aim to investigate the role of Kallikrein-related peptidase 6 (KLK6)-a secreted serine protease- in the progression of AD pathology, in mice. KLK6 has been shown to cleave APP and Aβ peptides, *in vitro.* Moreover, abnormal and excessive KLK6 expression have been reported in AD patients. To examine the role of KLK6, we crossed a well-characterized AD mouse model (5XFAD) with mice that lack KLK6 expression (KLK6-KO) and we compared the Aβ plaque load in the brain. To do so, brain hippocampal sections were analysed by immunohistochemistry at different time points following Aβ accumulation (2.5, 4 and 10 months). Our preliminary data indicate that both types of animals develop plaques which are detrimental for neuronal function. Plaques begin to grow at the initial stages as dense-core and evolve into a more diffused form at 10 months. KLK6-KO/5XFAD mice exhibit reduced plaque accumulation compared to 5XFAD mice. Moreover, the majority of Aβ plaques that developed in KLK6-KO/5XFAD mice was much more diffused in the stage of 10 months. In both animals, diffused plaques tend to have more microglia surrounding them compared to the dense-core plaques. Given that microglia, the brain's resident immune cells, are major players in Aβ plaque formation during the process of AD, we examined their phenotype in our model. By performing morphometric analysis in microglia surrounding the Aβ plaques, we concluded that microglia in KLK6-KO/5XFAD mice exhibit a higher level of activation compared to those in 5XFAD animals. Our findings suggest that KLK6 influences Aβ plaque accumulation in the brain, pointing to a potential novel target in AD progression.

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MO04-1

INVESTIGATING THE ROLE OF MENA IN THE FORMATION AND FUNCTION OF INHIBITORY SYNAPSES

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A hallmark of cortical circuit organization is the highly precise pattern of connectivity between neurons. This specificity is established by common cellular and molecular mechanisms like local mRNA translation and actin cytoskeleton remodeling, that dictate the precise placement of neurons and their synaptic connections. However, our understanding of the coordination between such mechanisms and the specific molecular components that underlie synapse formation and function, remains elusive. Previous work from our lab has established the dual role of the protein Mena in actin cytoskeleton remodeling and the regulation of local translation of specific mRNAs in developing neurons. We hypothesize that this dual function of Mena is conserved in adult neurons, and may be crucial for synapse formation and plasticity. To this end, we report Mena to be preferentially localized in inhibitory synapses (of cortical PV interneurons in particular), and also its tendency to co-immunopercipitate with several key organizers of inhibitory synapses and regulators of local translation. In good agreement with these findings and our initial hypothesis, we observe significant synaptic defects in mice with genetic ablation of Mena (Mena KO), including hindered formation of inhibitory synapses, hastened maturation of dendritic spines and formation of perineuronal nets around PV interneurons. Based on those observations, we speculate that Mena, along with its network of interneuron-specific interactors, may be implicated in the molecular and structural organization of inhibitory synapses. Therefore, our data uncover novel mechanistic insights into synapse biology, and contribute to our understanding of inhibitory synapse establishment and potential function.

MO04-2

GEMININ AND GEMC1 INFLUENCE THE DYNAMICS OF NEURAL STEM CELLS AND EPENDYMAL CELLS IN THE ADULT SUBVENTRICULAR ZONE

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The adult subventricular zone (SVZ) is a highly specialized neurogenic niche where neural stem cells (NSCs) and multiciliated ependymal cells (ECs) work in concert to sustain neural regeneration and niche integrity. NSCs generate neurons and glia, while ECs provide structural and trophic support. The fine-tuned balance between these cell populations is essential for proper SVZ function, yet the regulatory mechanisms guiding their fate decisions remain poorly defined.

In this study, we demonstrate that Geminin family members, Geminin and GemC1, play essential roles in regulating the equilibrium between neural stem cells (NSCs) and ependymal cells (ECs). Our results show that the loss of Geminin leads to an increase in EC production at the expense of NSCs, causing the remaining NSCs to shift towards self-renewal while decreasing differentiation. This transition is accompanied by a higher rate of cell cycle re-entry and increased S-phase activity in NSC progenitors. Moreover, Geminin-deficient SVZ colonies seem to shift toward neuronal lineage. Transcriptomic profiling further reveals that Geminin regulates critical pathways involved in NSC activation and neurogenesis. These findings underscore the importance of Geminin in preserving the balance between NSC maintenance and differentiation within the SVZ. Additionally, we find that the absence of GemC1 results in a shift toward an NSC-like phenotype, with a corresponding increase in NSC proliferation and neurogenesis in the postnatal SVZ. Chromatin analysis of GemC1 knockout cells reveals significant changes in chromatin organization, further supporting their NSC identity.

Together, these results reveal that Geminin and GemC1 are antagonizing regulators of NSC and EC fate, ensuring a tightly controlled equilibrium between these lineages. Disruption of this balance alters the SVZ cell composition, highlighting a fundamental mechanism that links NSC dynamics to niche composition and neurogenesis in the adult brain.

MO04-3

THE ROLE OF MENA IN THE FORMATION AND FUNCTION OF RNA GRANULES IN ADULT AXONS

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Two major types of RNA granules that are found in neuronal axons, Stress Granules (SGs) and Processing Bodies (PBs) are characterized as pools of untranslated mRNAs, which upon proper environmental stimulation disassemble to allow translation or degradation of their transcripts. This property makes them crucial for axon biology, as they can influence key intrinsic axonal properties, including local translation (LT). Granted that LT is one of the first processes activated after nerve injury, RNA granules could significantly affect the ability of axons to regenerate. However, their regulation in axons remains poorly understood.

We recently identified a dual role for Mena (mammalian homolog of Enah) in axonal LT and actin cytoskeleton dynamics. Mena forms a ribonucleoprotein complex (Mena-RNP) that regulates the translation of specific mRNAs in developing and adult axons, and is essential for LT and regeneration. Although the exact mechanism of Mena function remains unclear, our data show a correlation between Mena and RNA granules, suggesting a potential role in their dynamic assembly and disassembly.

To investigate these mechanisms, we will employ mice with genetic ablation of Mena, to explore its role in SG and PB distribution and molecular organization in sciatic nerve axons. We will use microscopy to visualize the levels and allocation of key proteins involved in RNA granule dynamics and LT, both in naïve and injured sciatic nerves (*ex vivo*), as well as in dorsal root ganglion (DRG) neuronal cultures (*in vitro*). Moreover, RNA granules will be isolated and high throughput assays will assess a) the molecular content of the granules (proteomics and transcriptomics), and b) the potential interactions between Mena and RNA granule proteins (co-IP and proteomics) in naïve and injured axons. These experiments will evaluate how Mena influences RNA granule assembly, disassembly, and transcript release for LT. Finally, sciatic nerve injury models in mice will validate our findings *in vivo*, revealing the functional relevance of Mena and RNA granules.

Overall, our work seeks to shed light on RNA granule biology and the molecular mechanisms behind the regulatory roles of Mena in axonal LT and regeneration after injury.

MO04-4

MODULATION OF GEMC1 EXPRESSION INDUCES WIDESPREAD CHROMATIN CHANGES

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In the developing telencephalon, neural stem cells *NSC) and ependymal cells (EC) originate from embryonic radial glial cells (RGCs) which serve as the primary progenitors during neurogenesis, giving rise to both neurons and glia. A subset of RGCs transforms into quiescent neural stem cells in the subventricular zone, where they remain dormant but can become reactivated to generate neurons and glia throughout life, in neurogenic niches. Another subset of RGCs commits to the ependymal lineage during late embryonic development. Ependymal cells are ciliated epithelial cells located on the ventricular surface of the brain, playing a vital role in cerebrospinal fluid circulation and brain homeostasis. This study aims to assess the effect of the presence of GemC1 in the chromatin organization of ependymal cells related promoters. We have demonstrated that two proteins from the Geminin superfamily, GemC1 and McIdas, are the upmost regulators of the molecular pathway that determines ependymal cell fate. Previous studies showed that in the absence of GemC1, RGCs are driven to NSC fate acquisition while GemC1 overexpression programmed neural stem cells towards the ependyma. We analyzed the chromatin landscape of GemC1KO/KO radial glial cells using ATAC-seq. Our findings revealed that ependymal-associated genes exhibited a closed chromatin state, while NSC-related genes had twice as many promoters with increased accessibility, suggesting that GemC1 modulation regulates radial glial cell fate and ablation of GemC1 enhanced the chromatin accessibility in NSC-linked promoters. Geminin, a direct heterodimer antagonist of GemC1 and McIdas, has been characterized as an important epigenetic regulator through its interaction with the Polycomb and the SWI/SNF chromatin remodeling complex. To investigate if the protein suppresses the ependymal fate differentiation by inducing histone modifications that activate NSC-related genes while repressing EC genes, we will perform ChIP assays in HEK293T cells transfected with GemC1, Geminin, or both, analyzing epigenetic marks (H3K27Ac, H3K27me3) at ependymal-associated gene promoters using real-time PCR. Our data support the notion that fate decisions between NSCs and ependymal cells are closely interconnected and offer new insights into the understanding of the fate decisions of post-natal neural progenitor cells in the mammal brain.

MO04-5

Immunohistological study of the distribution of nerve fibers in the ovine nipple

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The nipple is the anatomical structure initiating and allowing milk let-down. Studies from several species emphasize the distribution, origin and importance of nipple innervation and our work aims at doing so in the ovine nipple.

Healthy, both lactating and non-lactating sheep were euthanized with injectable anesthesia overdose and perfused intracardially with cold 4% paraformaldehyde in phosphate buffer solution 0,1M. Nipples were excised, post-fixed and embedded in paraffin blocks. Ribbons were cut at 5 microns, and consecutive sections were used for histological and immunohistochemical staining. The former included Hematoxylin and Eosin and Masson's trichrome staining to visualize normal histological structures, and for the latter, primary antibodies raised against calcitonin gene-related peptide (CGRP), substance p (SP), and transient receptor potential vanilloid-1 (TRPV1), and secondary fluorescent antibodies were utilized.

Our preliminary results found immunopositivity in the nipple of lactating and non-lactating sheep for CGRP, SP, and TRPV1. Fluorescence was more evident in the nipples of the lactating sheep, and both states had fluorescence in similar structures: vessels, beneath the surface of the epithelium and in stratum corneum, hair follicles, lactiferous ducts and some fluorescence was found in sebaceous and sweat gland epithelial cells. TRPV1 was more abundant than the other markers, while SP was sparse. TRPV1 was the only marker observed in keratinocytes of the epidermis. Notably, the alveoli of the mammary gland were positive for TRPV1 and CGRP but not SP. Smooth muscle did not appear to be positive for any marker tested.

We conclude that the nerve fibers are distributed among multiple structures of the nipple and the presence of neuropeptides such as CGRP and SP must play a role in the milk ejection reflex or local modulation of the tissue. Coupled with the presence of TRPV1, a receptor for painful stimuli, it is indicated that nociception plays a key role in ovine nipple function.

We hope that our work will aid in the improvement of sheep milking by utilizing the optimal functionality of these neuronal components.

MO04-6

THERAPEUTIC FXN EXPRESSION VIA HEMATOPOIETIC STEM CELL GENE THERAPY FOR FRIEDREICH'S ATAXIA

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Friedreich's Ataxia or FRDA is a rare neurodegenerative and multisystemic disease caused by FXN gene mutations. While its monogenic nature makes it ideal for gene therapy, efficient transgene delivery to multiple tissues is challenging. To address this, recent studies employed Hematopoietic Stem Cells (HSCs), harnessing their potential to differentiate into immune myeloid cells that fuse with diseased tissues to transfer mitochondria and/or functional FXN protein. Additionally, erythroid cells' abundance and systemic distribution were recently harnessed for high-level production of therapeutic proteins through gene-modified HSCs, in a non-hematopoietic disease context. Our goal is to employ autologous, genetically modified HSCs for a therapeutic outcome in FRDA. To fine-tune FXN expression we developed FXN-encoding lentiviral vectors with enhancers of differential potency and cell specificity, restricting expression in erythroid and myeloid hematopoietic lineages. These vectors were assessed ex vivo in hematopoietic progenitors from FRDA mice. More specifically, Lin- bone marrow cells were transduced with FXN-cDNA vectors and subsequently differentiated towards myeloid and erythroid lineages. All vectors increased FXN expression in a VCN-dependent manner, without altering cells' clonogenic capacity or ex vivo multilineage differentiation ability. Of all tested vectors, the combination of an erythroid and a panmyeloid enhancer produced an optimal expression profile with high erythroid and median myeloid expression patterns. Our results highlight the potential of genetically modified HSCs to provide a safe and effective therapeutic strategy for Friedreich's Ataxia, paving the way for future studies translating this approach into clinical practice.

MO04-7

NF1 EXON 51 SKIPPING IN PRIMARY NEURONS REVEALS NOVEL PROPERTIES OF NEUROFIBROMIN ISOFORMS IN CYTOSKELETON DYNAMICS

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Neurofibromin is a versatile protein functioning as a RasGAP and as a microtubule-associated protein (MAP), encoded by the *NF1* gene. Inherited or de novo mutations of *NF1*, cause Neurofibromatosis type 1 (NF-1), a disorder with variable clinical symptoms, primarily involving the CNS. The symptoms range from learning difficulties to glioblastomas, with no clear genotype-phenotype correlations. This complexity is further compounded by developmentally regulated alternative splicing of exons 31 and 51, which modulate neurofibromin's RasGAP potency and MAP activity through its Nuclear Localization Sequence (NLS), respectively, producing isoforms with distinct cellular roles. Our previous studies provided evidence that NLS and ΔNLS are different MAPs, differentially affecting cytoplasmic MTs, centrosomal assembly, and positioning for cell movement and migration, as well as astral microtubule MT assembly, and spindle positioning, leading to abnormal chromosome congression and segregation. In the present study, we used Splice-switching antisense oligonucleotides to induce exon51 skipping in chick-embryo primary neurons, generating ΔNLS neurofibromins. Confocal imaging analysis revealed that ΔNLS neurofibromins significantly alter F-actin distribution, leading to distinct growth cone morphology characterized by extensive protrusion of filopodia and retracting lamellipodia. Fluctuation of F-actin organization was further linked to dysregulation of cytoskeletal and synaptic genes, as identified through RNA sequencing analysis, affecting the phosphorylated state of the actin-binding protein cofilin. Additionally, the unique association of ΔNLS neurofibromins with myosin II kinase, MYLK, alters the levels of p-myosin in treated cells, highlighting the role of neurofibromin in regulating cytoskeletal dynamics through both Ras and Rho signalling pathways. This research bridges molecular mechanisms with cellular outcomes, providing novel insights into the role of neurofibromin isoforms in neuronal differentiation and advancing our understanding of neural development in NF-1.

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MO04-8

THE ROLE OF VITAMIN D3 IN EMBRYONIC HIPPOCAMPAL CELLS

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Vitamin D3 (VD3) is a fat-soluble prohormone synthesized in the skin via UVB exposure and absorbed from the diet. Besides its classical role in calcium homeostasis and bone metabolism, VD3 exerts neuroprotective effects in the central nervous system. The hippocampus, essential for learning and memory, expresses vitamin D receptors (VDRs) and is particularly sensitive to VD3.

This study investigated the effects of VD3 on neuronal growth and differentiation using the embryonic hippocampal cell line HN9.10e. Cells were divided into control and VD3-treated groups, exposed to varying concentrations, and monitored at 24, 48, and 72 hours. Morphometric analysis focused on soma size and dendritic length.

VD3 treatment significantly promoted neuronal growth, as shown by increased soma size and dendritic extension, with the most pronounced effects after 48 hours at higher concentrations, indicating a dose-dependent response. Interestingly, treated cells also displayed more elaborate neurite branching patterns, suggesting that VD3 not only promotes neuronal growth but also facilitates the establishment of a more complex neuronal network. This observation could refl ect enhanced cytoskeletal dynamics and synaptogenesis, processes often regulated by neurotrophic factors. Our results support the hypothesis that VD3 enhances neurogenesis, possibly through interactions with Nerve Growth Factor (NGF) and Brain-Derived Neurotrophic Factor (BDNF), which are essential for neuronal survival, growth, and synaptic plasticity.

In conclusion, VD3 supports neuronal development in embryonic hippocampal cells, highlighting its potential role in cognitive function and its relevance in preventing neurodegenerative diseases. Further research is needed to explore its molecular mechanisms and therapeutic potential.

MO04-9

SELENIUM INFLUENCE ON GENE EXPRESSION IN MOUSE BRAIN AND LIVER TISSUE

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Selenium is an essential microelement involved in antioxidant defence, redox regulation, and cellular signalling. Both its inorganic form, sodium selenite (Na_2SeO_3), and organic form, selenomethionine (SeMet), influence gene expression related to oxidative stress, apoptosis, and cell survival. The brain and liver, being highly vulnerable to oxidative stress, are critical tissues in understanding selenium's biological effects.

This study evaluates the impact of sodium selenite and selenomethionine on gene expression in these tissues, focusing on genes involved in redox regulation (MsrB1), apoptosis (Caspase-3), and cell cycle control (GADD45).

The study was performed on 4-6-week-old BALB/c mice, which were divided into 4 experimental groups, that were given solutions of different concentrations of SeMet or Na_2SeO_3 (0.2 and 0.4 mg Se/kg body weight) for 8 weeks, and a control group that had free access to tap water. Then, mRNA was extracted from mouse liver and brain tissues and used to synthesize cDNA for RT-PCR analysis.

The results show that MsrB1 expression in both brain and liver was higher in mice receiving 0.2 mg Se/kg of either SeMet or Na_2SeO_3 compared to the control group and those receiving 0.4 mg Se/kg. Caspase-3 expression in brain and liver was increased in mice receiving 0.2 mg Se/kg of Na_2SeO_3, but lower in those receiving 0.2 mg Se/kg of SeMet compared to the control group and those given 0.4 mg Se/kg of either selenium form. GADD45 expression in the brain and liver was elevated in mice given 0.2 mg Se/kg of Na_2SeO_3, while in SeMet-treated mice, GADD45 expression in the liver increased with higher concentrations, whereas in the brain, it decreased with increasing SeMet dosage. Overall, Na_2SeO_3 may promote stronger oxidative stress defence and apoptosis regulation, while SeMet could be more selective in its influence on apoptosis and cell cycle control, depending on tissue type.

MO05-1

The Role of Palmitoylation in Regulating Kainate Receptor Trafficking and Function

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Abstract

Kainate receptors (KARs) are key regulators of neuronal excitability and synaptic transmission, and their dysregulation causes several disorders, such as epilepsy and autism. The surface expression of tetrameric KARs is crucial for regulating synaptic excitation and is tightly controlled, in part, by post-translational modifications (PTMs) of one of the most highly expressed subunits, GluK2. We previously demonstrated that palmitoylation basally regulates the surface expression of KARs. Upon agonist activation of GluK2-containing KARs, depalmitoylation occurs, leading to the phosphorylation of GluK2 at S868. This phosphorylation subsequently promotes SUMOylation at K886, triggering receptor endocytosis. However, there is limited knowledge regarding how different palmitoylation sites on GluK2 regulate KAR trafficking. Additionally, whether there is a more intricate interplay between individual palmitoylated cysteines, phosphorylation, and SUMOylation in orchestrating KAR trafficking remains unclear.

Here, we used a library of site-specific GluK2 mutants to investigate the interrelationship between GluK2 PTMs and their impact on KAR surface expression. We show that GluK2 is basally palmitoylated and that this modification decreases upon kainate (KA) stimulation. Moreover, a non-palmitoylatable C858A GluK2 mutant exhibits enhanced S868 phosphorylation and SUMOylation, whereas the C871A mutant shows no phosphorylation. Our findings indicate that GluK2 palmitoylation bidirectionally contributes to stabilizing KAR surface expression. Furthermore, dynamic depalmitoylation of C858 promotes downstream phosphorylation and SUMOylation, mediating activity-dependent KAR endocytosis.

MO05-2

Unveiling the biochemical secrets of ST3GAL3: a multi-approach characterization of the glycosphingolipid pathway

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ST3GAL3 is a sialyltransferase that transfer sialic acid to the C-3 position of galactose of glycoproteins and gangliosides. Patients carrying inactive ST3GAL3 variants present neurological symptoms and express normal levels of circulating CA19.9. Enzymatic studies showed that ST3GAL3 uses both Galβ1,3GlcNAc (lacto-series) and the Galβ1,3GalNAc (ganglio-series) glycosphingolipids, suggesting that it generates of a definite pool of minor but essential brain gangliosides.

Cloned ST3GAL3 cDNA variants were transfected into HEK-293T cells to measure sialyltransferase activity through LC-MS/MS. ST3GAL3-KO and β4GALNT1-KO HEK-293T and HCT-15 cells were also obtained through CRISPR-Cas9 genome editing and characterized by end-point RT-PCR. Plasma samples from patients were analyzed by ultra-sensitive HR-LC-MS/MS .

Enzyme activity assays indicated that almost all pathogenic ST3GAL3 variants completely lack activity. cDNAs encompassing the whole coding sequence of ST3GAL3 and B4GALNT1, respectively, were detected upon reverse transcription of RNA extracted from parental HEK-293T and HCT-15 cells but not by the respective CRISPR-Cas9 clones, indicating actual gene KO.

Semi-quantitative LC-MS/MS analysis detected lower levels of sLc4 (Siaα2,3Galβ1,3GlcNAcβ1,4Galβ1,4Glc-Cer)/GM1b (Siaα2,3Galβ1,3GalNAcβ1,4Galβ1,4Glc-Cer), in plasma patients when compared to controls.

We are going to determine by LC-MS/MS the glycosphingolipid profile of the obtained KO cells in steady-state and upon metabolic flux performed after seeding cells with deuterated precursors, and to study variants directly in fibroblasts and plasma samples from two other patients.

Together with recent literature data showing no relevant alteration of sialylated glycoproteins in neurons differentiated from patient induced-pluripotent stem cells, our

data corroborate the hypothesis that ST3GAL3 plays a crucial role in ganglioside biosynthesis.

MO05-3

A NOVEL CONGENITAL DISORDER OF GLYCOSYLATION IS LINKED TO B4GALT5 INACTIVATION AND GLYCOSPHINGOLIPID DEFICIENCY

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A 7-year-old Italian girl with developmental delay, intellectual disability, microcephaly, and bilateral cataracts carried compound heterozygous B4GALT5 variants (Decipher ID 412221). B4GALT5 encodes a galactosyltransferase producing lactosylceramide (LacCer), precursor of glycosphingolipids. We aimed to determine if these variants cause a novel CDG, even if B4GALT6, structurally similar to B4GALT5, has been suggested to compensate for LacCer synthesis in mouse models. However, we hypothesized that B4GALT6 fails to fully rescue B4GALT5 function in humans. To test this, human B4GALT5 cDNA was cloned, and patient variants introduced via site-directed mutagenesis. B4GALT6 (human and mouse) was also cloned. Constructs were transfected into HEK-293T cells, including B4GALT5-KO and B4GALT5/T6 double KO generated via CRISPR-Cas9. We assessed enzyme activity in vitro, protein expression via western blot, and glycosphingolipid profiles in cells and patient serum via LC/MS. In-silico models were also generated. Mutant B4GALT5 constructs showed no enzyme activity. Mouse B4GALT6 was kinetically distinct, being unsaturable by GlcCer, while human B4GALT6 exhibited low expression, which improved by modifying its N-terminal. LC/MS revealed a significant LacCer loss and ceramide monohexoside accumulation in patient serum. B4GALT5-KO cells retained ~30% glycosphingolipids, that were lost in B4GALT5/T6 double KO HEK-293T cells. Our data indicate that B4GALT5 variants are virtually inactive and human B4GALT6 is kinetically much less efficient than B4GALT5 and bears N-terminal sequence apparently critical for protein translation or stability. Although further cases need to be recognized to confirm the identification of this new disorder, our data corroborate the hypothesis that B4GALT5 activity is necessary in humans and not in mice due to inefficient rescuing by B4GALT6.

MO05-4

The COBRA-Assay: A NanoBRET based biosensor for tracking CERT activation and oligomerisation

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In Alzheimer's disease (AD), downregulation of ceramide transfer protein (CERT) leads to ceramide accumulation, driving neurodegeneration. Restoring CERT activity could counteract this accumulation, making it a promising therapeutic target. However, while CERT is known to undergo both activation and oligomerisation, the relationship between these processes remains poorly understood. Existing methods rely on indirect, static measurements, limiting our ability to study how CERT functions in real time.

To address this, we aim to develop the COBRA-assay (CERT Oligomerisation and BRET-based Activity assay), a dual NanoBRET-based biosensor for real-time monitoring of CERT activity and oligomerisation in living cells. By fusing NanoLuciferase (NLuc) to CERT's PH domain and a fluorescent acceptor to the START domain, the assay detects conformational changes upon activation. A second BRET assay, using distinct fluorescent acceptors, measures oligomerisation via PH domain clustering. By integrating these readouts, COBRA enables a direct, quantitative assessment of CERT dynamics.

Understanding how CERT activation and oligomerisation relate could provide new insights into ceramide metabolism and its dysregulation in AD. COBRA-assay could offers a valuable tool to study CERT regulation and may aid in identifying new therapeutic strategies targeting lipid homeostasis in neurodegenerative disease.

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TU01-1

INVESTIGATING p.A53T-ALPHA-SYNUCLEIN-INDUCED ASTROCYTIC SENESCENCE IN A PARKINSON'S DISEASE iPSC MODEL

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Cellular senescence is characterized by irreversible cell cycle arrest, resistance to cell death, and a pro-inflammatory phenotype known as the senescence-associated secretory phenotype (SASP). While a normal feature of aging, senescence can be triggered by environmental and cellular stressors. Increasing evidence suggests that senescent astrocytes contribute to neurodegenerative disorders, including Parkinson's Disease (PD). PD, is characterized by α-synuclein (αSyn) aggregation and progressive loss of dopaminergic neurons in the substantia nigra, leading to motor and non-motor symptoms. Previous studies provide evidence of senescence in both post-mortem PD brains and experimental models. A major genetic risk factor for early-onset and severe PD is the p.A53T mutation in αSyn (*SNCA*^{G209A}). Our recent work with p.A53T-αSyn patient-derived induced pluripotent stem cells (iPSC) demonstrated that PD astrocytes exhibit pathological protein aggregation, disturbed autophagy, and deleterious effects on dopaminergic neuron health. Here, we investigated whether the p.A53T mutation drives astrocytic senescence, thereby contributing to PD pathology. To this end, we compared senescence traits in PD and healthy iPSC-derived astrocytes. Our results show that PD astrocytes exhibit elevated p21 transcription, enlarged cell and nuclear sizes, increased lysosome content, and enhanced autofluorescence, a hallmark of senescence. These findings indicate that the p.A53T-αSyn mutation drives astrocytic senescence, potentially contributing to PD neuropathology. Ongoing experiments aim to validate the senescent phenotype of mutant astrocytes and investigate SASP factors as paracrine mediators of neuronal dysfunction. Ultimately, our goal is to explore senomorphic molecules for their ability to reverse or delay PD progression by targeting astrocytic senescence.

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TU01-2

THE ROLE OF SMALL EXTRACELLULAR VESICLES IN A-SYNUCLEIN TRANSMISSION

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Small extracellular vesicles (sEVs) have recently emerged as key factors in cellular communication in both physiological and pathological brain processes, particularly in synucleinopathies. Our recent work indicates that brain-derived sEVs are internalized by glial cells (microglia and astrocytes) through macropinocytosis and are sorted to endolysosomes for subsequent processing. The current study aims to examine the intracellular trafficking pathway of sEVs in glial cells linked with the sEV-associated α-synuclein (α-Syn) transmission. Glial primary cultures were incubated with DiI-stained mouse brain-derived sEVs, in the absence or presence of recombinant fibrillar human α-Syn (pre-formed fibrils, PFFs). The internalization, degradation and trafficking pathways in cells treated with pharmacological reagents that block the major endocytic pathways along with exosomal biogenesis, were analyzed by immunofluorescence, using the Imaris analysis software. PFFs were internalized by both microglia and astrocytes at early time points of PFF incubation. In microglia, PFF uptake occurred faster than astrocytes. In the presence of sEVs, a delay in the PFF uptake was observed in both glial cell types. Treatment with dynasore, that inhibits dynamin-dependent endocytosis, affected PFF uptake only in the absence of sEVs. Inhibition of exosomal biogenesis seems to influence PFFs internalization. sEV-associated PFFs seemed to utilize macropinocytosis and/or phagocytosis as the main pathway of endocytosis. Degradation of PFFs in the presence of sEVs in astrocytes is sufficient even after 3h. Fibrillar α-Syn when associated with sEVs enters the endolysosomal pathway and is targeted to the lysosome for subsequent degradation. Our data indicate that brain-derived sEVs serve as scavengers and mediate a rather slow cell-to-glia transfer of pathological α-Syn which is targeted to the endolysosomal pathway, suggesting a beneficial role in glia-mediated clearance of toxic protein aggregates, present in many neurodegenerative disorders.

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TU01-3

Linking cellular senescence and alpha-synuclein driven pathologies in Parkinson's disease

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Senescence, an important age-related process, is extensively studied in the periphery; however, it remains less explored in the central nervous system. Increasing evidence indicates a link between senescence and alpha-synuclein (a-Syn) induced toxicity in Parkinson's disease (PD), yet the precise interplay between them remains unclear. In this study, we aim to study in depth the interconnection of these two processes utilizing novel neuronal cell lines with dosage dependent overexpression of wild type (WT) or A53T mutated a-Syn to clarify a causative link between senescence and a-Syn induced toxicity.

We created novel neuronal cell models in SH SY5Y neuroblastoma and ReNCell VM neural progenitor cell lines, stably overexpressing GFP-tagged a-Syn either WT or its A53T mutant version via lentiviral transduction. Cells were then sorted into two subpopulations each based on GFP expression to obtain dosage dependent expression (low and high) of a-Syn. These cell models were then characterized for a-Syn overexpression at mRNA and protein levels along with investigating the cytotoxic effects of altered a-Syn levels. Next, we analyzed the senescence-associated changes by performing senescence-associated β-galactosidase staining, quantifying the levels of prominent senescence markers p16INK4a and/or p21CIP1 at mRNA and protein levels, also through immunofluorescence correlating the induction of senescence and overload of WT/A53T a-Syn in both SH and ReNCell VM cells.

We successfully generated and characterized two novel neuronal cell lines overexpressing WT/A53T a-Syn and employed them to comparatively investigate senescence-associated changes in relation with alterations in a-Syn levels.

Results from our study shed light on the previously unexplored connection between senescence and a-Syn induced toxicity in PD. Importantly, our approach further enables us to monitor these changes upon differentiating these models to dopaminergic neurons.

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TU01-4

FMRP REGULATES ALPHA-SYNUCLEIN ACCUMULATION THROUGH DUAL CONTROL OF PROTEIN DEGRADATION AND TRANSLATION

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The accumulation of alpha-synuclein (SNCA) is a key contributor to the pathogenesis of Parkinson's disease (PD) and other synucleinopathies. Understanding and targeting the molecular pathways regulating SNCA expression and degradation may offer new therapeutic strategies to mitigate its pathological aggregation and cellular toxicity.

This study investigated how Fragile X Messenger Ribonuclear Protein (FMRP), an RNA-binding protein involved in mRNA stability, translation, and synaptic plasticity, regulates SNCA expression and protein degradation pathways in neuroblastoma SK-N-SH cells.

Overexpression of FMRP reduced global translation rates and enhanced ubiquitin-dependent degradation through both the N-end rule pathway and the ubiquitin-fusion degradation system. In contrast, FMRP suppressed macroautophagy and decreased chaperone-mediated autophagy receptor LAMP2A levels.

Mechanistic analysis revealed that FMRP preferentially binds to the proximal SNCA 3'UTR, decreasing total *SNCA* pre/mRNA levels while promoting cap-independent *SNCA* translation. The deregulation of these degradation pathways, combined with FMRP-driven translational control, leads to the accumulation of monomeric and aggregated SNCA forms, the latter being associated with cellular toxicity and Lewy body pathology.

These findings identify FMRP as a dual regulator of SNCA protein expression and degradation, with potential implications for synucleinopathy pathogenesis and therapeutic targeting.

TU01-5

EXTRACELLULAR VESICLE DERIVED MIR-101-3P AND MIR-30B-5P AS INDICATORS OF PARKINSON'S DISEASE PROGRESSION

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Parkinson's disease (PD) is a chronic neurodegenerative disorder primarily affecting movement due to the progressive degeneration of dopaminergic neurons. It is characterized by motor symptoms such as tremors, bradykinesia and rigidity, which worsen over time. Extracellular vesicles (EVs) facilitate intercellular communication by transporting biomolecules, including microRNAs (miRNAs), which may reflect disease state and progression. Changes in EV miRNA expression could serve as valuable biomarkers for assessing PD severity and guiding treatment approaches. This study investigated the expression levels of specific EV miRNAs in the blood serum of PD patients at different stages of disease severity.

EV miRNAs were extracted from blood serum, converted into cDNA, and analyzed using RT-PCR. The study included 88 PD patients with varying symptom severity. miRNA expression was assessed in relation to motor impairments such as bradykinesia, tremor, rigidity, dyskinesia, gait disturbances, and the "on-off" phenomenon, as well as disease onset and duration. Statistical analysis was conducted using GraphPad Prism 8.

Among the analyzed EV miRNAs, miR-101-3p and miR-30b-5p showed a significant decline in expression as motor symptoms worsened. Patients experiencing more severe bradykinesia, tremor, balance, "on-off" phenomenon and gait difficulties exhibited the most pronounced downregulation of these miRNAs. Statistical analysis revealed a strong negative correlation between miRNA expression and general motor symptom severity, with miR-101-3p showing $r = -0.362$ ($p < 0.01$) and miR-30b-5p exhibiting $r = -0.411$ ($p < 0.001$). However, no significant association was observed between miRNA expression and disease onset or duration.

This study suggests that EV miRNAs could serve as biomarkers for Parkinson's disease severity. The downregulation of miR-101-3p and miR-30b-5p correlates with worsening symptoms, highlighting their potential in disease monitoring and targeted treatment strategies.

TU01-6

Subcellular and biochemical characterization of the novel A30G α-Synuclein mutant in Parkinson's Disease

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A30G is a novel heterozygous mutation of the SNCA gene, recently identified in five affected individuals of three non-related Greek families. This mutation is responsible for a Parkinson's Disease phenotype, including prominent non-motor symptoms. Biochemical analysis in isolated systems revealed that the A30G SNCA mutation altered the alpha-helical structure of the protein, perturbed membrane binding and promoted fibril formation. However, the effects of this mutation in a cellular context have not been assessed. Our main aim is to unravel the biochemical properties of the A30G mutant in a cellular context, deciphering the mechanisms governing toxicity, seeding and aggregation, leading to a better understanding of PD pathogenesis. Using WT α-synuclein recombinant fibrils (PFFs) in neuronally differentiated SH-SY5Y neuroblastoma cells with stable overexpression of mutant A30G α-synuclein, we examined the seeding and aggregation of endogenous α-synuclein by immunofluorescence and immunoblotting assays. The overexpression of the A30G mutant protein resulted in the accumulation of insoluble α-synuclein species, partially phosphorylated at pS129, as well as in axonal retraction by 6 days and later neuronal death. Immunofluorescence and immunoblotting experiments showed that upon PFF addition, seeding and aggregation of endogenous A30G α-synuclein occurred within 4 days of PFF incubation. The overexpression of A30G α-synuclein results in the accumulation of insoluble α-synuclein species and consequently in cell death in a neuronal cell system, while PFF-induced seeding and aggregation of endogenous A30G α-synuclein also occur at an accelerate pace compared to that previously reported for the WT protein. These results highlight the increased aggregation and toxicity propensity of the mutant A30G α-synuclein in a cellular context.

TU01-7

Pharmacological inhibition of Mirk/Dyrk1B kinase decreases Tau phosphorylation at T212 residue and confers neuroprotection in THY-Tau22 cortical neurons.

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Abstract

Abnormal hyperphosphorylation of the microtubule-associated protein Tau is a hallmark of Alzheimer's disease and related tauopathies. Accumulation of hyperphosphorylated and aggregated Tau results to the formation of intracellular neurofibrillary tangles (NFTs), which are accumulated in the brain, and mainly are localized into the neurogenic niches, resulting to impaired adult neurogenesis. Tau phosphorylation is regulated by a balance between Tau kinases and phosphatases. Disruption of this equilibrium is at the origin of abnormal Tau hyperphosphorylation and contributes to Tau aggregation. Previously, we have demonstrated that Mirk/Dyrk1B kinase is highly expressed at the neurogenic niches of the dentate gyrus (DG) and along the SVZ-RMS-OB pathway and marks all stages of adult hippocampal neurogenesis. In this study, we aim to investigate the role of Mirk/Dyrk1B in Tau pathology, using THY-Tau22 mice. First, we examined the expression of Mirk/Dyrk1B and of its regulatory neurogenic microRNA, miR-9 in THY-Tau22 brain. We found that both Mirk/Dyrk1B and miR-9 mRNA expression is significantly reduced in the brain of THY-Tau22 mice during aging and progression of Tau pathology. Especially, Mirk/Dyrk1B and miR-9 mRNA expression is reduced by 50%, 60%, 80% and 80% and 35%, 60%, 60% and 80% respectively at the brain of 3, 6, 9 and 12 months old THY-Tau22 mice, when compared to the control mice. Moreover, Tau protein expression is increased during neurodegeneration progression in the brain of THY-Tau22 mice. Next, we examined the effect of Mirk/Dyrk1B kinase in Tau phosphorylation. The pharmacological inhibition of Mirk/Dyrk1B kinase activity, by using AZ191 inhibitor, in E14.5 THY-Tau22 cortical neurons results in the decreasing by 2-fold of Tau phosphorylation at T212 residue when compared to non-treated neurons. In addition, treatment of E14.5 THY-Tau22 cortical neurons with AZ191 exhibit a neurotrophic effect, by decreasing by 1.58-fold the number of Bax^+ pro-apoptotic THY-Tau22 cortical neurons.

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TU01-8

Validation of KLK6 as a new therapeutic target for Parkinson's Disease

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Alpha-Synuclein (α-Syn) is a presynaptic neuronal protein genetically and biochemically implicated in a group of neurodegenerative diseases, termed synucleinopathies. Aberrant forms of α-Syn have been linked to the development of Parkinson's disease (PD) and have been shown to facilitate the transmission of PD pathology to neighboring healthy cells. Consequently, effective clearance of extracellular α-Syn is crucial for slowing down or halting PD. Kallikrein-6 (KLK-6) is a serine protease, abundantly expressed throughout the central nervous system and secreted into the extracellular space. KLK-6 has been implicated in the proteolytic clearance of extracellular α-Syn, although its role in the formation of α-Syn fragments and their effect on toxicity remain unclear. In this project, the role of KLK-6 as a potential therapeutic target for PD was investigated, using the α-Syn preformed fibril (PFF) mouse model. In this model, intrastriatal inoculation with human PFFs results in α-Syn pathology along the nigro-srtiatal axis, neuro-inflammation and subsequent neuronal demise. The impact of KLK-6 was validated *in vivo*, following AAV-mediated expression of the protease in the ventral midbrain of PFF-inoculated mice. Our data show that KLK-6 expression ameliorates PFF-induced phospho-α-Syn pathology. In addition, different PFF strains, produced by α-Syn fragments following KLK6 proteolysis, exhibited reduced pathogenic potential, when injected unilaterally in the right dorsal striatum of mice. Both results underlie a promising therapeutic potential of the serine protease.

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TU01-9

MONITORING THE ROLE OF EXTRACELLULAR VESICLES IN THE PRECIPITATING IMPACT OF CHRONIC STRESS IN TAU BRAIN PATHOLOGY

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Neurodegenerative progression of Alzheimer's disease (AD) is tightly related to Tau brain pathology. In light of clinical studies suggesting chronic, psychological stress as precipitating factor of AD, our previous experimental studies showed that chronic stress triggers the accumulation of pathological forms of Tau, facilitating its spreading; yet the precise mechanisms underlying this process remain unclear. As extracellular vesicles (EVs), particularly exosomes, play a crucial role in the spreading of Tau pathology, we next monitor the impact of stress in brain EVs secretion and cargo. We found that chronic stress exacerbates Tau brain pathology in Tau Tg mice, leading to an increase of brain-derived EVs carrying different forms of pathological Tau. Additionally, the proteomic composition of brain EVs under stress conditions mirrors stress-induced changes in the brain, supporting their potential as biomarkers. On-going analysis of brain-derived EVs in plasma of AD clinical cohort will provide novel knowledge about the biomarker potential of brain EVs and their interplay with stress.

TU01-10

OPTIMIZATION OF ALPHA-SYNUCLEIN SEEDING AMPLIFICATION ASSAY EXPLOITING RECOMBINANT MONOMERIC PROTEIN FROM DIFFERENT SOURCES

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Synucleinopathies, including Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy bodies (DLB), are characterized by the aggregation of misfolded alpha-synuclein (aSyn), which seeds monomeric aSyn, driving disease progression. Although clinical and imaging biomarkers assist in distinguishing these disorders from other neurological conditions, a reliable wet biomarker is still lacking.

The alpha-Synuclein Seeding Amplification Assay (aSyn-SAA) offers high sensitivity and specificity in detecting aSyn pathology in body fluids and tissues, particularly cerebrospinal fluid (CSF). The assay exploits the ability of aSyn aggregates to seed recombinant monomeric aSyn, with Thioflavin T fluorescence measured in a shaking fluorescent plate reader. However, full validation is needed before clinical implementation.

We aim to establish the assay in-house, a challenge encountered in many research and clinical settings. Various experimental conditions were tested, modifying key parameters—particularly the source of recombinant monomeric aSyn as a substrate— in the presence/absence of preformed fibrils (PFFs) as seeds. In some cases, we achieved low background seeding in the absence of PFFs and robust, dose-dependent seeding with PFFs. However, further standardization is required, as the quality and reliability of recombinant aSyn sources remain critical. Despite these challenges, the assay shows great promise for research and clinical applications, including early premanifest disease stages.

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TU01-11

MICROGLIAL TREM2 SIGNALLING IN HIPSC-DERIVED MODELS OF ASYNUCLEIN-MEDIATED NEURODEGENERATION AND NEUROINFLAMMATION

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Parkinson's disease (PD) is considered to be caused by a combination of genetic and environmental factors that may synergistically propagate neuronal death in the diseased central nervous system (CNS). Besides the well-established αSynuclein (αSyn)-induced neurodegeneration that characterizes PD, the disease pathophysiology has been linked to aberrant microglia-regulated neuroinflammation, and there is evidence to suggest that inflammatory triggers including viral infections increase the risk of developing PD. Microglia, the immune cells of the brain, are crucial for antiviral defence and the microglial triggering receptor expressed on myeloid cells 2 (TREM2) has been shown to regulate virus replication and pathogenesis, accelerating neurodegenerative processes or mediating neuroprotection. However, whether genetic or virus-induced microglia activation and subsequent TREM2 activity alterations predispose to post-infection PD or other neurological disorders is underexplored. To address this, we generated human iPSC-derived microglia and neurons that harbour G209A, a point αSyn gene mutation that results in the expression and accumulation of pathological A53T (p.A53T), TREM2$^{-/-}$ and healthy microglia. We then assessed how activation with Poly I:C, a viral mimic, affects TREM2 activity and TREM2-dependent microglia functions in healthy and PD patient-derived microglia. We found that pathological aSYN expression affects microglia responsiveness to Poly I:C, TREM2 signalling and downstream functions including phagocytosis. Our data provide insights into how TREM2 regulates microglia functions and neuronal health upon viral infection in healthy and diseased CNS, extending findings to mechanisms that link infectivity with late-appearing neurodegenerative diseases.

Funding
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TU01-12

THE EFFECTS OF CHRONIC STRESS ON THE HIPPOCAMPAL TRANSCRIPTOME IN A IN VIVO ALPHA-SYNUCLEIN PARKINSON'S DISEASE ANIMAL MODEL

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An emerging connection between chronic stress and the pathogenesis of Parkinson's disease (PD) is gaining increased attention. Despite the plethora of evidence linking the presynaptic neuronal protein alpha-synuclein (asyn) to PD pathogenesis, it remains unclear whether stress system dysfunction is present in PD, if asyn is involved, and if both together promote neurodegeneration. To explore these questions, we evaluated stress axis function in transgenic rats overexpressing full-length human asyn (asyn BAC rats) and performed multi-level stress and PD phenotyping following a protocol of chronic unpredictable stress (CRUST) exposure in a 2x2 experimental design. While the asyn BAC rats are characterized by HPA axis dysregulation, chronic stress further intensifies nigrostriatal degeneration, disrupts dopamine metabolism and neurotransmission and asyn truncation. Moreover in BAC rats, CRUST and human asyn disrupt genes and cellular pathways linked to neuronal development, synaptic function, and neurotransmitter signaling in the hippocampus, indicating impaired brain connectivity and synaptic function—archetypal features of neurodegenerative diseases. Chronic stress further amplifies these disruptions, leading to compensatory upregulation of mitochondrial and detoxification pathways, highlighting increased cellular stress suggesting that the combination of stress and asyn pathology accelerates synaptic dysfunction and the progression of PD. Overall, our findings from hippocampal RNA sequencing, DEG validation, and pathway enrichment analysis suggest that SNCA overexpression, in combination with CRUST exposure, induces transcriptomic alterations strongly linked to PD pathology. Taken together, our findings provide evidence that elevated glucocorticoids can contribute to asyn-induced neurodegeneration, ultimately triggering, ultimately leading to phenoconversion from a prodromal to an overt motor PD phenotype

TU01-13

Different mutations in the MAPT gene induce distinct neuropathological effects in a cellular model of tauopathy.

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Tauopathies are neurodegenerative diseases characterized by the accumulation of misfolded tau protein in brain aggregates, and present with significant clinical heterogeneity. Notably, more than 60 mutations in the tau-encoding MAPT gene, which induce distinct biological effects on tau function and/or 4R/3R tau isoforms ratio, have been linked to various manifestations of familial frontotemporal dementia (FTD). Understanding whether different tau mutations share common or specific neurotoxic mechanisms is crucial for developing targeted therapies. Our previous data showed that an increased 4R/3R tau ratio leads to neurodegeneration by disrupting mitochondrial function, affecting bioenergetics and calcium homeostasis. Here, we investigated whether other tau mutations causing other biological effects similarly impair cellular homeostasis. To this end, we used a model of undifferentiated and retinoic acid-differentiated SH-SY5Y cells overexpressing different tau isoforms and mutations. Our results indicate that some mutations/isoforms induce specific molecular signatures related to cell death, mitochondrial bioenergetics and oxidative stress. These findings suggest that disease-modifying therapies for tauopathies should be tailored to specific tau alterations to achieve optimal efficacy.

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TU01-14

NON-INVASIVE DOWN-REGULATION OF ENDOGENOUS MURINE ALPHA-SYNUCLEIN VIA AAV.PHP.EB-GFP CAPSIDS AS A NOVEL THERAPEUTIC APPROACH FOR ALPHA-SYNUCLEINOPATHIES

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Alpha-Synuclein (aSyn) aggregation in specific brain regions is a hallmark of Parkinson's disease (PD) and related synucleinopathies. The propagation of aSyn has been suggested to be the underlying mechanism by which aggregates spread throughout the brain. The human aSyn preformed fibril (PFF) striatal injection model recapitulates key disease characteristics, including aSyn aggregation, propagation, dopaminergic cell loss, and behavioral deficits. We investigated whether non-invasive endogenous aSyn down-regulation via AAV.PHP.eB-GFP capsids mitigates aSyn pathology.
PHP.eB-GFP AAVs carrying shRNA or microRNA targeting murine Snca (encoding aSyn) or scrambled controls were intravenously administered to 8-week-old male C57/Bl6 mice. One week later, human aSyn PFFs were unilaterally injected into the right dorsal striatum. Three months post-injection, behavioral and histochemical analyses were performed to assess aSyn down-regulation effects.
Intravenous administration of PHP.eB-GFP AAVs resulted in widespread GFP transduction, including the substantia nigra. Both Snca-targeted approaches (shRNA and microRNA) significantly reduced aSyn levels in transduced dopaminergic neurons, decreased Ser129-phosphorylated aSyn, and mitigated dopaminergic cell and terminal loss in the nigrostriatal axis. These improvements were reflected in motor phenotype.
PHP.eB-GFP-shRNA- or microRNA-targeted Snca capsids efficiently downregulated endogenous aSyn and hampered aSyn pathology propagation, supporting their potential as non-invasive therapeutic interventions for PD and related synucleinopathies.

TU02-1

LONG-TERM EFFECTS OF ADOLESCENT 5F-MDMB-PICA INTRAVENOUS SELF-ADMINISTRATION (IVSA): NEUROBEHAVIORAL CONSEQUENCES AND MEDIAL PREFRONTAL CORTEX DYSFUNCTION IN ADULT MICE

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Synthetic cannabinoids (SC) are the largest group of new psychoactive substances monitored worldwide (EMCDDA, 2022). 5F-MDMB-PICA is a recent SC classified as a potent full agonist at CB1/CB2 receptors able to activate the mesolimbic dopamine (DA) transmission in adolescent but not adult mice (Musa et al, 2020). Here, we have studied its reinforcing effects in adolescent mice and characterized the neurochemical and behavioral effects induced in the same animals at adulthood. First, by self-administering varying doses (1-5 μg/kg/25μl) in an intravenous self-administration (IVSA) paradigm, we observed an inverted U-shaped trend. The dose of 2.5 μg/kg/25ul elicited behavior aimed at obtaining the substance, as measured by different response ratios (FR1-3, PR). Adult mice exposed to 5F-MDMB-PICA during adolescence showed behavioral and neurochemical differences compared to controls, including a propensity for aggression and reduced social interaction, an anhedonic state, and delayed mPFC DA response to olfactory stress as estimated by in vivo brain microdialysis. In addition, the analysis of the excitatory neuronal activity in the mPFC, by using the fiber photometry technique and calcium sensors, revealed diminished responses to aversive stimuli in 5F-MDMB-PICA-exposed mice, compared to controls. These results correlate with the neurochemical analyses indicating disrupted DA signaling. This study provides the first evidence that 5F-MDMB-PICA IVSA is acquired and sustained by adolescent mice at lower doses than that of the prototypical SC JWH-018 (Margiani et al., 2023), confirming a higher abuse liability of this newer SC. Moreover, 5F-MDMB-PICA IVSA during adolescence induced long-term behavioral and neurochemical changes confirming the detrimental consequences related to the use of SC during adolescence.

TU02-2

CANNABINOID ADMINISTRATION IMPROVED MOTOR FUNCTION IN MALES AND MEMORY IN FEMALES IN A PRECLINICAL MODEL OF NEONATAL BRAIN INJURY

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Therapeutic hypothermia (TH) is the standard treatment for babies who suffer from perinatal asphyxia. However, TH is not effective in all infants, some of them surviving with neurological sequelae. Further, sex may affect both brain vulnerability and the therapeutic effect of TH. Cannabinoids have emerged as promising candidates for the treatment of neonatal brain injury, but their long-term effects in combination with TH and sex dimorphic responses are still unknown.

On postnatal day 7 (P7), 54 Sprague Dawley rats underwent hypoxia-ischemia (HI) by unilateral ligation of the left common carotid artery and exposure to hypoxia for 2 hours (8% Oxygen - 92% Nitrogen). Rats were randomly assigned to: HI (non-treated; 6 Males / 7 Females), HI+TH (cooled at 32.5-33°C for 3 hours; 6M/6F), HI+TH+URB447 (cooled+treated with a single 1mg/kg i.p. dose of URB447; 5M/6F). Non-HI pups served as Sham (8M/10F). Cylinder, NOR, rotarod and T-maze neurobehavioral tests were performed at P42 and P90, and animals were sacrificed at P90 for brain histological analysis.

In the HI group, male pups obtained worse performances in cylinder and rotarod ($p<0.05$ vs Sham), whereas females in all tests. However, histological analysis was worse in males, showing relevant tissue loss ($p<0.001$ vs Sham) and neuropathological scores ($p<0.01$ vs Sham) in all brain areas assessed. TH-alone did not improve neurobehavioral tests nor brain injury in either sex. The combined therapy of TH+URB improved neurobehavioral results in males (cylinder $p<0.01$ vs HI; rotarod $p<0.05$ vs HI) and females (NOR $p<0.05$ vs HI; T-maze $p<0.05$ vs HI) and was the only treatment able to ameliorate histological brain injury in cortex ($p<0.05$ vs HI).

Our results suggest that HI may differentially affect males and females, with the combined therapy of TH and the cannabinoid URB447 being able to improve motor function in males and memory in females.

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TU02-3

PERIPHERALLY-RESTRICTED NON-ADDICTIVE CANNABINOIDS FOR ORAL CANCER PAIN TREATMENT

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Oral cancer pain is a significant clinical challenge with a clear unmet need for efficacious treatments that lack the respiratory side effects and abuse potential of standard-of-care opioid analgesics. Here we describe a milestone-driven plan of SAR-informed scaffold-based synthesis and high-throughput *in vitro* screens that assess target activity, peripheral selectivity, and metabolic stability to discover peripherally-restricted cannabinoids (PRCBs) that target the CB1 receptor. In Year 1, we used industry-standard *in vitro* assays to assess efficacy and potency at human and mouse CB1/CB2Rs, solubility, blood-brain barrier (BBB) penetration, and cytotoxicity to further inform PRCB optimization. Efficacy of 149 new analogs (registered with NIH) at hCB1R (compared to CP55,940 @100%) ranged from -2.5% to 126%. BBB penetrability of new analogs in the hMDR1-MDCK assay ranged from high (undesirable) to low (desirable). Efficacy/permeability data were used by chemists to inform design and synthesis of compounds with improved solubility, CB1R efficacy, and maximized peripheral restriction. Only 2/149 new analogs showed significant toxicity in the nanomolar range. In Years 2-4, we will profile the optimized analogs for *in vivo* oral cancer pain suppression, central side effects, pharmacokinetics, target engagement, addiction liability, and safety pharmacology. The best candidate will advance to full scale-up, IND-enabling studies, followed by IND application and approval. These studies are prerequisite to conducting a first-in-man Phase I, single group, double-blind, placebo-controlled, dose-escalation trial of an optimal PRCB for oral cancer pain to determine its safety, PK, PD and dose-effect, and to confirm target engagement. Supported by NIH grant NS128148.

TU02-4

NEUROPROTECTIVE EFFECTS OF CANNABIDIOL IN ACRYLAMIDE-INDUCED NEUROTOXICITY: IMPACT ON OXIDATIVE STRESS, INFLAMMATION, AND CHOLINERGIC ACTIVITY IN MICE

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The neuroprotective potential of cannabidiol (CBD) was assessed in a mouse model of acrylamide-induced neurotoxicity. Male C57BL/6 mice were randomly assigned to four groups: Control (Con), Acrylamide (AA), Cannabidiol (CBD), and a combination treatment (AA+CBD).

The AA group received acrylamide (10 mg/kg, i.p.) daily for 5 days. In the CBD and AA+CBD groups, CBD was administered i.p. at a dose of 10 mg/kg per day for 10 days. The CBD was administrated (10 mg/kg, i.p.) for a 10-day period in CBD and AA+CBD group. In the AA+CBD group, acrylamide (10 mg/kg, i.p.) was co-administered during the last 5 days of CBD treatment. Behavioral outcomes were analyzed using the open field test, revealing that CBD mitigated anxiety-like behavior induced by acrylamide, enhancing movement and center exploration. Further, CBD treatment modulated oxidative stress responses, reducing MDA levels and partially restoring antioxidant markers (GSH, SOD, and CAT) in the hippocampus and striatum. Inflammatory markers were also assessed, revealing that acrylamide elevated pro-inflammatory cytokines TNF-α and IL-6. Notably, CBD co-treatment reduced TNF-α levels in the hippocampus and cortex and attenuated IL-6 levels in the cortex and striatum, suggesting an anti-inflammatory effect. Additionally, CBD modulated neuroplasticity by increasing BDNF levels in the hippocampus, counteracting the reduction caused by acrylamide. CBD also influenced cholinergic activity by restoring Ach levels and altering AChE activity across brain regions.

These findings suggest that CBD exhibits neuroprotective properties by reducing oxidative stress, inflammation, and cholinergic dysregulation, thereby offering a promising therapeutic approach for mitigating neurotoxicity and potentially treating neurodegenerative disorders.

TU02-5

MECHANISMS DRIVING TYPE 1 CANNABINOID RECEPTOR N-TERMINAL DIVERSITY

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The type 1 cannabinoid receptor (CB1) is a polyfunctional G-protein coupled receptor (GPCR) involved in brain processes such as reward, memory, pain perception, and stress-related responses. In neurons, CB1 is predominantly localized at the presynaptic membrane, where it detects endocannabinoids released from the postsynaptic neuron to downregulate neurotransmission. Additionally, CB1 can also be associated with mitochondrial membranes, where it regulates cellular respiration and energy production. Interestingly, deletion of the first 22 amino acids (DN22-CB1) prevents its mitochondrial localization, while preserving its other signalling properties, indicating that the CB1 N-terminus plays a role in the subcellular sorting of the receptor. Our data suggest that endogenous posttranscriptional mechanisms drive CB1 N-terminal diversity. Specifically, we show that in rat primary cultured neurons the CB1 N-terminal tail is cleaved by a disintegrin and metalloprotease-containing protein 10 (ADAM10). Additionally, we show that CB1 can be translated through alternative downstream start codons corresponding to methionines within its N-terminal tail, resulting in different posttranscriptional DN-CB1 variants. Understanding the mechanisms and functions of these variants will shed light on the regulatory framework underlying CB1 function and could contribute to future design of more targetted therapeutic approaches.

TU02-6

CANNABINOID ACTIONS ON SENSITIZED DURAL NOCICEPTORS IN A MOUSE MIGRAINE MODEL

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Migraine is a debilitating neurological disorder affecting ~15% of global population with greater prevalence in females. Cannabinoids acting on Gi/o-coupled cannabinoid 1 and 2 receptors (CB1Rs/ CB2Rs) alleviate migraine symptoms in humans but central side effects limit their use. Here we examine actions of a peripherally-restricted cannabinoid (PRCB) on behavioral and physiological parameters in a mouse migraine model. Female C57BL/6J mice (5-wks old) received pH-6.0 or pH-7.4 saline (4μL) supradural injections (at bregma). Periorbital allodynia symptoms were assessed by responses to von Frey hair stimulation. At 3-days post-injection, all mice were tested for latent sensitization by injection of pH-7.0 saline. In other groups of mice, the CB1R/CB2R agonist PRCB, PrNMI (5μM), was co-applied with pH-6.0 saline with or without the peripherally-restricted selective CB1R blocker, 18A (20μM) or the selective CB2R blocker, SR144528 (20μM). Patch recordings were obtained in fluorogold-labeled dural trigeminal ganglion neurons (dTGs) acutely isolated 3-days after fluorogold co-injection with pH-7.4 or pH-6.0 saline. Supradural co-administration of pH-6.0+PrNMI prevented the initial and latent allodynia. Co-administration of pH-6.0+PrNMI+18A or SR144528, abolished the analgesic effects of PrNMI. Post-treatment with pH-7.0+PrNMI did not prevent latent allodynia. Recordings revealed larger depolarizing responses to pH-6.0 perfusion in dTGs from pH-6.0-treated mice ($p=0.04$, t-test). PrNMI (1μM) decreased pH-6.0 responses in dTGs from pH-7.4-treated mice ($p=0.034$) but not pH-6.0-treated mice. Thus, PrNMI prevents acid-induced allodynia and latent sensitization by activating neuronal CB1Rs and non-neuronal CB2Rs. PrNMI post-treatment is ineffective due to sensitization of acid-sensitive ion channels and decreased CB1R effectiveness in dTGs from pH-6.0-treated mice.

TU02-7

The C-terminal tail of CB1R regulates its surface stability through interactions with SGIP1 and retromer.

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There is intense scientific, medical, and societal interest in the endocannabinoid system (ECS) because it modulates a wide and diverse range of brain functions including mood, appetite/energy expenditure, and learning and memory. However, because of the complex and pleotropic pharmacology of the ECS, cannabinoid drugs are associated with unwanted neurological and psychoactive side effects. These limitations drive the need for increased biochemical and cell biological understanding of the ECS to develop alternative approaches to modulate it without the use of direct cannabinoid agonists.

In neurons, the main ECS receptor, the cannabinoid type 1 receptor (CB1R), is located predominantly at the presynaptic terminal. CB1R activation by postsynaptically-released endocannabinoids dampens synaptic communication. Defining ways to alter the availability of functional CB1R at the presynaptic terminal could enhance or reduce ECS signalling while retaining physiological synaptic control mechanisms, thereby minimising off-target effects.

Here we examine how interactions of the C-terminal tail of CB1R (ctCB1R) with SGIP1 and retromer regulate CB1R expression at the plasma membrane. We show the *H9* domain binds to the endocytic adaptor protein SGIP1 to promote CB1R expression in the axonal membrane. Overexpression of SGIP1 increases CB1R axonal surface localisation while SGIP1 knockdown reduces axonal surface expression. Neither alter CB1R lacking the *H9* domain (CB1R-ΔH9). We also show that retromer recycles previously internalised CB1R back to the surface in conjunction with adaptor proteins sorting nexin 3 and/or sorting nexin 12 (SNX3/12). Knockdown of core retromer component VPS35 or SNX3/12 reduces both surface and total levels of CB1R in primary cortical cultures. In HEK cells, CB1R-WT, but not CB1R-ΔH9, co-IPs with GFP-VPS35.

This information will allow us to develop small peptide tools to stabilise/compete with SGIP1 and VPS35 binding to promote/reduce CB1R at the cell surface and modulate endocannabinoid signalling. We predict this indirect intervention could deliver the neuroprotective effect of CB1R without the need to apply exogenous cannabinoids and the associated negative side-effects.

TU02-8

ACUTE AND SUB-CHRONIC EFFECTS OF CANNABIDIOL ON BEHAVIORAL AND BIOCHEMICAL INDICATORS IN BRAIN REGIONS OF ADULT MICE

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Cannabidiol (CBD), a non-psychoactive compound from Cannabis sativa, has been increasingly studied for its potential neuroprotective, antidepressant, anti-inflammatory and anxiolytic properties. This study aimed to evaluate the acute and sub-chronic effects of cannabidiol (CBD) on anxiety-like behavior, locomotion, cholinergic system activity, and oxidative stress markers in adult male mice. Mice were divided into three groups: a control group, a sub-chronic treatment group receiving 5 mg/kg CBD for 10 days, and an acute treatment group receiving a single 20 mg/kg dose, both administered intraperitoneally. Behavioral assessments were conducted using the open field test, while acetylcholinesterase (AChE) activity was analyzed using Ellman's colorimetric method in both salt-soluble (SS) and detergent-soluble (DS) fractions. Oxidative stress was assessed by measuring superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde (MDA) levels. Results showed that sub-chronic CBD administration led to behavioral alterations indicative of anxiolytic effects, with no changes observed in the acute treatment group. Additionally, sub-chronic CBD administration significantly reduced AChE activity across all examined brain regions in both fractions while acute treatment only reduced AChE activity in the DS fraction. However, no significant alterations in oxidative stress markers were observed in either treatment group. In conclusion, sub-chronic CBD administration appears to modulate the cholinergic system and induce anxiolytic-like effects.

TU02-9

FROM ADOLESCENT TO AGED: THE AGE-DEPENDENT EFFECTS OF CANNABIDIOL ON ANXIETY, CHOLINERGIC ACTIVITY, AND OXIDATIVE STRESS

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Brain function undergoes significant changes across different life stages. Adolescence is characterized by ongoing neurodevelopment and increased susceptibility to stress-related disorders. In contrast, aging is associated with increased oxidative stress, and dysregulation of cholinergic function, contributing to neurodegenerative processes. Given these age-related changes, researching therapeutic agents like Cannabidiol (CBD) could help modulate aging effects. This study aimed to investigate the effects of CBD on anxiety-like behavior, acetylcholinesterase (AChE) activity, and oxidative stress markers in adolescent (1-month-old), adult (3-4 months old), and aged (13-14 months old) mice. Each age group was divided into a CBD-treated group (10mg/kg, 10%DMSO, 2% tween-80, intraperitoneally for 10 days) and a control group (saline, 10%DMSO, 2% tween-80). Behavioral analysis was conducted using the open field test. AChE activity was determined through Ellman's colorimetric method, while oxidative stress was assessed by analyzing the levels of glutathione (GSH), and malondialdehyde (MDA) in the hippocampus, cortex, and striatum. The results indicated higher anxiety levels in adolescent and aged mice compared to adults. CBD treatment induced an anxiolytic effect across all age groups. In control conditions, AChE activity was lower in adolescent and aged mice while CBD administration further reduced AChE activity in all age groups. Moreover, the aged group displayed increased MDA levels and decreased GSH levels compared to adults. CBD reduced MDA levels and increased GSH levels in the aged group but had no effect on redox markers in adults. These findings suggest age-related differences in anxiety, cholinergic activity, and oxidative stress. Furthermore, CBD exerts anxiolytic effects regardless of age and modulates both cholinergic function and oxidative stress in an age-dependent manner. In conclusion, CBD shows promise as a potential therapeutic agent for aging-related neurochemical and behavioral alterations.

TU02-10

NEUROPROTECTIVE EFFECTS OF *CANNABIS SATIVA* ON ALUMINIUM CHLORIDE (ALCL3)-INDUCED OXIDATIVE STRESS AND HISTOPATHOLOGICAL ALTERATIONS IN LONG EVANS RATS

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Aluminium is a ubiquitous environmental element resulting from natural and anthropogenic activities, known to adversely affect the nervous system. This study explores the neuroprotective potential of *Cannabis sativa*, an antioxidant and anti-inflammatory agent, against aluminium chloride (AlCl3)-induced cortico-hippocampal damage in Long Evans rats.

Twenty adult Long Evans rats (250–300 g) were randomly divided into four groups (N=5 per group):

- Group I (Control) received 2 mL/kg distilled water daily for 28 days.
- Group II received 1000 mg/kg body weight of AlCl3 daily for 21 days.
- Group III received 166.5 mg/kg ethanolic extract of *Cannabis sativa* (EESC) daily for 7 days.
- Group IV received 1000 mg/kg AlCl3 daily for 21 days, followed by 166.5 mg/kg EESC daily for 7 days.

Treatments were administered orally. AlCl3 was administered from days 1 to 21, while EESC was administered from days 22 to 28. After treatment, rats were euthanized, and their brains were collected for biochemical and histological analyses.

Oxidative stress levels showed no significant changes across all groups. However, EESC reversed histological damage in the motor cortex and CA3 region of the hippocampus induced by AlCl3 toxicity. Observed damage, such as vacuolation and pyknosis, was ameliorated by EESC treatment.

Cannabis sativa demonstrates histological neuroprotective properties against AlCl3-induced neurotoxicity, supporting its potential as a neuroprotective agent.

TU02-11

MODULATORY ROLE OF CANNABIDIOL ON THE BIOBEHAVIORAL PROFILE IN A RAT MODEL OF FRAGILE X SYNDROME

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Fragile X Syndrome (FXS) is a neurodevelopmental disorder, the most common cause of inherited intellectual disability and the leading monogenic autism spectrum disorder (ASD). FXS is primarily caused by the expansion of the CGG trinucleotide repeat in the 5′ untranslated region of the *FMR1* (Fragile X Messenger Ribonucleoprotein 1) gene. The *FMR1* knockout (KO) rat model reflects the symptomatology of FXS, exhibiting hyperactivity, deficits in learning and memory and impaired sociability. Cannabidiol (CBD) has been recently shown to mitigate cognitive deficits in the *FMR1* KO rat model, offering initial mechanistic insights into its therapeutic potential for neurodevelopmental disorders.
Our group has characterized the *FMR1* KO rat model, revealing altered excitability and local inhibition in the hippocampus, along with a distinct transcriptional profile, highlighting dysregulated hippocampal activity. In this study, we extend our findings to female rats. Thus, following acute CBD administration, we conducted behavioral experiments to evaluate motor activity, social interaction and cognitive functions in both sexes of *FMR1* KO and WT rats, and we assessed the glutamatergic receptor expression profile in distinct brain regions. Overall, our findings provide insights into CBD impact on the aberrant biobehavioral profile of KO as compared to WT rats and highlight sex differences that contribute to a better understanding of FXS pathology.

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TU03-1

SELECTIVE NKCC1 INHIBITION BY IAMA-6 FOR THE TREATMENT OF AUTISM AND FRAGILE X

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Maintaining a proper intracellular chloride (Cl) concentration in neurons is essential for the physiological development and function of the central nervous system. Accordingly, disruption of this balance, due to the aberrant expression of the Cl importer NKCC1 and/or exporter KCC2, is implicated in diverse brain conditions including autism spectrum disorders (ASD) such as idiopathic autism and Fragile X syndrome, Down syndrome (DS), and drug-resistant epilepsy (DRE). Notably, inhibiting NKCC1 has been shown to ameliorate core symptoms of these brain conditions in rodent models and/or clinical trials. However, current NKCC1 inhibitors also target the kidney Cl transporter NKCC2, leading to diuretic effects, which create critical issues with dosing and health concerns, limiting their potential as a viable therapy for long-term chronic treatment of these conditions. To overcome these issues, we developed a novel class of selective NKCC1 inhibitors, which effectively restore core brain-related symptoms in animal models of idiopathic autism, DS, and DRE, without inducing any diuretic and toxic effects. The lead compound from this class, IAMA-6, recently demonstrated safety and a favorable pharmacokinetic profile in a first-in-human Phase 1 clinical study in healthy volunteers. In this study, we evaluated the efficacy of IAMA-6 in improving behavioral and cognitive deficits in a mouse model of Fragile X, a rare genetic form of ASD. We found that systemic chronic treatment with IAMA-6 rescued behaviors related to autism and memory deficits in adult Fragile X mice. These new results further establish IAMA-6 as a promising therapeutic option for neurological conditions characterized by disrupted Cl homeostasis and expand its potential range of therapeutic indications.

TU03-2

Region-Related Differences in Short-Term Synaptic Plasticity and Synaptotagmin-7 in Male and Female Hippocampus of a Rat Model of Fragile X Syndrome

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Fragile X syndrome (FXS) is an intellectual developmental disorder characterized by deficits in the short-term processing of neural information. The primary cause of FXS is the loss of fragile X messenger ribonucleoprotein, which is profoundly involved in synaptic function and plasticity. Short-term synaptic plasticity (STSP) may play important roles in functions that are affected by FXS. Recent evidence points to the crucial involvement of the presynaptic calcium sensor synaptotagmin-7 in STSP. However, how the loss of FMRP affects STSP and Syt-7 have been insufficiently studied. Furthermore, males and females are affected differently by FXS, but the underlying mechanisms remain elusive. The aim of the present study was to investigate possible changes in STSP and the expression of Syt-7 in the dorsal (DH) and ventral (VH) hippocampus of adult males and females in a Fmr1-knockout rat model of FXS. We found that the paired-pulse ratio and frequency facilitation/depression, as well as the expression of Syt-7, are normal in adult KO males, but the PPR is increased in the ventral hippocampus of KO females. Furthermore, we found robust region-dependent difference in the STSP and a twofold higher level of Syt-7 in the dorsal compared to the ventral hippocampus in the males of both genotypes and in the WT females. These results point to the susceptibility of the female ventral hippocampus to FMRP loss. Importantly, the different levels of Syt-7 suggest that Syt-7 may play a pivotal role in defining the striking differences in STSP along the long axis of the hippocampus.

TU03-3

Negr1: a new target converging into core impaired processes in autism spectrum disorders

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Autism spectrum disorders (ASDs) are a group of medical conditions with different etiologies that originate during neurodevelopment. Although hundreds of diverse gene variants have been implicated in the pathogenesis of ASD, all ASDs are characterized by common core symptoms (i.e. social impairment and repetitive behaviors). Nowadays there is no effective treatment for core behavior impairments in autism and numerous key mechanisms remain unknown. Here, we describe a cleavable cell-adhesion molecule (Negr1) as causative of core ASD behaviors when up-regulated in Wild type (WT) animals, and we indicate Negr1 as a potential convergent molecule dysregulated in ASD. Indeed, up-regulation of Negr1 in the prefrontal cortex (PFC) was sufficient to induce ASD core behaviors in WT mice and brain morphological deficits. By specific overexpression of the soluble Negr1 form in the PFC of WT animals we demonstrated that the soluble form of Negr1 is sufficient and necessary to cause social deficits in mice. Supporting the relevance of the latter finding, we also found that Negr1 is up-regulated in the PFC of five diverse mouse models of genetic ASD and in postmortem PFC samples of people with ASD and Fragile X.

Altogether, our results on the causal link between Negr1 upregulation and ASD core behaviors in mice together with Negr1 dysregulation in diverse ASD mouse models may explain how a wide variety of ASD genetic variants converge into a unique core group of impaired processes during brain development and of behavioral phenotypes. Our results also suggest regulation of Negr1 pathway and its cleavage as a new target for the design of future therapeutic approaches.

TU03-4

THE EFFECTS OF EARLY POSTNATAL NOISE EXPOSURE ON THE DEVELOPMENT OF PERINEURONAL NETS IN RAT CENTRAL AUDITORY SYSTEM

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In the rat auditory system, a period of increased vulnerability to external stimuli (critical period, CP) starts with the onset of hearing at postnatal day 12 (PD 12) and ends around three weeks later. It is expected that the closure of the CP is paralleled with the maturation of perineuronal nets (PNNs). The exposure to loud sound during the CP can significantly affect the morphology and electrophysiology of neurons in the rat central auditory system. Whether the exposure can also change the pattern of PNN maturation remains unknown. Rat pups of Long-Evans strain were exposed at PD 14 to a 125 dB SPL broad-band noise for 8 min. The changes of PNNs were evaluated in the brain sections of inferior colliculus (IC), medial geniculate body (MGB), and auditory cortex (AC), stained for Wisteria floribunda agglutinin in the exposed rats from PD 14 to PD 106, and compared with non-exposed controls. In the IC, PNNs were visible already at PD 14 in a greater number in exposed than control animals. The number of PNNs continued to increase during the development, but the difference between exposed and control groups remained to be present. In the MGB, nets appeared only in the medial part – relatively lately and in limited numbers. In the AC, no PNNs were discernible until PD 21, when they were again more expressed in the exposed animals, particularly in the deep layers. The difference remained significant in PD 28, but has diminished during further development. To summarize, we confirmed that PNNs develop in the AC later than in the IC, some nets were found even in the MGB. Further, we found that the development of PNNs appeared to be more accelerated in the noise-exposed animals than in the non-exposed controls. These results suggest that noise exposure may lead to a premature closing of the CP window, thus limiting the plasticity of early postnatal development in the central auditory system.

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TU03-5

Unraveling the Interaction between TBP and STUB1 in the Pathogenesis of SCA17-DI

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Spinocerebellar ataxia type 17 (SCA17) is a neurodegenerative disease characterized by a range of neurologic and psychiatric symptoms. The etiology of SCA17 involves dysfunction and loss of neuronal cells, particularly affecting Purkinje cells. The key factor contributing to disease development is the length of the variable CAG/CAA triplet repeats in the coding sequence of the TATA-box binding protein (*TBP*) gene. In the general population alleles with up to 41 repeats are prevalent, while expansions beyond 47 repeats are associated with full penetrance. At the protein level, pathogenic alleles are characterized by the presence of polyglutamine expansions, which lead to the formation of nuclear aggregates. In individuals with alleles containing 41-46 repeats, incomplete penetrance has been observed. In those cases, the contribution of a heterozygous mutation in the *STUB1* gene was found to be important, resulting in a digenic variant of SCA17 (SCA17-DI). *STUB1* encodes the E3 ubiquitin ligase CHIP, which is thought to be involved in the proteasomal degradation of the TBP protein.

To further investigate the pathomechanism underlying SCA17-DI, we developed iPSC lines derived from fibroblasts from donors with different genetic backgrounds: a patient with the monogenic form of SCA17, a healthy donor with an intermediate *TBP* allele, and a patient with SCA17-DI carrying a mutation in *STUB1*. These iPSC lines, along with healthy control, are subsequently differentiated into Purkinje cells. This disease model allows us to study the interaction between TBP and STUB1 involved in the pathogenesis of SCA17-DI. Furthermore, preliminary results of gene expression analysis related to protein homeostasis and autophagy suggest putative disease-related changes in patient-derived primary cell lines.

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TU03-6

Studying cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) using patient-derived brain organoids

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Neurological disorders are a leading cause of disability, affecting millions of people worldwide. Among them, cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a condition marked by late-onset symptoms, including cerebellar, bilateral vestibular, and somatosensory deficits. Recently, biallelic repeat expansions were identified within the replication factor C subunit 1 (*RFC1*) gene in CANVAS patients. Given the role of *RFC1* in normal DNA replication and repair, we hypothesize that within the nervous system, its mutation may lead to molecular and cellular changes that present even in developmental stages. Thus, our study aims to investigate the earliest mechanisms implicated in CANVAS manifestation. To this end, we identified two Greek ataxia patients with CANVAS, isolated and expanded PBMCs from the two patients and two healthy donors and reprogrammed all lines into iPSCs. The genetic integrity and pluripotent capacity of the iPSCs were examined and these lines were used to generate brain organoids (BOs). Patient-derived BOs exhibited reduced size compared to control, indicating a possible defect in neurodevelopment, even during early stages. Utilizing these BOs, we are investigating *RFC1*'s role in neural progenitors' proliferation, neuronal migration, differentiation and maturation. In addition, DNA damage levels will be assessed and a non-hypothesis-driven high-throughput approach to its etiopathology will be applied. Our findings will contribute to a better understanding of CANVAS pathophysiology and the development of novel diagnostic and therapeutic approaches.

TU03-7

THE ALTERNATIVE ANDROGEN RECEPTOR ISOFORM (AR-A) PROTECTS AGAINST TOXICITY OF MUTANT ANDROGEN RECEPTOR WITH THE ELONGATED POLYGLUTAMINE TRACT CAUSATIVE OF SPINAL AND BULBAR MUSCULAR ATROPHY (SBMA)

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Spinal and bulbar muscular atrophy (SBMA) is an X-linked neurodegenerative disease caused by a CAG repeat expansion in the androgen receptor (AR) gene. This mutation leads to the translation of an elongated polyglutamine (polyQ) tract, and the receptor becomes toxic upon testosterone activation. Since current therapeutic approaches primarily focus on androgen reduction and AR modulation, leading to severe endocrine side effects, new therapeutic approaches became relevant to counteract the pathology. Different start codons (AUGs) are involved in AR translation. An internal AUG is located downstream of the CAG repeat leading to the translation of a shorter isoform (AR-A), devoid of the polyQ tract that should not aggregate and should exhibit at least a partial androgenic transcriptional activity in response to ligand binding.

Initial analysis revealed that AR-A is predominantly expressed in the CNS rather than androgen-sensitive tissues. Furthermore, evaluation of AR-A behavior showed that this isoform cannot aggregate but retains partial androgenic activity. Since ARpolyQ and AR-A can heterodimerize, by testing the effect of AR-A on ARpolyQ behavior, we showed that AR-A has a pro-solubilizing effect on ARpolyQ aggregates and that the heterodimers retained their transcriptional activity. Finally, to confirm our in vitro data, we evaluated AR-A effect in a *Drosophila Melanogaster* based in vivo model of SBMA. Data obtained demonstrated the absence of eye degeneration in AR-A expressing flies which is instead present in ARpolyQ expressing flies. Moreover, it has been observed a reduction of eye degeneration in flies expressing both ARpolyQ and AR-A corroborating our hypothesis of the AR-A pro-solubilizing effect.

Altogether, our results demonstrate that an induction of AR-A expression may have a role in protecting against ARpolyQ aggregation and toxicity and could be a relevant alternative therapeutic approach to overcome the issues related to currently available therapy.

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TU03-8

BRAIN INSULIN RESISTANCE AS A HALLMARK OF ACCELERATED AGING IN DOWN SYNDROME

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Background
Aging is a major health challenge, increasing susceptibility to neurodegenerative diseases such as Alzheimer's disease (AD). Brain insulin resistance (bIR) contributes to AD and neurodegeneration. Down syndrome (DS), a model of accelerated aging, presents early metabolic dysfunctions. We previously demonstrated bIR in children with DS. Here, we investigate whether these alterations persist in adults.

Methods
Neuronal-derived extracellular vesicles (nEVs), "liquid biopsies" for brain-related pathology, were isolated from plasma of sex- and age-matched DS individuals (20-40 years, n=35) and controls (n=30) from Gemelli Hospital. nEVs were characterized for protein content, size, and concentration. bIR markers were quantified using a multiplex approach (BioRad), while circulating AD biomarkers were analyzed with SIMOA (Quanterix).

Results
nEVs from DS individuals showed significantly elevated bIR markers, including pIRS1S636 (p=.01), pAKTS473 (p=.04), pGSK3βS9 (p=.0001), pmTORS2448 (p=.002), pP70S6KT389 (p=.04), pS6S235/S236 (p=.0001), and pBADS136 (p=.0001), vs controls. AD markers showed a reduced Aβ42/40 ratio (p=.0001) and increased NfL (p=.0001) and GFAP (p=.006), while pTau217 remained unchanged (p=.14). Multivariate analyses confirmed age-related accumulation of bIR markers in DS, independent of AD pathology.

Conclusions
Our findings confirm that bIR is an early and persistent feature of DS, independent of AD pathology. This suggests that insulin signaling impairments contribute to neurodegeneration in DS, reinforcing its role as a model of accelerated aging. Targeting bIR in DS may offer new strategies to mitigate cognitive decline.

TU03-9

Decoding Primary Cilia Function in Human Cortical Development and Disease

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The cerebral cortex is among the most intricate structures in mammals, and disruptions in its development lead to severe brain disorders, like malformations of cortical development (MCDs). Dysfunction of the primary cilium (PC), a small organelle acting as the cell's "antenna", leads to corticogenesis defects in mice as it regulates neural progenitor cell (NPC) function and neuronal migration. However, its role in human brain development remains largely unknown. Notably, mutations in centrosomal or ciliary-associated genes have been linked to MCD subtypes, including microcephaly, periventricular heterotopia, and polymicrogyria, emphasizing the importance of PC function in human corticogenesis. Our study explores the impact of PC on cortical development and MCDs. By analyzing single-cell RNA sequencing datasets from human and animal models, we identified species-specific differences in PC-related gene expression. To assess PC function, we ectopically manipulated candidate genes in the developing mouse cortex and human brain organoids. Our results reveal changes in NPC and neuronal distribution, likely caused by alterations in PC positioning, disruption of apical anchoring and cellular delamination. To further investigate PC dysfunction in MCDs, we are generating PC-mutated brain organoids using the CRISPR/Cas9 system. These findings provide insight into PC's role in human corticogenesis and the mechanisms underlying MCDs.

TU03-10

A novel drug reduces motor impairments and premature death in a *Grin2d* mouse model for developmental and epileptic encephalopathy

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Developmental and Epileptic Encephalopathies (DEEs) are devastating neurodevelopmental disorders caused by genetic mutations, leading to global developmental delays, intractable epilepsy, motor impairments, and premature death. GRIN2D c.1999G>A is a notable de novo DEE-causing variant which results in a gain-of-function alteration of the N-methyl D-aspartate receptor subunit GluN2D, leading to severe neurological impairments. Current treatments unsuccessfully focus primarily on managing epileptic seizures, and they fail to address associated cognitive and motor impairments. This study investigated the therapeutic potential of Compound A, a cholesterol 24-hydroxylase inhibitor, on a *Grin2D* Val667Ile mouse model that mirrors the human phenotype. Mice were treated with Compound A from birth or weaning age, and their motor performance, cognitive function, seizure burden, and survival rates were evaluated. Treatment significantly extended the lifespan of both early- and late-treated mice, with improved motor coordination and balance, as shown by increased time on the RotaRod. Compound A also improved cognitive performance, correcting hyperactivity and nest building abilities. Electroencephalogram (EEG) recordings revealed that treatment altered brain activity by rescuing reduced delta power, reflecting improvements in both epileptic and non-epileptic brain function. These results suggest that Compound A not only reduces epileptic activity but also addresses broader cognitive and motor deficits, providing a comprehensive therapeutic approach. In contrast to current DEE treatments, which primarily target epilepsy, Compound A offers a promising new avenue for improving the overall quality of life for patients with GRIN2D-related DEEs.

TU03-11

PARP INHIBITORS AS A NEW POSSIBLE THERAPEUTIC STRATEGY IN COHESIN-DRIVEN MEDULLOBLASTOMA

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Medulloblastoma (MB) is a cerebellar embryonic tumor, probably due to neural progenitor-impaired differentiation. However, the underlying genetics and predisposing factors are still unclear. The cohesin complex role in cerebellum development, gene expression regulation, and DNA repair suggests this structure as a possible oncogenic candidate. Among cohesins, STAG2 is the most frequently affected in cancers, and its loss of function is believed to promote tumorigenesis by altering DNA architecture.
By interrogating cBioPortal database, we found *STAG2* variants in 2% of MB patients. Thus, we studied the impact of *STAG2* silencing on cell function and DNA damage in cellular models. Then, we exploited 3D cerebellum organoids to study the effect of STAG2 deficiency on its development. Last, the *in vivo* fly model obtained by *SA1* silencing (ortholog of *STAG2*), was characterized to assess tumor-related phenotypic features by investigating neural precursor differentiation and flies' viability.
In vitro, we highlighted that *STAG2*-deficient cells present increased DNA damage, while *STAG2*-silenced cerebellum organoids exhibit a deregulated expression of genes related to cerebellar development. *In vivo,* we showed a defective differentiation of neuroblasts during larvae development and mass formation within adult fly brains with a lowered life expectancy consistent with the malignant phenotype. These models aim to unveil the contribution of STAG2 in MB oncogenesis and represent a basis for possible targeted therapy testing. Interestingly, we applied PARP inhibitors (PARPi), a class of drugs recently implemented in BRCA1/2 breast tumor treatment and trials for AML-carrying cohesin variants, in our *in vivo SA1*-deficient model, resulting in a significant life expectancy improvement. Overall, by demonstrating the involvement of STAG2 in MB, it will be possible to deepen the knowledge of MB pathogenesis and set the ground for the potential therapeutic use of PARPi.

TU03-12

MORPHOPHEN: A JOINT EUROPEAN MASTER DEGREE ON HUMAN DISEASES MODELS MORPHOLOGICAL PHENOTYPING

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The scientific community currently lacks knowledge and expert human resources in mouse anatomy and pathology at a time when morphological phenotyping of the mouse as a model for discovery and mechanistic research, purpose-driven in vivo translational studies, and preclinical validation for human therapeutics is increasing.

To address this situation, the European Commission funded the European Joint Master degree in "Human Diseases Models Morphological Phenotyping" (MorphoPHEN) (https://morphophen.eu). MorphoPHEN includes training in anatomy, imaging technologies, pathology, and machine learning as it applies to automated image analysis. MorphoPHEN's objective is to develop and implement a novel European and global training programme on mouse morphological phenotyping that surges capacity for robust, reproducible, and truly comparative analysis and understanding of data generated using the mouse as a model. This master degree combines the excellence and expertise at four institutions: Autonomous University of Barcelona, the University of Naples Federico II and its Biotechnology Advanced Center (CEINGE), the Aristotle University of Thessaloniki, and the Veterinary School at Lisbon.

The MorphoPHEN consortium is supported by a multidisciplinary network of associated partners by varied, pivotal ways. The University of Cambridge pioneer in developing mouse ontologies and informatics supports MorphoPHEN. Furthermore, five major European and North American mouse clinics (ICS, MRC-Harwell, HMGU, CCP, ant TCP) equipped with integrated facilities for production and characterization of genetically engineered mouse models are also associated. Finally, the veterinary pathology core at St Jude Children's Research Hospital, and a company leader in the field of preclinical imaging (Visualsonics) are also essential partners, providing MorphoPHEN with a unique and global position to teach a common and fundamental skill set and required knowledge to validate morphologically mouse models of human diseases.

TU03-13

ALTERATIONS IN MITOCHONDRIA DYNAMICS IN THE HIPPOCAMPUS OF TRAP1 MUTANT MICE, A NOVEL MODEL OF ASD

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Neuronal cells depend on mitochondrial activity to maintain membrane excitability, neurotransmission and synaptic plasticity. The AMP-activated protein kinase (AMPK) signalling pathway is a key regulator of cellular energy homeostasis and has been implicated in mitochondrial dynamics. The dynamics of these organelles, in particular fission/fusion, determine their morphology, with the result that they adapt to the metabolic needs of cells. Additionally, phosphorylation of the main protein involved in the regulation of mitochondrial dynamics- DRP1 is essential for the regulation of mitochondrial dynamics, synapse maturation, synaptic transmission and plasticity, and learning and memory.

A mutation (*p.Q639**) in the *TRAP1* gene encoding the mitochondrial chaperone was identified in an ASD patient whose monozygotic twin brother was unaffected. This chaperone belongs to the HSP-90 family of proteins involved in protection against oxidative stress and regulation of the cell's metabolism. A novel model of ASD- *Trap1 p.Q641* knock-in* mouse model carrying the identical mutation was generated using CRISPR-Cas9. We observed sex-specific deficits in the sociability of novel *Trap1 p.Q641* knock-in* mice.

Here, we aimed to investigate of mitochondria dynamics of *Trap1* male and female mice in the hippocampus of Trap1 (mutant and wild-type). To achieve this, we isolated mitochondria from the hippocampi of *Trap1* mutant and WT mice and assessed the levels and phosphorylation status of proteins involved in fission and fusion in both sexes. In males, we observed downregulated levels of some proteins involved in fission, whereas the level of Mitofusin2 was increased. AMPK signalling connected with the regulation of mitochondrial dynamics was analysed.

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TU04-1

OVEREXPRESSION OF *MCIDAS* LEADS TO DIRECT REPROGRAMMING OF HUMAN REACTIVE ASTROCYTES TO THE EPENDYMAL LINEAGE

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Hydrocephalus is a neurological disorder characterized by enlarged ventricles filled with cerebrospinal fluid (CSF) that cannot get absorbed adequately. Most common interventions include surgical placement of stunts with poor quality of life which accommodate the manifestations of the disorder but not the underlying cause

A common characteristic of hydrocephalus is the destroyed ventricular lining where ependymal cells appear, preserving the CSF flow with the coordinated movement of hundreds of cilia on their apical surface. Previous results from our team have characterized *MCIDAS* as one of the master regulators of ependymal cell differentiation during early stages of embryogenesis. Recent published research has shown that the overexpression of *MCIDAS* direct reprograms cortical murine astrocytes into functional ependymal cells containing beating cilia. Astrocytes were selected due to the astrocytic scarring occurring in hydrocephalic ventricles but without being able to functionally replace ependymal cell loss due to the absence of cilia. However, the mode of gene delivery has been challenging due to the low transduction efficiency the lentiviral system provides and the random genome integration. Therefore, the use of Adeno-associated viruses (AAVs) is currently being investigated.

To try and recapitulate the human phenotype, we have acquired human astrocytes and hypothesized that by overexpressing *MCIDAS* with the use of AAVs the direct reprogramming to ependymal lineage will be more efficient. In addition, assessing the reactivity of the human astrocytes following a seven-day IL1β and TNFα treatment can serve as a steppingstone for further reprogramming those inflamed cells and therefore when the astrocytic scarring occurs the reprogrammed astrocytes will be able to functionally replace the damaged ependymal cells.

The multifactorial nature of the disorder, congenital and environmental, has proved challenging the emerge of an effective treatment. Therefore, our research strongly suggests that *MCIDAS* can serve as a good candidate for new therapeutic approaches against neurological disorders such as hydrocephalus.

TU04-2

KETOGENIC ACTIVITY OF CULTURED HUMAN ASTROCYTES

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Ketone bodies, acetoacetate and 3-hydroxybutyrate (3HB), play metabolic, signaling, and regulatory roles in brain, with putative neuroprotective effect. Out of cells in brain parenchyma, astrocytes possess the enzymatic machinery necessary for ketogenesis, and therefore, enabling them to convert suitable substrates into ketone bodies. Astrocyte-derived ketone bodies are considered to serve a role of energy substrate or source of acetyl-moieties for neurons and other glial cells such as oligodendroglia. Since, animal astrocytes were shown to convert leucine to ketone bodies, our recent results indicate that human cultured astrocytes possess the capability to generate substantial amounts of ketone bodies from the broader range of substrates.

The ability of cultured human astrocytes to metabolize the carbon skeleton of purely ketogenic amino acids, i.e., leucine and lysine, was assessed by analysis of culture media by LC-MS, NMR and enzymatic assays. The increased level of either leucine or lysine promoted generation of 3HB in manner regulated by glucose availability. Indeed, hyperglycemia stimulated irreversible catabolism of leucine and production of ketone bodies.

These findings collectively highlight the active involvement of human astrocytes in ketone body production and their significant role in supporting neuronal function through metabolic adaptations. The ability of astrocytes to utilize leucine and lysine as substrates for ketogenesis not only underscores their metabolic flexibility but also suggests potential therapeutic avenues for conditions characterized by impaired energy metabolism. In addition, the observed effect of hyperglycemia to stimulate the production of ketone bodies by cultured astrocytes is in contrast to starvation induced ketogenesis by hepatocytes. Our results point to possibility that by hyperglycemia stimulated metabolic changes may contribute to neurological damage associated with common pathology, as is diabetes mellitus.

TU04-3

CONSEQUENCES OF INHIBITION OF SYNOVIOLIN ENZYMATIC ACTIVITY ON THE SURVIVAL AND RESPONSE OF CELL DERIVED FROM BRAIN TUMORS AND ASTROCYTES.

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Neurodegenerative diseases and cancer are the most serious problems of human medicine, which significantly affect the quality and length of life. ER associated degradation (ERAD) and unfolded protein response (UPR) are two key mechanisms of protein quality control in cell. Disturbance in the ER functions leads to ER stress associated with accumulation of unfolded proteins that has to be eliminated by ERAD to secure cell survival.

The main purpose of the project was to analyze the effect of SYVN1 inhibition on survival and response of brain tumor cell lines - SH-SY5Y, T98G, U87 as well as astrocytes NHA and K1884. We used a specific inhibitor of enzymatic activity LS-102.

We found that inhibition of SYVN1 leads to decreased survival of all examined cell lines. Astrocytes K1884 exhibit the highest sensitivity to LS-102, while T98G glioblastoma cells were the most resistant to LS-102. We also observed a significant increase in SYVN1 expression. Treatment of T98G cells results in activation of the IRE1α-XBP1 pathway and subsequent activation of IRE1α ribonuclease activity leading to splicing of XBP1 into XBP1s. In U87 cells treated with LS-102, SYVN1 was overexpressed but IRE1α-XBP1 pathway was not activated.

Our results showed that T98G and U87 cells respond to SYVN1 inhibition through increased expression of SYVN1. We assume that increased expression of SYVN1 as an essential protein of ERAD represents a certain compensatory mechanism by which the cells try to cope with the inhibition of SYVN1 activity.

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TU04-4

TRANSCRIPTIONAL MECHANISMS CONTROLLING THE MIR-124/ISX9 INSTRUCTED DIRECT REPROGRAMMING OF MOUSE CORTICAL ASTROCYTES TO INDUCED NEURONS

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Direct neurogenic reprogramming of astrocytes is a promising in vivo therapeutic approach aiming at alleviating neuronal loss, which is a hallmark of neurodegeneration and brain injury.
We have previously shown that miR-124 is an efficient driver of the astrocytic fate switch towards an immature neuronal fate in vitro and in vivo in a mouse model of cortical trauma, whereas the addition of the neurogenic compound ISX9 leads to induced neurons' (iNs) functional maturation in vitro and increased survival in vivo.
In this study, we aimed to dissect the transcriptional mechanisms through which miR-124 instructs the astrocytic fate switch and along with ISX9 establishes the neuronal fate. For this, using the RNA-seq data from our previous study, we constructed the transcriptional regulatory network (TRN) established by miR-124 and further expanded by ISX9 and also estimated the core transcription factors (TFs) of these networks employing the betweenness centrality analysis. Among the top TFs that exhibited high activity in both networks was the DNA demethylase, Tet1. Silencing of Tet1 using siRNAs greatly reduced reprogramming efficiency and iNs' differentiation. Further on, we revealed a coregulatory role between Tet1 and Lin28a – among the top TFs in miR-124+ISX9 network – in the ISX9-induced upregulation of maturation genes with synaptic function.
Concluding, in this work we have unveiled novel transcriptional mechanisms that instruct the direct reprogramming of astrocytes by miR-124 and ISX9 to iNs and revealed the pivotal role of Tet1 in neurogenic reprogramming.
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TU04-5

Paracrine effects of astrocytes on neurons in an iPSC-derived Parkinson's disease model with the p.A53T-αSyn mutation

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Parkinson's disease (PD) is characterized by the loss of midbrain dopaminergic neurons and the presence of α-synuclein (αSyn) inclusions, named Lewy bodies and neurites. Approximately 10% of cases are linked to mutations in specific genes, such as the p.A53T-αSyn mutation. Despite intensive research on neuronal dysfunction, the role of astrocytes in PD remains underexplored. Recently, we showed that A53T induced pluripotent stem cell (iPSC)-derived astrocytes exhibit intrinsic defects and contribute to non-cell-autonomous neurodegeneration in astrocyte-neuron co-cultures. By contrast, healthy astrocytes mitigate neuronal pathology and accumulation of αSyn aggregates. Astrocytes may modulate neuronal autophagy, yet little is known about their role in aggrephagy and how this affects neuronal pathophysiology. To address this, we treated iPSC-derived dopaminergic (DA) neurons with conditioned medium from iPSC-derived ventral midbrain astrocytes. Our results revealed that healthy astrocyte-conditioned medium (H-ACM) reduced αSyn aggregates in A53T DA neurons and enhanced their viability. Conversely, PD astrocyte conditioned medium (PD-ACM) decreased the viability of healthy DA neurons, suggesting a neurotoxic effect of A53T astrocytes. Ongoing experiments will elucidate the effects of H- or PD-ACM on neuronal function using rabies virus-based retrograde monosynaptic tracing, electrophysiological recordings in multi-electrode array and Ca^{2+} imaging. In parallel, we are investigating the paracrine effects of healthy and PD astrocytes on global and neuronal selective autophagy. In summary, our findings underscore the detrimental role of A53T astrocytes in PD pathology, and highlight the neuroprotective potential of H-ACM. These results suggest that astrocytic paracrine mediators may serve as therapeutic targets for PD.

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TU04-6

S-100+ CELL POPULATION DIVERSITY IN THE DEVELOPING HUMAN NEOCORTEX

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S-100β is widely used as a glial marker with soma/endfeet location in normal brain astrocytes and a prognostic marker of glioblastoma. It is also referred to express in developing oligodendrocytes.

The dorsal gliogenic wave in the human fetal telencephalon turns on at the end of early fetal period. It possesses astroglial marker manifestation, including the S-100β, in the ventricular/subventricular (VZ/SVZ) transient zones after 17th weeks of gestation. In contrast to other astroglial markers, such as GFAP and ALDH1L1, which first appearing and further expansion align with the dorsal gliogenic wave, S-100β cells present in the human developing cortex as early as pre-fetal stage. Furthermore, S-100β+ cell distribution at these stages is close to the OLIG2 and differ from the SOX9 marker patterns at the pre-fetal and early fetal stages.

The autopsies (a brain hemisphaeria) from the thirteen human fetuses at the stages from 11th gestational week to birth were used in the study. Double-labeling experiments with the S-100β, OLIG2, SOX10 and SOX9 markers revealed coexistence of the S-100β+/OLIG2+ and S-100β+/SOX10+ double-labeled cell populations with the S-100β+/SOX9+ one in the subplate and cortical plate at the all stages from the end of the early to late fetal periods and only at the late fetal period in the SVZ and intermediate transient zone, also as in the newborn white and gray matter. The single and double-labeling distribution patterns of the used markers at the different developmental stages are also provided.

TU04-7

GLIOBLASTOMA ORGAN ON CHIP: TO CHARACTERIZE SECRETOME AND CANCER MASS INTERPLAY

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Glioblastoma (GBM) is a highly aggressive and challenging-to-treat brain tumor characterized by poor prognosis as a consequence of therapeutic resistance and relapse after treatment. The therapeutic resistance could depend on residing glioblastoma stem cells (GSCs) in the cancer mass, forming tumor niches that support GSCs quiescent status and include hypoxic tumor microenvironment (TME).

Cancer cells release many growth factors and cytokines in TME, resulting in altered secretoma composition. This secretome released by GSCs in TME contributes to maintaining their pluripotent, conferring them self-renewal ability and exerting a pivotal role in invasiveness and intra-brain tumor spreading, as well as in the resistance to chemotherapeutic treatments.

The present investigation aimed to set up an innovative *in vitro* microfluidic dynamic system to understand the secretome and cancer mass interplay.

The *in vitro* microfluidic dynamic model was assessed by using the MIVO® single-organ platform to culture GBM immortalized cell lines or patient-derived GBM organoids.

In the preliminary step, we have cultured immortalized GBM cells (U87MG and A172) in a compartment physically separated through a porous permeable membrane from the fluid flow compartment to emulate the microcirculation of secreted cells within the TME. The circulating compartment is accessible, allowing us to monitor and quantify the changes occurring in the TME. The closed-loop fluidic circuit pumps the culture medium through the lower chamber at a rate of 2.3 mL/min, simulating the capillary flow rate (0.1 cm/s), and mimicking the circulatory system disseminating the GBM secretome.

Preliminary results have shown that secretome composition derived from immortalized GBM cells exposed to a hypoxic mimetic agent, deferoxamine, showed increased levels of vascular endothelial growth factor (VEGF) and interleukin (IL)-1β as compared to the control group.

Considering that niches undergo dynamic alterations following therapeutic treatment, the validation of this new system able to mirror TME could be useful for deeply understanding the behavior of GSCs during therapeutic intervention. Notably, the optimized system could be useful for performing preliminary screening of therapeutic agents.

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TU04-8

A-SYNUCLEIN OLIGOMERS POTENTIATE NEUROINFLAMMATORY NF-KB ACTIVITY AND INDUCE $CA_v3.2$ CALCIUM SIGNALING IN ASTROCYTES

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α-Synuclein aggregation has been linked with sustained neuroinflammation in Parkinson's Disease (PD), aggravating neuronal degeneration; however, there is still a lack of critical information about the structural identity of the α-synuclein conformers that activate microglia and/or astrocytes and the molecular pathways involved. To investigate the role of α-synuclein conformers in the development and maintenance of neuroinflammation, we combined experimental approaches in a variety of preclinical models such as primary quiescent microglia and astrocytes, post-mortem brain tissues from PD patients and A53T α-synuclein transgenic mice that recapitulate key features of PD-related inflammatory responses in the absence of cell death. We found that the presence of SDS-resistant hyper-phosphorylated α-synuclein oligomers, but not monomers, was correlated with sustained inflammatory responses, such as elevated levels of endogenous antibodies and cytokines and microglial activation. Similar oligomeric α-synuclein species were found in post-mortem human brain samples of PD patients but not control individuals. Detailed analysis revealed a decrease in $Iba1^{Low}/CD68^{Low}$ microglia and robust alterations in astrocyte number and morphology including process retraction. Our data indicated an activation of the p38/ATF2 signaling pathway mostly in microglia and a sustained induction of the NF-κB pathway in astrocytes of A53T mice. The sustained NF-κB activity triggered the upregulation of astrocytic T-type $Ca_v3.2$ Ca^{2+} channels, altering the astrocytic secretome and promoting the secretion of IGFBPL1, an IGF-1 binding protein with anti-inflammatory potential. Our work supports a causative link between the neuron-produced α-synuclein oligomers and sustained neuroinflammation *in vivo* and highlights the recruitment of astrocytic $Ca_v3.2$ channels as a potential neuroprotective mediator against the α-synuclein-induced neuroinflammation.

TU04-9

ASTROCYTES, VIA RTP801, CONTRIBUTE TO COGNITIVE DECLINE BY DISRUPTING GABAERGIC-REGULATED CONNECTIVITY AND DRIVING NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a common form of dementia characterized by a gradual decline in cognitive abilities. The condition involves neuroinflammation, which is triggered by the accumulation of amyloid beta (Aβ) aggregates. This inflammation worsens AD pathology, leading to synaptic dysfunction and neuron death. Astrocytes and microglia contribute to this process by releasing pro-inflammatory molecules that exacerbate neuronal damage. RTP801 is a protein involved in neuroinflammation and regulates mTOR/Akt signaling. Its levels increase in AD, correlating with the severity of the disease and cognitive decline. Moreover, silencing RTP801 in hippocampal neurons in the 5xFAD mouse model of AD prevents cognitive decline and reduces neuroinflammation. Here, we assessed whether astrocytic RTP801 contributes to the disease outcome. Hence, RTP801 was silenced specifically in hippocampal astrocytes in the 5xFAD mouse and we observed that spatial memory and anxiety were recovered. MRS (Magnetic resonance spectroscopy) and resting-state functional connectivity analyses showed that silencing RTP801 in hippocampal astrocytes prevented the loss of the GABAergic signaling and therefore, preserving connectivity. Furthermore, silencing RTP801 in 5xFAD hippocampal astrocytes reduced microgliosis and astrogliosis, as well as the protein levels of some inflammasome effectors. Hence, we conclude that astrocytic RTP801 is contributing to cognitive decline by affecting GABAergic-regulated connectivity, and the inflammatory response in the pathogenic context of AD. Targeting astrocytic RTP801 may offer therapeutic potential in managing AD progression.

WE01-1

DISSECTING THE COMPLEX NEURON-MICROGLIA INTERPLAY IN THE PRECIPITATING ROLE OF CHRONIC STRESS ON TAU-RELATED HIPPOCAMPAL MALPLASTICITY

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Hippocampus is one of most affected brain areas in Alzheimer's disease (AD), with Tau pathology propagating to hippocampus via dentate gyrus (DG). Thus, DG plays a critical role in the vulnerability of hippocampus to AD neuropathology. Chronic stress, an AD risk factor, precipitates Tau pathology; however, and despite that stress affects both neurons and microglia, no study has dissected the contribution of these DG cell types, and the interplay among them, in mechanisms through which stress damages DG in Tau brain pathology. We used P310L-Tau Tg mice with conditional deletion of glucocorticoid receptor (GR) in brain neurons (P310L-neuroGR^{KO}) or microglia (P310L-microGR^{KO}) in order to block specifically the stress signaling. Our results showed that the stress-driven neurogenesis suppression in P301L mice was also found in stressed P310L-microGR^{KO}, but not in P310L-neuroGR^{KO} mice, suggesting a role for GR signaling in neurons, but not in microglia. Analysis of mature neurons demonstrated that the stress-induced decrease of their dendritic length and spine density in P310L-GR mice was blocked in lack of GR in microglia, highlighting an essential role of microglial activation in stress-evoked neuronal atrophy. DG microglia complexity analysis showed that stress caused microglia hyper-ramification which was blocked in P310L-microGR^{KO} mice, with an opposite effect (de-ramification) in stressed P301L-neuroGR^{KO} mice. AI-based clustering in resting, hyper-ramified and reactive microglia categories suggest that microglia require their (microglia) GR signaling for their stress-driven transition to the reactive state, while microglia transition to hyper-ramified state is dependent on neuronal GR signaling and the corresponding 'signal' that microglia receive from neurons under stressful conditions. Our findings provide novel insights towards the dissection of the complex neuronal-microglial interplay in the precipitating role of chronic stress in DG malplasticity in AD brain.

WE01-2

Microglia-specific phenotypic and functional changes upon neuronal expression of familial Parkinson's disease p.A53T-alpha synuclein

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Parkinson's disease (PD) is the second most common neurodegenerative disorder (ND), characterized by diverse clinical manifestations. Synaptic dysfunction, a hallmark of most NDs, arises from structural and functional synaptic changes that can act as both a cause and a consequence of the disease. Recent evidence suggests a neurodevelopmental origin for synaptic dysfunction, challenging the long-held view that it emerges as a late-stage phenomenon. These findings underscore the role of inappropriate glial interactions, not only in adulthood but also during critical embryonic and postnatal developmental stages, where microglia are known to play pivotal roles.

In this study, we employ the M83 mouse model, which overexpresses human p.A53T-alpha Synuclein (p.A53TαSyn) in neurons, a mutation linked to autosomal dominant familial PD. We conduct a comprehensive morphological analysis of microglia across multiple brain regions at key developmental and disease stages: embryonic (E14–E17), early postnatal (P7–P10), young adult (P30), older adult (4–5 months), and symptomatic (>1 year). Notably, transcriptomic analysis of microglia from M83 and wild-type (WT) mice reveals over 600 differentially expressed genes (DEGs) at P7, well before the onset of any observable symptoms. This highlights the remarkably early molecular alterations in microglial signaling pathways associated with PD pathogenesis.

This study leverages the p.A53TαSyn familial PD model to investigate neuron-microglia interactions during the earliest and presymptomatic phases of PD. By adopting a non-neurocentric approach, we aim to elucidate the role of microglia in disease progression, define disease-associated cellular subpopulations, and identify potential therapeutic targets.

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WE01-3

The intracellular organelles of microglia are altered by inhibiting colony-stimulating factor 1 receptor

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Microglia, the resident immune cells of the central nervous system, play vital roles in the proper brain development, maturation and maintenance. One of its key cell-surface receptors, colony-stimulating factor 1 receptor (CSF1R), is essential for microglial survival, proliferation and functions. In recent years, pharmacological inhibitors of CSF1R such as Plexxikon 5622 (PLX5622) have become a major research focus due to its ability to deplete microglia *in vivo,* aiding researchers in uncovering core functions of these resident brain microphages in rodent models. However, the early effects of PLX5622 on the microglial organelles have so far been largely unexplored. Therefore, the aim of this study is to analyze the response of a 10 μM PLX5622 exposure on immortalized rodent (BV2) and human (HMC3) microglial cell lines compared to DMSO-treated cells utilizing a combination of live-cell imaging assays and transmission electron microscopy. Using live-cell labelling of mitochondria (TMRE, Mitotracker) and lysosomes (Lysotracker, Lysosensor), we found a decrease in lysosomal pH concomitant with an increase in lysosomal mass at 6 and 24 hours in BV2 cells, and a decrease in lysosomal pH at 24 and 48 hours in HMC3 cells in PLX-treated cells compared to their controls, along with an increase in mitochondrial membrane potential and mass at 24 hours in BV2 cells and mitochondrial membrane potential at 24 hours in HMC3 cells. Ultrastructural analyses of the mitochondrial alterations (e.g., elongation, membrane disruption, cristae enlargement, area) and other intracellular organelles (lysosomes, endoplasmic reticulum) of BV2 and HMC3 will further elucidate the effect of PLX5622 on cultured microglial cell lines. The present study highlights the response of PLX5622 on microglial organelles and will help reshape the perspective of its application in the neuroimmunology field.

WE01-4

In vivo study of microglia BIN1 deletion on mouse brain under homeostatic and neuroinflammatory conditions

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Genome-Wide Association Studies have identified SNPs strongly associated with an increased risk of developing AD. SNPs in the locus harboring Bridging Integrator 1 (Bin1) gene show the strongest association with AD, after ApoE. BIN1 is an adaptor protein that is implicated in cell membrane modelling dynamics and expressed in microglia. Although BIN1 implication in neuronal dysfunction has been studied, its role in microglia remains elusive. To this end, we developed a double transgenic Cx3CR1$^{Cre\text{-}ERT2}$//Bin1$^{fl/fl}$ mouse, in which Bin1 is knocked-out in microglia and injected LPS, to investigate the effect of microglia BIN1 deletion under homeostatic and inflammatory conditions. SnRNA-Seq analysis of adult mouse cortices indicated that a number of signaling pathways are differently impacted in Bin1cKO microglia following LPS administration. Bin1 deletion resulted in the enrichment of microglia cell subpopulations exhibiting enhanced proliferative capacity and IFN-type I-mediated inflammatory response following LPS treatment, findings that were confirmed by subsequent real time RT-PCR and immunohistochemical analysis. Moreover, FACs analysis indicated increased CD11c^{+} microglia, while immunofluorescence/morphometric analysis revealed that microglia exhibits a more activated phenotype with enhanced CD68 expression, along with appearance of a hyper-ramified morphology with increased number of intersections and convex hull volume.

WE01-5

B-LYMPHOCYTES-DERIVED EXTRACELLULAR VESICLES INFLUENCE MICROGLIAL ACTIVATION: INSIGHTS INTO EPSTEIN BARR VIRUS ROLE IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) pathophysiology begins peripherally, where immune cells migrate into the CNS, cross the blood-brain barrier, and trigger an autoimmune attack. Epstein-Barr virus (EBV) may be a key environmental trigger. Extracellular vesicles (EVs) influence CNS inflammation and immune modulation, possibly carrying EBV molecules. Understanding EV-microglia interactions is crucial for elucidating their role in MS. This study aims to develop an experimental model to investigate EVs-microglia interactions.

ARH77 (EBV+) and OPM2 are B-lymphocytes derived cell lines whose EVs were isolated through ultracentrifugation. HMC3 microglial cell line were treated with 2*10*e*8 EVs/100000 cells for 24h. RT-qPCR was performed to assess the expression of IL-1β, IL-6, IL-8, TNFα and CD68. Mean Cq and fold change was calculated.

Characterization of EVs revealed an increase in mode and mean size of ARH77-EVs compared to OPM2-EVs ($p<0.05$). ARH77-EVs are carriers of EBV genetic material.

Inflammatory phenotype of HMC3 upon stimulation with EVs has been evaluated: there was a significant and general decrease of IL-1β, IL-6, TNFα and CD68 ($p<0.05$) in both treatments compared to untreated cells. Interestingly, stimulation with ARH77-EVs resulted in an increase of IL-8 expression compared to that of OPM2-EVs stimulation and to untreated cells ($p<0.05$).

Stimulation of HMC3 microglial cell lines with B lymphocytes-derived EVs switched microglia towards an anti-inflammatory phenotype. The sole increase of IL-8 in ARH77-EVs treated HMC3 might be related to the activation of specific pathways by EBV genetic material carried by EVs. Its role, as that of anti-inflammatory cytokines, will be further investigated.

WE01-6

Alzheimer's Disease risk factor BIN1: Linking microglial deficiency to neuroinflammation and regulation of Adult Hippocampal Neurogenesis

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Bridging Integrator 1 (BIN1) is a key genetic risk factor for Late-Onset Alzheimer's Disease (LOAD). While BIN1's role in neuronal functions is well-documented, its role in microglia remains underexplored. Given the central role of microglia in neuroinflammation and Adult Hippocampal Neurogenesis (AHN)—both key aspects of Alzheimer's pathology—studying BIN1 in this context may shed light on disease progression. To address this, we use a conditional double transgenic mouse model with microglia-specific BIN1 knockout to investigate its hippocampal function.

Aligned with snRNA-Seq data from our group in the cortex, real-time qPCR of LPS-treated hippocampi lacking microglial BIN1 shows upregulation of type I interferon pathway genes. This upregulation occurs alongside a notable decrease in inflammatory chemokines and complement genes, indicating a complex interplay between interferon signaling and inflammatory responses in the hippocampus under conditions of microglial BIN1 deficiency. Morphometric analysis reveals a hyper-ramified microglial phenotype, indicating an intermediate activation state. Additionally, neuroinflammation in BIN1cKO mice increases proliferating microglia, without overall microglial population expansion. Interestingly, BIN1 loss under homeostasis led to an increase in DCX+ neuroblasts in the Subgranular Zone, suggesting that BIN1 plays a role in regulating AHN.

Our findings indicate that the loss of BIN1 in hippocampal microglia under inflammatory conditions drives the expansion of proliferative and IFN-I-responsive, reactive microglial subpopulations. Additionally, microglial BIN1 deficiency independently modulates both phagocytic capacity of microglial cells and neurogenesis, with the molecular mechanisms underlying these effects being still under investigation.

WE01-7

Unveiling TREM2 mediated microglia to neuron crosstalk in neurodevelopment

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Despite the traditional view of the brain as an immunologically privileged organ, a continuous crosstalk between microglia, the resident immune cells of the brain, and neurons is critical shaping neuronal circuits during development. Disruptions in this bidirectional communication can contribute to many developmental disorders, including autism and schizophrenia.

Among the receptors expressed by microglia, the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) has garnered significant attention for its pivotal role in modulating neurodevelopment. TREM2, a phagocytic receptor uniquely expressed by microglia in the brain, and risk factor of late-onset Alzheimer's Disease (AD), has been shown to control the process of synaptic refinement and influences the metabolic properties of developing neurons. Therefore, dysfunctional TREM2 causes imbalances during early neuronal maturation phases. These neurodevelopmental defects have a striking impact on the adult brain, possibly making it a more sensitive target for insults occurring during adulthood and aging. However, the exact mechanisms by which TREM2 signalling impacts early neuronal development and maturation remains unclear.

In this project, I aim to elucidate the mechanisms by which TREM2 modulates neuronal development. To do so, I addressed the different scenarios by which TREM2 could impact neuronal cell development. By using trans-well and/or microglial conditioned media, I first assessed the effect of soluble factors or microglia-derived extracellular vesicles in this process. Next, by using microglia and neuron co-culture, I evaluated the impact of cell-to-cell contact in early neuronal development and identified new potential TREM2 ligands present on the target cells. Ultimately, I validated the role of such interaction on neuronal signalling pathways linked to neurodevelopment.

Altogether, this project aims at shedding light on the processes by which microglia impact brain homeostasis but also at providing new insights potentially leading to the discovery of therapeutic targets in brain diseases where microglia and TREM2 are primarily involved.

WE01-8

EFFECT OF *LRRK2* G2019S MUTATION ON MICROGLIAL FUNCTIONALITY

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The G2019S mutation in the *LRRK2* gene is a significant genetic risk factor for Parkinson's disease (PD). Increasing evidence suggests that the innate immune system plays a crucial role in PD progression, with LRRK2 being highly expressed in microglial cells. However, how this mutation affects microglial function remains largely unknown. This study investigates the impact of LRRK2 on microglial homeostasis and inflammatory responses.

To explore this, we utilized microglial cells derived from pluripotent stem cells carrying the LRRK2-G2019S mutation. Transcriptomic analysis revealed substantial gene expression changes, particularly in pathways related to lipid metabolism and phagolysosomal function. Functional experiments demonstrated impaired degradation of engulfed material, as LRRK2-G2019S microglia exhibited defective processing of myelin debris and beads during phagocytosis.

To further elucidate these alterations, we are currently examining the underlying molecular mechanisms. Our approach involves both primary microglial cultures and in vivo studies using a LRRK2-G2019S knock-in model. In these models, microglia are exposed to pro-inflammatory stimuli, such as lipopolysaccharide (LPS) and interferon-gamma (IFNγ), to assess how the mutation modifies their immune response.

Overall, our findings suggest that lipid metabolism dysregulation caused by the LRRK2-G2019S mutation disrupts microglial function, affecting both their homeostatic and inflammatory roles. These insights contribute to a deeper understanding of how LRRK2 dysfunction leads to microglial dysregulation and its potential role in PD pathogenesis.

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WE01-9

DISRUPTED IL-33 SIGNALING IMPAIRS MICROGLIAL SYNAPTIC PRUNING IN FRAGILE X SYNDROME

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Fragile X Syndrome (FXS) is a neurodevelopmental disorder caused by the loss of Fragile X Mental Retardation Protein (FMRP), an RNA-binding protein that regulates the translation of key synaptic mRNAs. While the role of FMRP in neurons has been extensively studied, its function in non-neuronal cells remains largely unexplored. Our preliminary data reveal a significant reduction in IL-33 levels in the Fmr1 KO mouse model. IL-33, primarily released by astrocytes, plays a crucial role in microglial activation and synaptic remodeling. We observed that IL-33 downregulation leads to decreased PU.1/Spi1 and TREM2 expression, two key regulators of microglial function. This disruption correlates with impaired microglia-mediated synaptic pruning, contributing to the excessive dendritic spine density characteristic of FXS.

These findings suggest that IL-33 is a critical modulator of microglial activity in FXS, and its dysregulation may underlie defective synaptic refinement. By elucidating the molecular interplay between astrocyte-derived IL-33 and microglial pruning mechanisms, this study aims to uncover novel therapeutic targets for restoring synaptic homeostasis in FXS.

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WE01-10

INVESTIGATION OF THE ROLE OF MICROGLIA IN CNS REPAIR VIA MICROGLIAL DELETION

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This study investigates the role of microglia in CNS repair following demyelination using a targeted microglial depletion approach. Previous research suggests that microglia actively support remyelination, but the way is yet to be clarified. To investigate their role, therapeutic depletion of microglia was conducted via the CSF1R inhibitor, PLX3397, in a cuprizone-induced demyelination mouse model of multiple sclerosis. Here we aimed to assess the impact of microglial ablation on remyelination efficacy, astrocyte activity, and CNS recovery in both white matter (WM) and cortical gray matter (GM). To induce demyelination, C57BL/6 mice were fed a cuprizone diet for six weeks, followed by a two-week recovery phase. PLX3397 was administered starting at week 5 (peak of demyelination) to the end of recovery 2 weeks after cuprizone withdrawal (week 6+2), at doses of 290 mg/kg (standard dose) and 500 mg/kg (high dose) in diet, to deplete microglia. Immunohistochemical analysis was performed on brain sections from the corpus callosum and somatosensory cortex to measure the response of microglia (Iba1), myelin (MBP), and astrocytes (GFAP). Image analysis was employed to compare microglia depletion effects on remyelination. Quantitative analyses revealed reduced myelin MBP immunoreactivity and lower astrocyte GFAP density in PLX3397-treated groups compared to controls in the WM and GM. These findings highlight the vital role of microglia in promoting remyelination and maintaining astrocyte activity following injury. Therapeutic microglial depletion with PLX3397 impairs remyelination in both WM and GM, indicating that microglia support is integral to myelin repair mechanisms. This research provides insights into microglia-targeted therapeutic strategies for demyelinating diseases, emphasizing the need for approaches that preserve or enhance microglial function for enhancing remyelination.

Reference: "Boutou A, Roufagalas I, Politopoulou K, et al. Microglia regulate cortical remyelination via TNFR1-dependent phenotypic polarization. Cell Rep. 2024;43(11):114894. doi:10.1016/j.celrep.2024.114894"

WE02-1

EVOLUTIONARY AND FUNCTIONAL INSIGHTS INTO HUMAN GDH2: STRUCTURAL ADAPTATIONS AND METABOLIC REGULATION

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Glutamate dehydrogenase (GDH) plays a key role in cellular metabolism by catalyzing the reversible conversion of glutamate to α-ketoglutarate and ammonia, linking amino acid and carbohydrate metabolism. In humans, two GDH isoenzymes, hGDH1 and hGDH2, are encoded by the *GLUD1* and *GLUD2* genes, respectively. *GLUD2* arose via *GLUD1* retrotransposition in the common ancestor of modern apes. Both isoenzymes are implicated in metabolic, neoplastic, and neurodegenerative disorders. While the three-dimensional structures of hGDH1 and hGDH2 have been experimentally determined, the evolutionary trajectory of GDH2 structural modifications in primates remains unclear.

To address this, we used AlphaFold Colab to predict and compare GDH2 structural conformations across modern apes and extinct primate ancestors. We also assessed the functional impact of amino acid substitutions that emerged during primate evolution. Our analysis identified the common ancestor of modern apes as a pivotal point in GDH2's structural evolution. Notably, two key substitutions—Arg443Ser and Gly456Ala—had significant functional consequences early in evolution, while later modifications fine-tuned enzyme properties, facilitating adaptation to primate-specific metabolic demands.

To explore GDH2 regulation, we performed molecular docking and enzymatic assays to evaluate its interaction with dicarboxylic acid metabolites. Docking studies, validated against bovine GDH, identified high-affinity ligands: Thapsate (-6.3 kcal/mol), Traumatate (-6.3 kcal/mol), Azelex (-5.8 kcal/mol), Sebacate (-5.6 kcal/mol), and Suberate (-5.6 kcal/mol). Enzymatic assays revealed distinct inhibitory effects on hGDH1/2, with IC_{50} values and Hill coefficients indicating differences in affinity and cooperative binding. Notably, thapsic acid exhibited stronger binding to hGDH1, while azelaic, sebacic, and suberic acids preferentially inhibited hGDH2, suggesting these metabolites act as competitive inhibitors of hGDH2.

These findings provide insights into both the structural evolution and potential regulatory mechanisms of hGDH2, shedding light on its role in human metabolism and disease.

WE02-2

CTBP1 controls glucose metabolism to sustain energy homeostasis during neuronal activity

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The brain's high energy demands, driven by synaptic transmission, are met by activity-dependent tuning of metabolic processes in neurons and glia. Neuronal activity stimulates glial glycolysis, providing lactate to fuel synaptic mitochondria. C-terminal binding protein 1 (CTBP1) is a metabolic sensor that responds to neuronal activity-induced fluctuations in NAD+/NADH levels. While CTBP1 regulates gene expression reprogramming in the nucleus, neuronal activity directs its localization to the presynapse, where it plays a key role in synaptic recycling. In cancer, CTBP1 controls glycolysis in response to metabolic demands. Here, we investigated the role of CTBP1 in glia-neuron metabolic communication, where neuronal oxidative phosphorylation (OXPHOS) is tightly coupled to and regulated by glial glycolysis. Seahorse metabolic measurements revealed that *Ctbp1* knockout (KO) led to reduced hippocampal glycolysis and OXPHOS but did not affect evoked field potentials. qRT-PCR analysis showed aberrant expression of glycolytic and mitochondrial genes, though mitochondrial morphology remained unchanged. However, under metabolic stress, hippocampal slices from KO mice exhibited a dramatic reduction in evoked field potentials. Furthermore, replacing glucose with pyruvate preserved synaptic activity in wild-type (WT) hippocampus but failed to sustain evoked field potentials in *Ctbp1* KO slices. Our findings demonstrate that CTBP1 is essential for maintaining glycolysis and OXPHOS in the hippocampus, highlighting its crucial role in energy homeostasis and synaptic function. Moving forward, we aim to dissect the molecular mechanisms underlying CTBP1-mediated metabolic adaptation by performing metabolomic profiling of hippocampal tissue. Our study will provide deeper insights into the metabolic control of synaptic function and may uncover potential therapeutic targets for neurodegenerative disorders.

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WE02-3

LYSOSOMAL ACCUMULATION OF GLUCOSYLCERAMIDE IN THE ONSET OF NEURODEGENERATION: EXPLORING MITOCHONDRIAL AND METABOLIC DYSFUNCTIONS

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β-glucocerebrosidase (GCase) is a lysosomal enzyme responsible for the catabolism of glucosylceramide (GlcCer). Its deficiency causes the lysosomal accumulation of GlcCer, leading to neurodegeneration. To investigate the molecular mechanism linking GCase deficiency and the consequent GlcCer accumulation with neurodegeneration, we developed an in vitro model represented by iPSCs-derived dopaminergic neurons chronically treated with conduritol-B-epoxide (CBE), a specific GCase inhibitor. CBE-treated neurons present neurodegenerative features and are characterized by accumulation of GlcCer within lysosomes and at plasma membrane (PM) level, as a consequence of the aberrant activation of lysosomal exocytosis, altering PM lipid and protein composition. Given lysosomes' role in macromolecule recycling and mitochondrial turnover, we examined the impact of lysosomal dysfunction on metabolic homeostasis. Seahorse XF Real-time ATP Rate and Mito stress tests revealed increased mitochondrial (mit) activity and mit ATP production in CBE-treated neurons, likely to sustain the biosynthetic pathways since the lysosomal recycling activity is blocked. Moreover, by mitoSOX staining, we found that this increased mit activity was followed by an increased superoxide production. LC-MS/MS following [U-^{13}C_6]-glucose, [U-^{13}C_5]-glutamine, and [U-^{13}C_5]-valine administration, showed a reduction in glucose uptake and catabolism, and an increase in TCA cycle intermediates deriving from valine and glutamine in CBE-treated neurons respect to controls. CBE-treated neurons also showed higher amino acid levels, particularly branched-chain amino acids, likely to support energy production or protein synthesis. The obtained data let to speculate that lysosomal impairment mimics a starvation state that increases ATP demand, leading to mit stress and ROS production. Additionally, defects in glucose incorporation, likely linked to altered PM composition due to increased lysosomal exocytosis, force neurons to rely on alternative energy substrates, particularly amino acids, to meet their elevated energy demands.

WE02-4

Deciphering MiR-802 in Down Syndrome: From Insulin Resistance to Oxidative Stress

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Down syndrome (DS), or trisomy 21, is a genetic disorder characterized by a third copy of chromosome 21, leading to a wide range of pathological phenotypes. In the central nervous system, individuals with DS exhibit accelerated aging, increasing their risk of Alzheimer-like dementia. A key aspect of neurodegeneration is the interplay between metabolic dysfunctions, such as insulin resistance (IR), and redox imbalance, both contributing to cognitive decline. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression. Among the triplicated miRNAs on chromosome 21, miR-802 has been linked to IR in obesity and diabetes. Based on the "gene dosage hypothesis" of DS, this study aims to elucidate how miR-802 contributes to aberrant insulin signaling (IS) and its role in oxidative stress (OS) in brain IR pathophysiology.

MiR-802 expression and activation states of key IS components were assessed in (i) autopsy brains from DS, DS-AD, and age-matched controls, (ii) euploid and Ts65Dn mice (a DS model), and (iii) cell cultures transfected with miR-802 mimics to investigate its role in OS. Bioinformatic tools identified miR-802 target genes involved in IS, and oxidative stress markers were analyzed.

IS dysfunction worsening in the progression from DS to DS/AD, with similar findings in Ts65Dn mice, where IS dysregulation persists with aging and neurodegeneration. MiR-802 overexpression negatively regulates some mRNAs in the brain and impairs IS. Moreover, miR-802-induced IR was associated with increased OS, suggesting a mechanistic link. Identifying miR-802 targets in IS and redox homeostasis may inform novel therapeutic strategies to prevent or delay brain IR in DS.

WE02-5

WEAK NEURONAL GLYCOLYSIS SUSTAINS COGNITION AND ORGANISMAL FITNESS

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The energy cost of neuronal activity is mainly sustained by glucose. However, in an apparent paradox, neurons modestly metabolize glucose through glycolysis, a circumstance that can be accounted for by the constant degradation of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (Pfkfb3), a key glycolysis-promoting enzyme. To evaluate the *in vivo* physiological significance of this hypo-glycolytic metabolism, here we genetically engineered mice with their neurons transformed into active glycolytic cells through Pfkfb3 expression. *In vivo* molecular, biochemical, and metabolic flux analyses of these neurons revealed an accumulation of anomalous mitochondria, complex I disassembly, bioenergetic deficiency and mitochondrial redox stress. Notably, glycolysis-mediated NAD^+ reduction impaired sirtuin-dependent autophagy. Furthermore, these mice displayed cognitive decline and a metabolic syndrome that was mimicked by confining Pfkfb3 expression to hypothalamic neurons. Neuron-specific genetic ablation of mitochondrial redox stress or brain NAD^+ restoration corrected these behavioral alterations. Thus, the weak glycolytic nature of neurons is required to sustain higher-order organismal functions.

WE02-6

Loss of BVR-A fosters oxidative distress in the brain of mice fed with high-fat diet

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Background: Biliverdin reductase-A (BVR-A) plays a key role in different intracellular processes, including the regulation of cellular redox homeostasis. Previous studies from our lab have shown a reduction in BVR-A protein levels in neurodegenerative diseases, suggesting that alterations in this protein may represent a key molecular event in the progression of Alzheimer's disease (AD). Oxidative diStress (OS) is a condition that can contribute to synaptic dysfunction and cognitive decline in AD. Therefore, this study aimed to explore whether loss of BVR-A protein fosters OS in mice fed with high-fat diet (HFD), which is known to promote metabolic imbalances and the development of brain insulin resistance, typical conditions of AD and often associated with alterations in redox balance.

Methods: Prefrontal cortex and hippocampal samples were isolated from male and female C57Bl/6J wild-type (WT) and BVR-A knock-out (BVR-A$^{-/-}$) mice, fed a standard diet (SD) or a high-fat diet (HFD, 60% of calories from fat) for 1 or 8 weeks. Slot-blot analysis was used to assess the levels of OS markers, including protein carbonyls, 3-nitrotyrosine, and 4-hydroxy-2-nonenals.

Results: Eight weeks of HFD promoted significant metabolic alterations, but were not sufficient to trigger OS in the prefrontal cortex of either male or female mice. Intriguingly, BVR-A$^{-/-}$ mice exhibited elevated OS markers, suggesting that loss of BVR-A triggers these changes, even after 1 week of SD, and promotes a significant alteration of OS. This finding underscores the key antioxidant role of BVR-A.

Conclusions: Our results suggest that a short-term HFD does not induce an early increase of OS in the prefrontal cortex, and a longer diet period may be necessary to observe any alterations. However, the loss of BVR-A is sufficient to drive a rapid increase in OS, highlighting its critical role in cellular stress response. These findings support the hypothesis that BVR-A is a key protein, and its reduction may represent a crucial molecular event that leads to a series of cellular responses, including the deregulation of redox balance and increased oxidative damage, ultimately contributing to the progression of neurodegeneration.

WE02-7

Targeting nuclear receptor NR5A2 promotes neuronal survival and neuroprotection under oxidative stress

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Neurodegenerative diseases are characterized by the loss of structural and functional properties of groups of neurons and their subsequent death. Oxidative stress is a common underlying factor in many neurodegenerative disorders leading to neuronal dysregulation and death. The development of neuroprotective therapies that promote neuronal survival by reversing the damage of oxidative stress could provide novel therapeutic insights for nervous system-related diseases. Towards this direction, we focused on the orphan nuclear receptor NR5A2, which is known for inducing neurogenesis during development and maintaining neuronal properties in the adult brain. In this study we investigated the potential neuroprotective effect of pharmacological targeting of NR5A2 on neuronal cells that undergo oxidative stress. We demonstrate that the adenoviral overexpression of NR5A2 in *ex vivo* cultured murine cortical neurons promotes their survival under oxidative stress conditions. Knockdown of NR5A2 has the opposite effect on neuronal survival. Most importantly, dilauroyl phospatidylcholine (DLPC), a phospholipidic agonist of NR5A2, recapitulates the effect of the overexpression of NR5A2 in decreasing neuronal apoptosis under oxidative stress. RNA-seq analysis of DLPC treated neurons, unravel a panel of significant genes and pathways that are upregulated and downregulated, and are linked with neuroprotective processes. We validated which of these genes are altered significantly by DLPC and are crucial for neuronal apoptosis and neuroprotection. These findings could reveal the pathway(s) by which NR5A2 inhibits neuronal apoptosis and promotes neuronal survival in oxidative stress conditions. Finally, this work could suggest that NR5A2 agonist DLPC could be used as a neuroprotective treatment in neurodegenerative disorders.

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WE02-8

Astrocyte remodeling in acute hepatic encephalopathy – focus on mitochondria

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Hepatic encephalopathy (HE) following acute liver failure is a primary toxic astrocytopathy, although in-depth characterisation of underlying pathogenesis is far from complete. While glutamine accumulation may cause astrocyte swelling and subsequent clinical complications such as brain edema, details of astrocytes' morphological characterization, including mitochondria status and dynamics corelates, are lacking.

Using transmission electron and confocal fluorescent microscopy, we found complex morphological alterations of cortical astrocytes in mice with azoxymethane-induced acute HE (i.p. at 100 mg/kg of b.w.) and post-mortem cortical tissue of patients at grade IV of HE. In both mice and post-mortem human tissues astrocytic primary branches and soma demonstrated swelling, whereas leaflets exhibited atrophy quantified by the reduced area and decreased volume fraction. Moreover, astrocytic mitochondria showed morphological alterations, with an increased area of ~48% in acute HE mice and ~115% in HE patients, alongside inner membrane damage. In contrast, astrocytes in a mouse model of SN1 glutamine transporter silencing (vivo-morpholino) displayed swelling but intact mitochondrial morphology. To assess mitochondrial dynamics, we analyzed fusion-related mitofusin 2 (Mfn2) and the fission regulator Dynamin-related protein 1 (DRP1). Immunohistochemistry revealed a 79% increase in DRP1 and a slight Mfn2 rise in astrocytes from acute HE mice, unlike in the SN1 silencing model (a model of glutamine accumulation in astrocytes).

While a direct documentation of the role of glutamine accumulation in the development of edema and mitochondrial dynamics changes remains to be analyzed in the future studies, this report is to our knowledge the first to demonstrate 1) astrocyte morphology remodeling in acute HE that encompasses swollen soma and primary branches, and reduced complexity of distal arborisation 2) swollen mitochondria and altered mitochondria dynamics documented with visualization of crucial proteins (DRP, Mfn2) in acute HE, but not in vivo-morpholino model implicating that selective glutamine accumulation, is not sufficient factor to affect mitochondria dynamics in HE pathology.

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WE02-9

Consumption of proline as energy substrate in astrocytes

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The proteinogenic amino acid proline is commonly known for its special structure as a secondary amino acid. Although proline is involved in a variety of cellular processes including autophagy and neurotransmission, very little is known about the proline metabolism in brain cells. To investigate the proline metabolism of brain astrocytes, we have studied the ability of cultured primary rat astrocytes to consume and utilize extracellular proline as an energy substrate. The presence of proline completely prevented the loss in cellular ATP content in glucose-deprived astrocytes during a 24 h incubation. Analysis of the concentration-dependency of this effect revealed that proline in low concentrations was even better suited to maintain a high ATP content in starved astrocytes than glucose or lactate as demonstrated by applied concentrations that were found to maintain a half maximal ATP content (around 320 µM for proline, 550 µM for glucose and 950 µM for lactate). In the absence of glucose and lactate, astrocytes consumed proline proportional to time in a concentration-dependent saturable manner with half-maximal velocity found for 500 µM proline and with a maximal consumption velocity of 130 nmol/(h x mg). Both consumption of proline and ATP maintenance by proline were inhibited in a concentration-dependent manner by tetrahydro-2 furoric acid, an inhibitor of proline dehydrogenase, the first enzyme in mitochondrial proline catabolism. In addition, proline consumption was lowered in a concentration-dependent manner by the presence of other exogenous energy substrates including glucose and lactate. These data demonstrate that astrocytes have the potential to efficiently metabolize proline by mitochondrial metabolism. This proline metabolism may have important functions in astrocytes, especially under conditions of impaired supply of the other energy substrates.

WE02-10

BRAIN GLUCOSE AND LACTATE METABOLISM FOLLOWING LIPOPOLYSACCHARIDE ADMINISTRATION AND CEREBIOME TREATMENT

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Fatigue is a common consequence of both systemic infection and chronic stress, and recent theories posit that disrupted brain metabolism could be a mechanism underlying this effect. Exposure to an immune challenge, like the administration of lipopolysaccharide (LPS) in rodents, is a common method of eliciting an immune response and sickness behaviours. It is unclear, however, if immune activation and sickness behaviours following infection are accompanied by fluctuations in the brain's main metabolic substrate, glucose, and its alternate energy source, lactate, and whether they can be mitigated by probiotic consumption. Male and female CD-1 mice received a bilateral cannulation surgery at 4 weeks or 8 weeks. to allow for the *in vivo* measurement of glucose and lactate via biosensing electrodes in the hippocampus. Following recovery, mice received either a probiotic formula or water for one week starting at either 5 weeks of age (pubertal group) or at 9 weeks of age (adult group). At 6 weeks (pubertal group) or 10 weeks (adult group) of age, all mice received either a saline or LPS injection and *in vivo* glucose and lactate measurements in the dorsal hippocampus began, continuing for 48 hours. Recordings did not show robust age and sex differences in baseline glucose and lactate concentrations, but cerebral lactate levels appear to steadily decrease from puberty to adulthood in both male and female control groups. LPS-treated adult mice showed consistently higher glucose concentrations across time than their saline-treated counterparts, but this effect was completely opposite in the pubertal groups. Additionally, probiotics appear to have a protective effect against LPS-induced increases in lactate concentration across sexes in adulthood. This study demonstrates the ability of probiotics to compensate for the disrupted hippocampal lactate levels that follow an immune challenge. These findings are particularly relevant for our understanding of the long-term impact of infection, such as that seen in Long COVID.

WE02-11

MUTATION IN MITOCHONDRIAL CHAPERONE TRAP1 IN MICE AFFECTS THE NUMBER OF SYNAPTIC MITOCHONDRIA

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Brain consumes about 20% of total body energy and synapses are the regions of the neuron with the highest demand for mitochondrial ATP production. In our research, we focus on examining the role of TRAP1, mitochondrial chaperonin from HSP90 family in the brain. Although TRAP1 was discovered more than 20 years ago a detailed understanding of its function remains elusive.

Recently, we generated knock-in Trap1 p.Q641* mice that displayed ASD-related behavioral abnormalities that were more pronounced in males than in females. Further studies have shown that Trap1 p.Q641* mutation also resulted in the decreased number of presynaptic mitochondria evaluated with 3D reconstruction of images obtained with serial block face scanning electron microscopy. We also found significant differences in mitochondrial respiration: an increase in the activity of respiratory complex I and decrease in the activity of respiratory complex II in Trap1 p.Q641* mice as compared to WT mice.

Thus, the TRAP1 p.Q639* mutation is the first example of a monogenic ASD caused by impaired mitochondrial protein homeostasis.

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WE02-12

TRAP1 MUTANT MICE, A NOVEL MODEL OF ASD, SHOW SEX-SPECIFIC IMPAIRMENT OF SOCIAL BEHAVIOR AND DYSREGULATED MITOCHONDRIAL METABOLISM IN THE BRAIN

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There is increasing evidence of mitochondrial dysfunction in autism spectrum disorders (ASD), but the causal relationships are unclear. In an ASD patient whose identical twin was unaffected, we identified a postzygotic mosaic mutation p.Q639* in the *TRAP1* gene, which encodes a mitochondrial chaperone of the HSP90 family. Additional screening of 176 unrelated ASD probands revealed an identical *TRAP1* variant in a male patient who had inherited it from a healthy mother.

Notably, newly generated knock-in *Trap1* p.Q641* mice display ASD-related behavioral abnormalities that are more pronounced in males. Accordingly, *Trap1* p.Q641* mutation also resulted in sex-specific changes in synaptic plasticity.

The functional mitochondrial phenotyping of synaptoneurosomes isolated from mouse brains (cortex and hippocampus) of male and female *Trap1* mice revealed differences in the use of the tricarboxylic acid cycle substrates in males but not in females. Next, targeted metabolomics was performed to asess levels of amino acids in the brains of mutant and WT mice. Our preliminary data suggests that the levels glutamate and GABA are decreased in the hippocampus of male mutant mice, but not in females. Finally, the levels of NAD/NADH and NADP/NADPH were assessed as a readout for the energetic state and cellular redox state in the hippocampi of WT and mutant mice.

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WE03-1

Effects of minocycline and oxytocin on lipopolysaccharide-altered behaviors in male prairie voles

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The socially monogamous prairie vole (*Microtus ochrogaster*) provides an excellent opportunity to study the impact of social environments on the brain and behavior. Chronic social isolation increases anxiety-like behavior, impairs social affiliation, and elevates neuroinflammation in the brain of adult male prairie voles. We hypothesized that increased neuroinflammation in the brain may play a role in mediating altered behaviors associated with social isolation. In the present study, we injected male prairie voles with saline or lipopolysaccharide (LPS) – a cell wall component of gram-negative bacteria that induces neuroinflammation – and found that LPS treatment increased anxiety-like and impaired social affiliative behaviors, and elevated neuroinflammation in several brain regions associated with these behaviors. We then pretreated male voles with minocycline – an antibiotic with anti-inflammatory properties – and found that it prevented LPS effects on the brain neuroinflammation and the behaviors. Pretreatment of the toll-like receptor 4 antagonist also prevented the development of LPS-induced behavior. To determine whether LPS acts within the brain to exert its pro-inflammatory effects, we next performed intracerebroventricular administration of minocycline prior to peripheral LPS administration. Further, since oxytocin (OXT) in the brain has been implicated in both anxiety-like and social affiliative behaviors and has anti-inflammatory properties, a pretreatment with OXT into the brain was also performed. Collectively, our results demonstrate that LPS-induced changes in anxiety-like and social affiliative behaviors in the male prairie vole can be prevented by the actions of minocycline or OXT in the brain (supported by NIMH R01 125408).

WE03-2

GLUTAMATE-MEDIATED NEUROTOXICITY DRIVES DISEASE PROGRESSION IN SPINOCEREBELLAR ATAXIAS

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Polyglutamine spinocerebellar ataxias (SCA) are a group of 6 rare inherited neurodegenerative disorders characterized mainly by protein aggregation, cerebellar atrophy and severe loss of motor coordination. There is an urgent need to develop innovative therapies. Evidence has emerged indicating that hyperexcitability contributes to the development of these diseases. The activation of N-methyl-D-aspartate receptors (NMDAR) localized outside the synapse has been described as a major driver for neurodegeneration in neurological disorders. Whether NMDAR-mediated neurodegeneration contributes to cerebellar disorders such as SCA2 and SCA3 is unknown. We found a reduction in the levels of glutamate transporters in *postmortem* cerebellar slices from SCA2/SCA3 patients and SCA mouse models. These results suggest that glutamate spillover and consequently activation of extrasynaptic NMDAR might occur. We discovered that the interaction between NMDAR and an ion channel (TRPM4) underlies several types of neurodegenerative disorders. To evaluate the therapeutic potential of blockade of NMDAR/TRPM4 coupling, we expressed recombinant interface inhibitors that compete with endogenous NMDAR/TRPM4 binding in SCA2 and SCA3 mouse models. As a complementary therapeutic strategy, we evaluated the neuroprotective effects of a small molecule that blocks NMDAR/TRPM4 interaction. We analyzed the levels of neuroinflammation markers, insoluble aggregate deposition, and neuronal integrity. Both approaches resulted in a pronounced reduction of neuronal damage, protein aggregation and neuroinflammation in cerebellar and extracerebellar SCA2 and SCA3 mouse models. We analyzed the motor performance of these mice during four months in behavior tasks sensitive to cerebellar dysfunction. Both therapies drastically improved the performance of mouse models. These results reveal that glutamate-mediated degeneration occurs in cerebellar disorders and that NMDAR/TRPM4 interaction interface inhibitors are a viable therapeutic strategy to treat polyglutamine SCA disorders.

WE03-3

The role of miRNAs in the regulation of injury-induced neuronal regeneration

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microRNAs (miRNAs) are short non-coding RNA molecules that regulate gene expression and protein synthesis, playing fundamental roles in cellular processes, including neuronal repair and neuropathic pain following peripheral nerve injury (PNI). We have previously identified several miRNAs deregulated after PNI, which were further differentially involved in processes related to either neuropathic pain or neuronal regeneration.

miRNA knock-out mouse models were employed to explore the impact of specific miRNAs on neuronal repair mechanisms. Dorsal root ganglia (DRG), containing the sensory neuron cell bodies, were analyzed using mRNA sequencing and RT-qPCR to identify potential miRNA target genes. To assess functional effects, human neuron-like cells were transduced with inducible plasmids and viral vectors to overexpress selected miRNAs, followed by *in vitro* neurite outgrowth assays.

Loss of a specific miRNA resulted in diminished neuronal outgrowth *in vitro*, coinciding with a significant upregulation of genes implicated in axonal regeneration, cytoskeletal processes, epigenetic modifications, and ion channel regulation. Partial restoration of neurite outgrowth was observed upon inhibition of these genes. Additionally, inducible expression systems in SH-SY5Y cells successfully increased the miRNA expression to levels similar to those in injured sensory neurons. Future investigation will extend this approach to nociceptors derived from human induced pluripotent stem cells (iNocs) to elucidate miRNA functions in human neuronal regeneration.

These findings contribute to a deeper understanding of miRNA-driven neuroregeneration and may offer novel approaches for therapeutic interventions targeting nerve damage and neurodegenerative conditions.

WE03-4

INTRA-INSULAR CIRCUITRY REGULATES CONDITIONED IMMUNE RESPONSES IN MICE

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Conditioned immune responses (CIRs) are an example of associative learning where a conditioned stimulus (CS), such as saccharin, paired with an immunomodulatory unconditioned stimulus (UCS) like lipopolysaccharide (LPS), elicits both behavioral and immunological adaptations upon CS re-exposure. While the insular cortex (IC) is thought to be essential for taste learning and multisensory integration, its specific contribution to CIRs remains underexplored. We identify the anterior-posterior insular connectivity as a key regulator of CIR retrieval.

We employed a single-trial CIR paradigm in mice, demonstrating that re-exposure to the CS activates reciprocal anterior--posterior projections of the IC (aIC-pIC). Chemogenetic inhibition of aIC-to-pIC projections during retrieval disrupted conditioned taste aversion and attenuated monocyte activation and pro-inflammatory cytokine responses. Conversely, pIC-to-aIC inhibition modulated immune responses without affecting behavior. Electrophysiological analysis revealed a functional asymmetry in intrinsic neuronal excitability between the two pathways and distinct roles in CIR retrieval.

Our findings highlight the intra-insular circuitry as an integrator of sensory and immune information, with aIC-to-pIC projections mediating behavioral adaptations and bidirectional pathways modulating immune responses. These results provide a mechanistic framework for CIRs, offering insights into how the brain can fine-tune immune functions and paving the way for innovative therapeutic strategies targeting neuroimmune communication.

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WE03-5

Comparative analysis of different TBK1-ALS mutations in patient-derived motor neurons

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Heterozygous mutations in the TANK-binding kinase 1 (*TBK1*) gene lead to ALS. TBK1 is a pleiotropic kinase controlling, among others, the autophagic process. TBK1 contains four domains: a serine/threonine kinase domain (KD) located at its N-terminal, a ubiquitin-like domain (ULD) and two coiled-coil domains, CCD1 and CCD2. The *TBK1* loss-of-function variants cause an early truncation of the protein resulting in a decrease of TBK1 kinase activity and/or prevention of substrate-binding, for example autophagy adaptor proteins. This study has two major aims: 1) to characterize the cell-autonomous cellular and molecular effects of different TBK1 mutations in an ALS vulnerable neuronal population, and 2) to identify specific phenotypic markers associated with them. To this end, we re-programmed human iPSCs from primary blood mononuclear cells (PBMCs) from one ALS pre-manifest carrier of a heterozygous TBK1 mutation c.1760+1G>C (splice site mutation) and from two ALS affected members of a family with a TBK1 frameshift heterozygous mutation (c.78_79delAA/p.27Thrfs*2) and differentiated them into motor neurons. Additionally, we included a heterozygous and a homozygous line carrying the *TBK1* missense mutation p.E696K and their isogenic control. First, we asked whether this TBK1 missense mutation leads to defective autophagy. Immunofluorescence analysis in the homozygous motor neurons showed a significant increase in the accumulation of p62 positive autophagosomes, LAMP2 positive lysosomes and an increase in Galectin8 positive puncta, as a marker of damaged lysosomes, suggesting impaired lysosomal degradation. We characterised these puncta based on their size, number, and intensity, providing a possible readout to establish a high throughput screening platform for therapeutic compounds and to develop mutation-specific therapies.

WE03-6

WHEN CHRONIC STRESS MEETS TAU: DISSECTING THE INTERPLAY IN STRESS-GRANULE-RELATED RNA-BINDING PROTEIN DYNAMICS

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Recent advances in the pathogenesis of neurodegenerative diseases highlight the involvement of dysregulated RNA metabolism and RNA-binding protein (RBP) dynamics, with Tau protein—an RBP itself—being a central player in the related pathologies. Accumulating evidence further identifies chronic stress and disrupted glucocorticoid signalling as key accelerators of disease progression. Despite our prior work establishing the role of disease-related Stress Granules (SGs) in exacerbating the chronic stress-driven Tau pathology, it remains unclear how chronic stress alters RBP dynamics and whether these changes are differentially affected by normal versus pathological Tau. To address this question, we used 5–8-months-old P301L-Tau transgenic and Tau-knock-out (KO) mice, as well as their wild-type littermates, subjected to a chronic unpredictable stress protocol (CUS; 6 weeks of randomly assigned daily stressors). We performed transcriptional, molecular, proteomic and histological analyses to evaluate stress-induced changes in RBP behaviour. Our results show that chronic stress induces distinct alterations in SG-related RBPs (e.g., TIA1, TDP-43, G3BP1) marked by nucleocytoplasmic redistribution into both soluble ribonucleoprotein complexes and insoluble SGs. These stress-mediated changes in RBP expression and localization are differentially modulated by Tau's presence or pathological state, revealing both Tau-dependent and Tau-independent responses. Proteomic analysis further supports disruptions in protein-RNA interactions and ribonucleoprotein complex assembly, with distinct changes across different Tau genotypes. Overall, pathological Tau exacerbates these effects, amplifying stress-induced RBP dysregulation and SG induction, suggesting an RBP-driven mechanism underlying the previously described susceptibility to Tau-related hippocampal dysfunction.

WE03-7

INVESTIGATING THE AMPK-MFF PATHWAY TO MODULATE MITOCHONDRIAL DYNAMICS AS A TARGET FOR NEUROPROTECTION

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Cellular hallmarks of neurodegenerative diseases include loss of synapses, neuronal death, inflammation, energy homeostasis defects, oxidative stress, mitochondrial dysfunction, and aberrant proteostasis. AMP-activated kinase (AMPK) maintains energy homeostasis by responding to fluctuating ATP levels, with diverse targets ranging from changing the transcriptional program, promoting glucose uptake, inhibiting protein synthesis, inducing mitochondrial fission, mitophagy and autophagy. Evidence indicates that AMPK is hyperactivated in Alzheimer's disease, leading to mitochondrial fragmentation, mitophagy, and spine loss. In a variety of *in vitro* and *in vivo* neurodegenerative models, promoting mitochondrial elongation has been shown to be neuroprotective. A key protein facilitating mitochondrial division is the outer mitochondrial membrane protein mitochondrial fission factor (MFF), which is phosphorylated by AMPK at Ser155 and Ser172 to promote fission during bioenergetic stress. We have previously reported that MFF is a target of SUMOylation (small ubiquitin-like modifier) at Lys151, which is enhanced upon AMPK phosphorylation of MFF. Using primary hippocampal neurons, we show that the AMPK-MFF-phospho-SUMO pathway is required to maintain mitochondrial size, and that blocking MFF SUMOylation enhances neuronal mitochondrial length. We find that sustained AMPK activation, using the AMP analog AICAR, induces dendritic mitochondrial fragmentation, loss of pre- and post-synaptic proteins, and reduces spine density in primary neurons. Future work will examine the link between mitochondrial dysfunction, fragmentation, and loss of spines, and whether preventing AMPK-induced MFF SUMOylation under stress conditions can be targeted to prevent mitochondrial fragmentation and ultimately maintain mitochondrial function and synaptic integrity.

WE03-8

AZ191 INHIBITOR RESCUES EARLY AXONOPATHY OF THE EMBRYONIC SPINAL MOTOR NEURONS IN SOD1^{G93A} MICE

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Here, we used SOD1^{G93A} mice to investigate the role of Mirk/Dyrk1B kinase in Amyotrophic Lateral Sclerosis (ALS) and to evaluate the therapeutic effect of its specific inhibitor, AZ191. We previously demonstrated that in the embryonic chick spinal cord (SC), Mirk/Dyrk1B regulates the generation and survival of spinal motor neurons (SpMNs) and affects specifically the LMCm motor column which innervates ventrally the muscles of the limbs via Shh/Gli pathway. To perform the preclinical evaluation of AZ191 in ALS, we optimized a primary culture protocol for SpMNs enriched of them about 90%. We revealed that E12.5 SOD1^{G93A} SpMNs exhibit shorter by 1.53-fold and fragmented axons, indicating a severe axonopathy starting from the embryonic life. Pharmacological inhibition of Mirk/Dyrk1B by AZ191, increased both the axonal length of E12.5 WT and SOD1^{G93A} SpMNs by 1.48-fold and 2.11-fold respectively and restored completely the axonal phenotype of SOD1^{G93A} SpMNs. Moreover, AZ191 reduced the apoptosis of SOD1^{G93A} SpMNs by 5.46-fold, as indicated by Bax and Caspase-3 expression, suggesting a neurotrophic and anti-apoptotic effect of AZ191, in agreement with our previous observations in the embryonic chick spinal cord. Notably, AZ191 enhanced the autophagy both in E12.5 WT and SOD1^{G93A} SpMNs by 1.94-fold and 1.13-fold respectively, as indicated by LC3 expression. In addition, AZ191 decreased SOD1 accumulation on SOD1^{G93A} SpMNs by 1.86-fold when compared to non-treated SOD1^{G93A} SpMNs, due to increased autophagy and/or as an additional effect of AZ191 on SOD1^{G93A} protein misfolding and aggregation. Interestingly, Shh/Gli3 pathway was found down-regulated in E12.5 SOD1^{G93A} SC.

WE03-9

DAPAGLIFLOZIN-MEDIATED NEUROPROTECTION IN *IN VITRO* AND *IN VIVO* TOXICITY MODELS

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Dapagliflozin, a sodium-glucose cotransporter-2 (SGLT2) inhibitor widely used in diabetes treatment, has demonstrated neuroprotective effect in several experimental models. The present study aims to elucidate the impact of this factor on neuronal viability, dendritic morphology and cognitive function in *in vitro* and *in vivo* neurotoxicity models. *In vitro*, primary hippocampal neurons were exposed to H_2O_2-induced oxidative stress, resulting in a decrease in dendritic length and spine density. Furthermore, dapagliflozin treatment significantly altered dendritic field complexity and increased spine density in primary hippocampal neurons following the induction of oxidative-stress. *In vivo,* Wistar rats exposed to $AlCl_3$ displayed cognitive deficits, as evidenced by impaired performance in the Morris Water Maze and Y-maze tests. Dapagliflozin co-administration significantly improved performance in both tests, with treated rats displaying reduced latency in locating the hidden platform, increased quadrant crossings, and enhanced novel arm exploration. Immunohistochemical and morphological analyses on brain slices further indicated dapagliflozin-mediated neuroprotection. These findings suggest that dapagliflozin exerts neuroprotective effects by preserving dendritic architecture, reducing oxidative stress-induced apoptosis, and improving cognitive function, highlighting its potential as a therapeutic agent against neurodegenerative conditions.

WE03-10

CLUH maintains functional mitochondria and translation in motoneuronal axons and prevents peripheral neuropathy

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Transporting and translating mRNAs in axons is crucial for neuronal viability. Local synthesis of nuclear-encoded mitochondrial proteins protects long-lived axonal mitochondria from damage; however, the regulatory factors involved are largely unknown. We show that CLUH, which binds mRNAs encoding mitochondrial proteins, prevents peripheral neuropathy and motor deficits in the mouse. CLUH is enriched in the growth cone of developing spinal motoneurons and is required for their growth. The lack of CLUH affects the abundance of target mRNAs and the corresponding mitochondrial proteins more prominently in axons, leading to ATP deficits in the growth cone. CLUH interacts with ribosomal subunits, translation initiation, and ribosome recycling components and preserves axonal translation. Overexpression of the ribosome recycling factor ABCE1 rescues the mRNA and translation defects, as well as the growth cone size, in CLUH-deficient motoneurons. Thus, we demonstrate a role for CLUH in mitochondrial quality control and translational regulation in axons, which is essential for their development and long-term integrity and function.

WE03-11

THE EFFECT OF INDIVIDUAL TUDCA AND PBA AS WELL AS COMBINATIONAL TREATMENT IN SH-SY5Y CELLS

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The AMX0035 is currently evaluated in clinical trials of neurodegenerative diseases: wolfram syndrome (NCT05676034) and progressive supranuclear palsy (NCT06122662). It is a combination of the hydrophilic bile acid tauroursodeoxycholic acid (TUDCA) and sodium phenylbutyrate, a salt of an aromatic fatty acid, 4-phenylbutyrate (4-PBA). This coformulation is designed to reduce neuronal death by targeting endoplasmic reticulum stress and mitochondrial dysfunction, although the precise mode of action remains elusive. The impairment of mitochondrial function and abnormal morphology play a key role in the pathophysiology of neurodegenerative diseases.

Our objective was to investigate the effects of individual substances TUDCA and PBA, as well as combinational treatment (TUDCA + PBA) in human neuroblastoma cells (SH-SY5Y) - often used as an *in vitro* model for study of dopaminergic neuronal degeneration. We focus on functional parameters of mitochondria, such as mitochondrial respiration, ATP synthesis capacity, and mitochondrial morphology. TUDCA induced a significant change in mitochondrial respiration, a decrease in leak respiration that is associated with the positive impact of TUDCA on stabilisation of the mitochondrial membrane. Treatment with PBA resulted in significant increase of spare respiratory capacity, there is a correlation between cell resistance and enhanced cell survival. The impact of all cell treatments on the mitochondrial network indicated the possibility of changing mitochondrial morphology supporting mitochondrial fusion. In summary, PBA and combinational treatment significantly affect mitochondrial functions that could be associated with

their possible cytoprotective effects. TUDCA exhibited a possible cytoprotective effect; however, it involves another mechanism.

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WE03-12

Unraveling the molecular determinants of axonal regeneration: A comparative analysis of CNS and PNS axons

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Axon regeneration in the mature central nervous system (CNS) is highly restricted, leading to irreversible neuronal damage and permanent neurological deficits following traumatic injury. In contrast, mature peripheral nervous system (PNS) axons, as well as developing CNS axons, exhibit a remarkable ability to regrow and reconnect with their targets, often restoring function after nerve damage. Although the disparity in the regenerative capacity between these axonal populations has been primarily attributed to extrinsic factors for many years, recent studies highlight the crucial role of intrinsic RNA-related processes, such as axonal mRNA translation, in neuronal survival and regeneration. Indeed, elevated levels of local protein synthesis observed in mature PNS and developing CNS axons correlate with their enhanced regenerative potential. However, the precise intrinsic mechanisms and regulatory factors underlying the limited regeneration of mature CNS axons remain unclear.

This project aims to elucidate the molecular similarities and differences between developing CNS and adult CNS and PNS axons, which could explain their differential ability to respond to injury acutely, initiating the regenerative process. By employing high-throughput assays, we systematically compared their axonal transcriptomes and proteomes, identifying early responses to axotomy that occur independently of extrinsic influences or the distal somata. A key focus of our study is RNA-binding proteins and their potentially distinct roles across these axonal populations, and we have uncovered a battery of differentially expressed molecules, involved in multiple aspects of RNA metabolism in axons. Investigating these differences may provide insights into why adult CNS axons have a diminished capacity for local protein synthesis and regeneration.

Overall, our findings provide important insights into adult axon biology, and could help identify specific molecular factors that contribute to regeneration, paving the way for novel therapeutic strategies.

WE03-13

THE ROLE OF PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE IN DIABETIC KERATOPATHY

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Diabetic keratopathy (DK), a serious corneal complication of diabetes, is characterized by an overactive inflammatory condition resulting in slow wound healing, alteration of the corneal epithelial barrier and recurring ulcers. Pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide with widespread distribution throughout the body, and playing cytoprotective effects in the neural and non-neuronal parts of the eye, including the cornea. This study explores whether changes in the endogenous PACAP expression can concur for delayed epithelial wound healing in diabetic cornea and whether its administration can counteract hyperglycemia-induced inflammation. The expression of PACAP and its preferred receptor PAC1R was investigated in the cornea of normal and diabetic rats. The effect of the peptide against hyperglycemia-induced inflammation was analyzed in rabbit corneal epithelial cells (SIRC) grown in high glucose conditions. Moreover, to better resemble the natural conditions of corneal epithelium in vivo, an air-liquid interface (ALI) culture of the SIRC cell line was performed. Our results showed that in diabetic cornea the expression of PACAP and PAC1R is significantly decreased. The treatment with the peptide significantly reduced the levels of proinflammatory cytokines IL-1β and TNF-α. Moreover, PACAP improved epithelial morphology and corneal barrier thickness, suggesting its therapeutic potential in the treatment of DK.

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WE03-14

ABSENCE OF THE PERIPHERAL IMMUNE SYSTEM HIGHLY AFFECTS HIPPOCAMPAL NEUROGENESIS AT EARLY POSTNATAL STAGES

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Although the brain, unlike other organs, is separated from the peripheral immune machinery, it is considered to be an organ that actively intercommunicates with the peripheral immune system in order to obtain homeostatic support for its function. Immune cells of both innate and adaptive immune system can modulate the characteristics of neural stem cells (NSCs) and their progeny. The hippocampal dentate gyrus (DG) is a brain region that hosts a population of NSCs that remain across development and continue to generate new neurons throughout life. Although there is emerging evidence that immune system deficiency or malfunction leads to impaired adult hippocampal neurogenesis, the underlying mechanisms remain elusive. Our study aims to unravel whether this impairment is the result of a developmental deficit or an altered neurogenic niche in the adult hippocampus. To this end, we used as a model of study the immunodeficient NOD-SCID mice with defects in both innate and adaptive immunity. More specifically, we conducted a comparative study between NOD-SCID and control C57BL/6J mice at different developmental stages by analyzing the expression of specific markers of the neurogenic lineage combined with morphometrical criteria. Our results showed that NOD-SCID mice at postnatal day 21 (P21), P30, and in adult life (P60) have significantly reduced numbers of $GFAP^+$/radial glia-like NSCs and reduced DCX^+ neuronal precursors in the DG, compared to controls. Notably, when we compared the hippocampal neurogenic activity of NOD-SCID mice with that of $Rag1^{-/-}$ mice, an immunodeficient mouse model with intact innate immunity but complete lack of lymphocytes, we found that NOD-SCID mice have significantly reduced DCX+ neuronal precursors. This suggests that other components of the immune system besides lymphocytes are involved in the impairment of hippocampal neurogenesis. Overall, these findings expand our knowledge of the fundamental role of the immune system in the homeostasis of the nervous system and contribute to our understanding of the complex regulation of neurogenesis.

WE03-15

NEUROPROTECTION AND REPAIR FOLLOWING DAILY ORAL ADMINISTRATION OF 3,5-DIIODOTHYROPROPIONIC ACID (DITPA) IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.

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Multiple sclerosis (MS) is characterized by oligodendrocyte degeneration and demyelination, leading to neurological impairment. Monocarboxylate transporter 8 (MCT8) facilitates thyroid hormone (TH) entry into the CNS, promoting myelin production. However, neuroinflammation may impair MCT8 function in demyelinating lesions. Diiodothyropropionic acid (DITPA), a TH analog, bypasses MCT8 and is in clinical trials for MCT8 deficiency. We evaluated TH analogs', including DITPA, therapeutic potential in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS. Mice received various TH analogs including DITPA, TRIAC, LT3, or control treatment and we found that DITPA-treated mice exhibited reduced neurological symptoms and superior remyelination compared to other TH analogs. Transmission electron microscopy (TEM) and MRI confirmed myelin restoration, oligodendrocyte survival, and white matter preservation. DITPA reduced neurofilament light chain serum levels and neurotoxic Kynurenine pathway activation. Mechanistically, DITPA activated AKT-mTOR-PANK2 signalling, enhancing Coenzyme A metabolism and lipid synthesis, supporting neuroprotection and remyelination. These findings demonstrate that DITPA mitigates neuroinflammatory damage, promoting myelin repair and oligodendrocyte survival, highlighting its potential as a therapeutic candidate for MS.

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WE04-1

THE ROLE OF RGS4 PROTEIN IN K-OPIOID RECEPTOR MEDIATED AUTOPHAGY

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The κ-opioid receptor (κ-OR) is a G protein coupled receptor implicated in various biological responses including neurotransmission, pain perception and stress. κ-OR mediates its effects by coupling apart from Gi/o proteins to a variety of accessory proteins. We have previously shown that the Regulator of G protein Signalling 4 (RGS4) directly interacts with κ-OR and negatively modulates the receptor's signaling. Our previous, *in vitro* and *in vivo* studies also unraveled a novel mechanism via which RGS4 is implicated in neuronal outgrowth and differentiation and that κ-OR activation induces autophagy in neuronal cells under stress conditions. To investigate whether RGS4 exerts any effects in κ-OR-autophagy induction we measured initially the levels of key autophagic markers in Neuro-2A cells overexpressing RGS4. Our data demonstrated that RGS4 expression in Neuro-2A cells, stably expressing the κ -OR, resulted in a profound increase of LC3II levels compared to mock transfected ones upon receptor activation with U50,488H. Moreover, the levels of specific autophagic markers in primary neuronal cultures from wild type (WT) and RGS4$^{-/-}$ mice administered or not with U50,488H were altered. Proteomic analysis of hippocampal samples from WT and RGS4$^{-/-}$ mice treated with U50,488H or saline, revealed alterations in different biological processes such as autophagy, protein transport and other signaling pathways. Collectively, our data suggest a new putative role of RGS4 in autophagy via a mechanism that merits further investigation.

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WE04-2

ATAXIA TELANGIECTASIA MUTATED KINASE IN SYNAPTIC AUTOPHAGY

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Neurons are highly polarized, post-mitotic cells with extensive metabolic activity and rapid protein turnover. These features impose significant stress, highlighting the importance of protein quality control mechanisms at the synapse. Autophagy, the primary pathway for clearing damaged cytoplasmic content, plays a crucial role in maintaining neuronal health, particularly in a compartmentalized manner. However, the regulation of autophagy at the synapse remains poorly understood. Here, we aim to identify novel synapse-specific modulators of autophagy, focusing on ATM kinase, a protein mutated in Ataxia-Telangiectasia (AT), a syndrome characterized by cerebellar neurodegeneration. Although ATM kinase is known for its role in stress responses and autophagy induction in non-neuronal cells, its function in neurons is poorly understood. Using a highly specific inhibitor of ATM kinase, we assessed how core autophagic proteins are affected in primary hippocampal neurons. Our results show that ATM inhibition leads to aberrant autophagic flux, inefficient clearance of cargo, and abnormal trafficking of autophagosomes at synapses. Notably, we also observed that ATG9A, the sole transmembrane protein in the autophagic process, linking the synaptic vesicle cycle to synaptic autophagy, exhibits increased protein levels in presynaptic areas upon ATM inhibition. We hypothesize that ATM kinase may regulate the transport of autophagic vesicles at the synapse. Our findings suggest that ATM plays a critical role in synaptic autophagy regulation, and its dysregulation could inform therapeutic strategies for Ataxia-Telangiectasia and other neurodegenerative diseases linked to autophagy dysfunction.

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WE04-3

HSPA1A MODULATION FOR DEGRADATION OF TDP-25 THROUGH THE PROTEASOME IN MOTOR NEURON-LIKE CELLS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease affecting motor neurons. TAR DNA-binding protein 43 (TDP-43) cytoplasmic inclusions in these cells are a disease hallmark in up to 97% of ALS patients. Physiologically, TDP-43 primarily regulates RNA within the nucleus. However, pathogenic TDP-43 displays aberrant redistribution to the cytoplasm, fragmentation, and aggregation, overwhelming the proteostasis network. Thus, enhancing proteostasis is considered an approach to counter proteotoxic stress and cell death. Indeed, the overexpression of chaperones, such as HSPA1A, has shown promise in reducing protein aggregation. However, further study is required on how HSP70 impacts TDP-43 fragments like TDP-25.

Therefore, this study aims to explore the neuroprotective role of HSPA1A in cellular models of TDP-43 proteinopathy. Specifically, we investigated the effects of HSPA1A on TDP-25, focusing on degradation pathways in motor neuron-like cells.

We cultured NSC-34 and co-transfected them with HSPA1A and GFP-tagged TDP-25 to model TDP-43-related pathology. Using this model, we analysed protein expression and aggregation with the following techniques: western blot (WB), filter trap assay (FTA) and immunocytochemistry (ICC). We observed that HSP70 favours TDP-25 aggregate reduction. Subsequently, we examined degradation pathways with MG-132 and chloroquine. We observed that TDP-25 was degraded primarily via the proteasome, as demonstrated by the WB and FTA. Moreover, we utilised HSPA1A modulator JG-98 to regulate its activity. Interestingly, JG-98-treatment reduced TDP-25 fragment accumulation in the NP-40 insoluble fraction.

In summary, HSPA1A can degrade or prevent the formation of TDP-25 aggregates via the proteasome pathway, and JG-98 modulates its activity. By modulating HSP70 activity, we can apply this finding to *in vivo* disease models of TDP-43 proteinopathy, encouraging further research and potential therapeutic approaches in ALS.

WE05-1

Skipping meals and being anxious: What about mitochondria?

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Modern lifestyle promotes disordered eating patterns, such as skipping meals due to the long working hours or extreme intermittent fasting methods for greater weight loss, increasing eating disorders rates. Although anxiety and eating disorders are highly comorbid, the role of mitochondria in the co-regulation of dietary habits and anxiety remains largely unexplored. Here, we exposed male and female high anxiety-related behavior (HAB) and normal anxiety related behavior (NAB) mice to temporal food restriction, using a limited food access (LFA) protocol, according to which mice had 2h/day *ad libitum* access to food, while the control HAB group had *ad libitum* diet 24h/day. We then investigated the LFA effects on mouse behavior and hypothalamic proteome. Intriguingly, LFA-induced behavioral alterations were observed in HAB but not in NAB mice. HAB LFA female mice showed increased anxiety-like behavior compared to HAB female controls. In the hypothalamus of HAB LFA females, divergent proteomic signatures mostly associated with mitochondria were observed. Taken together, our findings indicate that mitochondrial pathways regulate co-occurring anxiety and eating disorders and may act as potential therapeutic targets for pertinent pathologies.

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WE05-2

PLPPR3 INFLUENCES ANXIETY BEHAVIORS AND INTERACTS WITH LYSOPHOSPHATIDIC ACID

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Phospholipid-phosphatase-related proteins (PLPPRs) are a five-member family of neuron-enriched, developmentally regulated membrane proteins that control axonal and dendritic filopodia formation and synaptic functions in the CNS. In glutamatergic synapses, postsynaptic PLPPR4 controls lysophosphatidic acid (LPA)-dependent glutamate release, and its deletion in Plppr4 KO mice leads to increased excitatory synaptic transmission and thus epileptic seizures. Plppr4 heterozygous mice show increased immobility after acute stress, indicating reduced resilience and vulnerability to stress. Previous studies have shown that presynaptic PLPPR3 may also act as an atypical regulator of LPA, however the exact mechanism remains unclear.
Aim of the present work is to characterize the behavioral aspects of Plppr3 KO mice and to study the molecular interactions of PLPPR3 with bioactive phospholipids, including LPA. We evaluated the effect of Plppr3 loss in animal behavior of male and female mice, through open field, Elevated Plus Maze (EPM) and marble burying behavioral tests. Utilizing Microscale Thermophoresis (MST), we investigated the physical interactions of PLPPR3 with specific lysophospholipids and sphingolipids.
Our results indicate that Plppr3 KO mice show increased exploratory behavior and locomotor activity in OFT and reduced anxiety-like behavior in EPM test compared to WT mice. Furthermore, the reduced anxiety phenotype of Plppr3 KO mice was supported by the marble burying test. Interestingly, our MST binding assays revealed a specific physical interaction between 18:1 LPA and PLPPR3 that is independent of the C2 extracellular loop. Collectively, our results suggest that deletion of Plppr3 leads to anxiolytic behaviors and that PLPPR3 interacts specifically with 18:1 LPA.

WE05-3

MOLECULAR UNDERPINNINGS OF CALORIC RESTRICTION ON A HIGH ANXIETY BACKGROUND: A MULTIOMICS APPROACH

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Anxiety disorders affect a third of the world population with women being twice more susceptible than men. Lifestyle, including dietary habits, has a profound effect on anxiety levels. Caloric restriction (CR) is an overall beneficial intervention that improves lifespan and exerts neuroprotective effects, according to which daily food intake is consistently reduced without causing malnutrition. To date, the molecular underpinnings of CR on a high anxiety background remain elusive. Here, we used the high anxiety-related behavior (HAB) mouse model of trait anxiety. Female HAB mice were subjected to a 30% CR protocol daily for 5 weeks. A battery of behavioral tests revealed that CR reduced anxiety-related behavior in HAB female mice. To disentangle the underlying molecular mechanisms of this anxiolytic effect, we investigated the neurochemical responses to CR in the hypothalamus, prefrontal cortex and hippocampus using quantitative proteomics, metabolomics and western blotting. CR altered glycolysis, antioxidant defense, oxidative phosphorylation, proteasome protein catabolism, fatty acid metabolism as well as the mitochondrial dynamics machinery in a region-specific manner in female HAB mice. Our data collectively demonstrate that reducing food intake in high anxiety has anxiolytic effects which are mediated by brain metabolic adaptations.

WE05-4

AN EVOLUTIONARY VIEW TO UNDERSTANDING AFFECTIVE STATES ACROSS SPECIES (AFFECT-EVO): A COST-ACTION IN PROGRESS

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Understanding the affective states (emotions and moods) of non-human animals is crucial to understand their needs, improve their welfare, and assess the effects of treatments for affective disorders in animals. Existing knowledge regarding affective states across species is limited and fragmented. For example, it is unclear: (1) whether and to what extent different affective states occur in different species; (2) if these states are expressed and experienced in similar ways by different species; (3) which physiological mechanisms of affective states are shared across species; and (4) which indicators of affective states are valid across more than one species.

AFFECT-EVO (https://www.cost.eu/actions/CA23106/) brings together an interdisciplinary network of scholars in philosophy, psychology, humanities, social, computational, and natural sciences, with relevant stakeholders from industry, advocacy organizations, and governments. This network will apply an evolutionary framework to evaluate collaboratively and systematically what we know about affective states in non-human animals.

For the fulfillment of the objectives, four working groups (WGs) have been created: (1) Advancing our fundamental understanding of affective states across species; (2) Societal impacts of understanding animals' affective states; (3) Affective state assessment in the service of improved animal welfare; (4) Improving treatments of affective problems across species.

Working group 1 has already started its tasks and results will be distributed among all other WGs of the action in order to improve our ability to interpret animal emotions. The presentation will describe the cost action, its aims and scopes and demonstrate the progress of the WG 1. It will also list the approaches including how the public and policy makers engage with the concept of affective states in animals and how this interacts with the implementation of new laws and policies that affect animals.

WE05-5

EVALUATING THE THERAPEUTIC EFFICACY OF G PROTEIN-COUPLED ESTROGEN RECEPTOR 1 (GPER1) ACTIVATION: INVESTIGATING SEX-SPECIFIC EFFECTS IN MOOD AND ANXIETY DISORDERS

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Mood and anxiety disorders are more prevalent in women, pose a significant societal burden. Recent evidence suggests that the G protein-coupled estrogen receptor 1 (GPER1) may play a key role in the development of these disorders, potentially acting through mechanisms similar to those of fast-acting treatments. In this study, we used adult Wistar rats of both sexes to examine the effects of the GPER1 agonist (G1), ketamine, and fluoxetine. Behavioral responses were assessed using a battery of behavioral tests. Neurochemical analysis via HPLC-ED was performed to quantify key neurotransmitters, metabolites, and amino acids. Additionally, prefrontal cortex and hippocampus were analysed with Western blot to evaluate protein expression, focusing on rapid signaling pathways and neuronal plasticity. To investigate GPER1's molecular pathways, intrahippocampal infusions of PI3K/Akt and ERK inhibitors were administered before the G1 agonist infusion. A separate cohort was subjected to chronic mild stress model of depression to assess the antidepressant potential of chronic GPER1 activation. Our results showed that G1 and ketamine co-administration induced anxiolytic-like behavior only in males. Systemic and intrahippocampal administration of the G1 agonist elicited anxiolytic/antidepressant effects exclusively in females, dependent on PI3K/Akt signaling pathway. Finally, CMS-induced anhedonia was reversed in both sexes after G1 chronic administration, highlighting its antidepressant potential. These findings support GPER1 as a promising target for developing novel treatments for mood disorders.

WE05-6

PANCREATIC CANCER-ASSOCIATED DEPRESSION (PCAD) IS LINKED TO ADULT NEUROGENESIS IMPAIRMENT

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Pancreatic cancer (PC) is a highly aggressive cancer and often linked to major depression (PCAD) before diagnosis. Our study investigates the mechanism behind this link by examining how PC impacts the brain to induce depression. For this purpose, we conducted behavioral tests on a PC mouse model created by orthotopically injecting human Panc-1 cells into immunocompromised NOD-SCID mice. Our results demonstrated that these mice exhibit a depressive-like phenotype as compared to sham-operated control mice. Brain serotonin levels in the injected mice were significantly altered as shown by HPLC analysis, and this alteration was also evident in a genetic mouse model of PC (Pdx1^{Cre}-AKras^{G12D}) that mimics human PC development by constitutively expressing the Kras^{G12D} mutation specifically in the pancreas. Interestingly, both PC mouse models exhibit impaired adult hippocampal neurogenesis (AN), as demonstrated by the reduced number of DCX^{+} cells found in the dentate gyrus of the hippocampus, compared to controls. Moreover, the pool of hippocampal neural stem cells (NSCs) was also reduced in both mouse models at a later stage of PC, as revealed by the number of GFAP^{+}/radial glia-like NSCs suggesting an impact of PC on AN. To evaluate the involvement of systemic factors on AN impairment during PC progression, we exposed wild-type hippocampal NSCs to serum from the PC mouse models, tthat sera reduced their proliferation and survival. A Luminex-based panel measured 20 key cytokines in serum from PC mice, revealing distinct increases in pro-inflammatory factors across two PC models. IL-6, MCP-1, and G-CSF were elevated in NOD-SCID mice with PC, while TNFa, IL-1b, IL-1a, and G-CSF were higher in Pdx1Cre-AKrasG12D mice. These findings suggest an adult neurogenesis-related mechanism for PCAD, highlighting specific pro-inflammatory factors as key players in this process.

LBA-01

STEAROIL-COA DESATURASE INHIBITION LEADS TO FATTY ACIDS NORMALIZATION AND IMPROVED DENDRITICS SPINES DENSITY IN THE HIPPOCAMPUS OF 5XFAD-AD MOUSE MODEL

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Alterations in brain lipids are a central feature of AD, nevertheless therapeutic strategies targeting brain lipid metabolism are still lacking. Our lab recently reported that a pharmacological inhibitor of the fatty acid enzyme, stearoyl-CoA desaturase (SCD), led to recovery of hippocampal synapses with associated improvements in learning and memory in 3xTg-AD mouse model. Here, we use the 5xFAD model to further delve into lipid metabolism disruptions in AD, and into the effect of the SCD inhibitor (SCDi) on fatty acids (FAs) alterations and synapse loss. Hippocampi from 5xFAD and NC mice were collected at 5 and 8 months of age for FAs profile, analysed by gas chromatography–flame ion detection (GC–FID), and IHC for β-amyloid, GFAP and Iba-1. SCDi was infused in 5xFAD and NC mice 5 MO, through an intracerebral ventricular osmotic pump for 28 days. Hippocampi were processed for GC-FID and Golgi staining for dendritic spines quantification. FAs alterations, appeared in female hippocampus at 5 months (together with plaque pathology and gliosis) and worsened by the age at 8MO, while males began to show alterations at 8MO. The desaturation index of the SCD enzyme associated with the conversion of palmitic to palmitoleic acid showed a significant increase in 5xFAD mice, notably at 8 months of age, but starting at 5MO in females.
Treating 5xFAD females' mice 5MO for 1 month with a SCDi improved dendritic spine density and normalized fatty acid levels. Taken together, data demonstrate that at symptomatic stages the central SCD inhibition has beneficial effects in a second mouse model of AD, 5xFAD characterized by a more aggressive and rapid progression of disease.

These findings identified SCD as a novel promising therapeutic target for AD.

LBA-02

EVALUATION OF THE NEUROCOMPATIBILITY OF NEW BIOMATERIALS WITH DIFFERENTIATED SH-SY5Y CELLS

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Currently, there is a steadily increasing demand for neurocompatible biomaterials due to their applications as stimulating electrodes, neural implants that record signal(s) or allow the connection between the brain and external devices (brain-computer interface). Nevertheless, this field remains underdeveloped. Here, we tested the neurocompatibility of silicon chips decorated with TiO_2, bionate, and parylene C, against SH-SY5Y cells, which are amenable to differentiation into "neuron-like" cells and constitute a well-established model for neurocompatibility studies. Initially, the surface of the materials was characterized with atomic force microscopy (AFM), scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX), contact angle measurement, and Raman microscopy. The resistance to corrosion was examined by incubating the materials in PBS (containing Cl^- at concentration comparable to physiological fluids) for 31 days. Subsequently, SH-SY5Y cells were drop-spotted on the surface of the materials and their adhesion and proliferation were assessed with SEM. Differentiation of SH-SY5Y was induced with 10 μM retinoic acid for 7 days and the process of differentiation was monitored at the microscopic level with SEM (*e.g.*, quantification of neurite extension, neurite body, etc.) and at the molecular level by immunofluorescence microscopy (for MAP2 protein and actin cytoskeleton). In addition, indirect cytotoxicity studies were carried out that showed no toxic effects. The results were directly compared to those obtained using tissue culture polystyrene as standard substrate for the growth and differentiation of SH-SY5Y. The bionate-coated Si chip was neurocompatible, while the TiO_2 coated chip was unstable and the TiO_2 layer stripped off. The parylene C could not support the adhesion of SH-SY5Y cells. Coating of the surface of parylene C decorated Si chip with collagen I allowed the adhesion of SH-SY5Y, thus, it improved neurocompatibility. In conclusion, this study forms the basis for the future development of implanted devices that encounter neural cells. Towards this direction, optimization of the decorated Si chips is currently underway.

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LBA-03

ASSESSMENT OF THE NEUROCOMPATIBILITY OF STAINLESS STEEL 316L WITH DIFFERENTIATED SH-SY5Y CELLS

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Stainless steel (SS) 316l is a well-established biomaterial with applications in cardiovascular surgery, in orthopaedics and in dentistry. Nevertheless, its potential neurocompatibility, a feature that is important for the construction of neuronal electrodes for applications in neuronal stimulation or brain-computer interfaces, has not been explored. Here, the neuroblastoma cell line SH-SY5Y was used to assess the potential of SS 316l to support cell growth and differentiation. First, the surface of SS 316l and the effects of UV-sterilization were investigated with scanning electron microscopy/electron-dispersive X-ray spectroscopy (SEM/EDX). Biocorrosion was tested in the presence of phosphate-buffer saline (PBS) for 31 days and analysis for wear metals with X-ray fluorescence (XRF). Subsequently, SH-SY5Y cells were deposited as drops of suspended cells in culture medium on the SS 316l surface and SH-SY5Y cell adhesion and proliferation were assessed with SEM and immunofluorescence microscopy. Consistent with cellular adhesion was the fact that SS 316l could be readily coated with the extracellular matrix component collagen I, determined with Raman microscopy. Differentiation of SH-SY5Y neuroblastoma cells to "neural-like cells" was induced with 10 μM retinoic acid for 7 days and the process of differentiation was monitored at the microscopic level with SEM (*e.g.*, quantification of neurite extension, neurite body etc.) and, at the molecular level, the expression of key neuronal differentiation markers (MAP2, NEFH, TUBB3) was confirmed and quantified by RT-qPCR. The results were directly compared with those obtained using tissue culture polystyrene as substrate for SH-SY5Y. Lack of cytotoxicity of SS 316l was also confirmed with indirect assays. Taken together, SS 316l appears a neuronal-compatible biomaterial that supports the adhesion, growth and differentiation of SH-SY5Y cells. This study supports the development of implanted devices based on SS 316, like electrodes to stimulate neuronal cells

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