SINS
ITALIAN SOCIETY FOR NEUROSCIENCE

DNSRO
Dipartimento Neuroscienze
Scienze Riproduttive
Odontostomatologiche
UNIVERSITA' DI NAPOLI FEDERICO II

UNIVERSITÀ DEGLI STUDI DI NAPOLI FEDERICO II

1st SINS YOUNG MEETING

Naples 2026

22nd - 23rd JUNE

CONNECTING YOUNG BRAINS TO ADVANCE NEUROSCIENCE

An event to bring together PhD students and postdocs under 35

DOUBLE INTERVIEW:
"What happens after the PhD?"

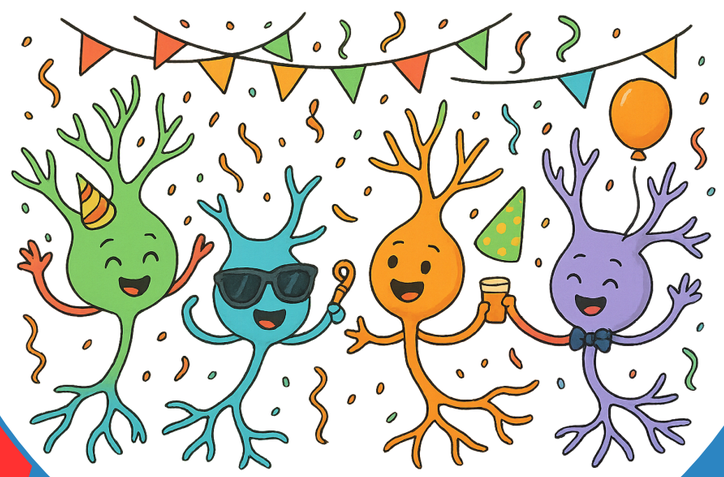
VENUE

CENTRO DI SERVIZIO DI ATENEO PER LE SCIENZE E TECNOLOGIE PER LA VITA (CESTEV)

Università di Napoli Federico II

Via Tommaso De Amicis, 95 – 80131 Naples, Italy

THE YOUNG SINS PARTY:
"Science by Day, NeuroVibes by Night"



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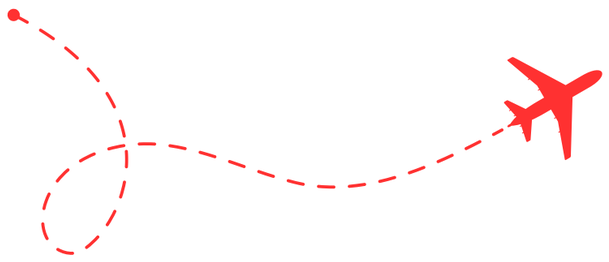
PARALLEL SYMPOSIA & ROUND TABLE:
"Neural ideas, Compact format, Macro insights"

CONGRESS VENUE

*The **1st SINS Young Meeting** will be held at the "**Centro di Servizio di Ateneo per le Scienze E Tecnologie per la Vita (CESTEV)**", Università di Napoli Federico II located in **Via Tommaso de Amicis, 95** - 80131 Naples, Italy*



ARRIVING BY PLANE

Nearest Airport: **Capodichino International Airport** (7 km from venue)

Directions from **Capodichino International Airport**

By Bus:

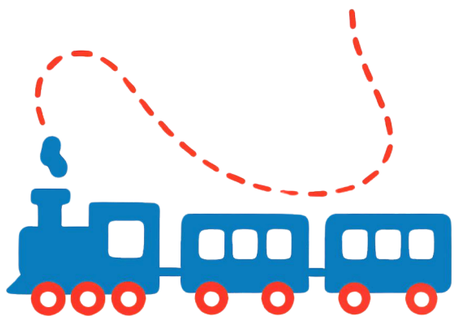
1. Take the Alibus from Arrivals and disembark at Napoli Piazza Garibaldi;
2. From Napoli Piazza Garibaldi, transfer to Line 1 metro to Policlinico Station (Piscinola direction), then walk 8 minutes to the venue.

Ticket Price: €5 (available on the alibus) + €1,50 (metro ticket)

By Taxi

Taxis are available in front of Arrivals.
Estimated Fare: ~ €25

ARRIVING BY TRAIN



Nearest Train Station: **Napoli Centrale** (3 km from venue)

Directions from **Napoli Centrale Station**:

By Metro

From Napoli Centrale, take the Line 1 metro to Policlinico Station, then walk 8 minutes to the venue.

By Taxi

Taxis are available at the station
Estimated Fare: ~ €20

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This event was organized by:

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Federica Antonelli, Istituto Italiano di Tecnologia – Genoa
Luigi Balasco, Università Cattolica del Sacro Cuore – Bolzano
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Detailed Program

DAY 1

08:30 - 09:00 **Arrival & Registration**

09:00 - 09:15 **Opening Remarks** (Main Hall)

09:15 - 10:35 **Session I "Decoding Nervous System Disorders: mechanisms, biomarkers and therapeutic opportunities"** (Main Hall)

Chairs: Dr. Serra Marcello & Dr. Coulomb Elea

09:15 - 09:25 **Dr. Galli Veronica, University of Trento**
Sex-Specific Behavioral Deficits and Regional Signaling Dysregulation in a Novel Pten T167N Knock-In Mouse Model of Autism Spectrum Disorder and Macrocephaly

09:25 - 09:35 **Dr. Abalai Raluca Elena, University of Turin**
Progressive Sensory Dysfunction driven by altered Skin-Nerve Remodeling in Flvcr1 P221S mice

09:35 - 09:45 **Dr. Gambelli Jasmine, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy**
Repetitive Transcranial Magnetic Stimulation Improves Motor and Non-Motor Symptoms and Modulates Inflammatory Markers in Parkinson's Disease

09:45 - 09:55 **Dr. Piemontese Monica, University of Modena and Reggio Emilia**
Optimized isolation and profiling of brain-derived extracellular vesicles in a 5XFAD mouse model of Alzheimer's disease

09:55 - 10:05 **Dr. Fontana Luca, University of Milano-Bicocca**
Differential Priming Approaches Shape the Molecular Cargo and Functional Activity of MSC-Derived Extracellular Vesicles

10:05 - 10:15 **Dr. Gorla Federica, University of Milan**
Modeling Alzheimer's disease co-pathology using patient-derived induced neurons

Detailed Program

10:15 - 10:25 **Dr. Mazzoni Giacomo, University of Genoa**
Nav1.6 is a key effector of PRRT2-linked cerebellar dysfunction

10:25 - 10:35 **Dr. Ciancio Raffaella, University of Naples "Federico II**
Sigma-1R: a key modulator of Ca2+ homeostasis and inter-organelle communication in preclinical models of Amyotrophic Lateral Sclerosis

10:35 - 11:05 **Coffee Break**

11:05 - 11:50 **Poster Session I**

11:50 - 12:00 **Sponsor Platinum Talk: Crisel Instruments** (Main Hall)

Dr. Niko Viggianiello, PhD

When science and technology team-up: a practical overview into microscopy and electrophysiology methodologies for in vitro and in vivo neuroscience experiments

12:00 - 12:10 **Città della Scienza meets SINS**

12:10 - 13:00 **Plenary Lecture** (Main Hall)

Prof. Simone Battaglia, PhD
Associate Professor in Cognitive Neuroscience
Department of Theoretical and Applied Sciences
eCampus University, Novedrate, Italy

New horizons in the neurobiology of human fear learning

13:00 - 14:00 **Lunch Break**

Detailed Program

14:00 - 16:00 **Session II "Molecular mechanisms and therapeutic targets in Disorders of the Nervous System"** (Main Hall)

Chairs: Dr. Chiacchierini Giulia & Dr. Carrillo Federica

14:00 - 14:15 **Dr. Basellini Milo, University of Milan**
Synucleinopathies predictors and where to find them

14:15 - 14:30 **Dr. Zupo Luca, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy**
A Treatable TEFM-Related Mitochondrial Disorder: Transcriptomic Evidence of CoQ10-Induced Metabolic Remodeling

14:30 - 14:45 **Dr. Penna Eduardo, University of Naples Federico II**
The 5-HT7R as a therapeutic target in synaptopathies

14:45 - 15:00 **Dr. Cherchi Laura, University of Milano-Bicocca**
Investigation of the supraspinal pain pathway in a rat model of paclitaxel induced peripheral neuropathy

15:00 - 15:15 **Dr. Servillo Federica, Università Cattolica del Sacro Cuore di Roma**
Modulation of IL-1β signaling rescues behavioral and synaptic deficits in an α-synuclein model of Parkinson's Disease

15:15 - 15:30 **Dr. Del Piano Filomena, University of Naples "Federico II**
Identification of microRNAs enhancing direct cell reprogramming into dopaminergic neurons

15:30 - 15:45 **Dr. Macchioni Giorgia, Sapienza University of Rome**
Early IL-17A blockade following maternal immune activation prevents autism-like brain and behavioral abnormalities in mice

15:45 - 16:00 **Dr. Melini Stefania, University of Naples Federico II**
A multitarget lipid–polyphenol strategy rescues diabesity associated neuropathy by rebalancing adipokines and rewiring neuroinflammation-microRNA networks

16:00 - 16:30 **Coffee Break**

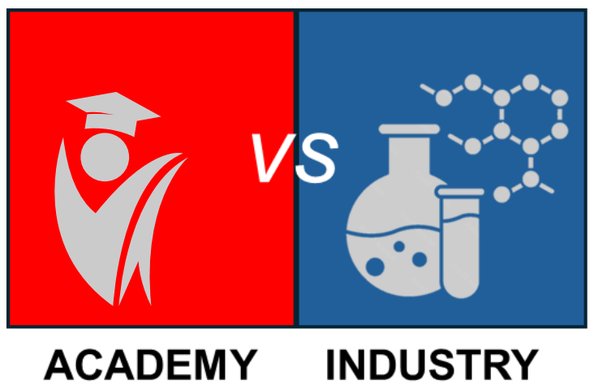
Detailed Program

16:30 - 17:15 **Poster Session II**

17:15 - 17:45 **Double Interview: Academia and Industry** (Main Hall)

Chairs: Dr. Cecilia Steinwurzel & Dr. Giuseppina Natale

"What happens after the PhD?"



17:45 - 19:00 **NeuroConnections: Aperitif & Networking**

21:00 - 01:00 **NeuroVibes in Bloom: SINS Young Social Night**
Where Synapses Meet in the Garden

PA·VE NAPOLI
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Detailed Program

DAY 2

09:10 - 10:30 **Session III “Integrative Neuroscience: From Molecules to Neural Networks"** (Main Hall)

Chairs: Dr. Francesca Mottarlini & Dr. Castellotti Serena

09:10 - 09:20 **Dr. Gazza Stefano, University of Genoa**
Nano-optoporation as a therapeutic strategy for the treatment of glioblastoma

09:20 - 09:30 **Dr. Caldarelli Matteo, Scuola Normale Superiore**
Pupillometry and Brain-wide c-fos mapping reveal multimodal networks underling emotional contagion in mice

09:30 - 09:40 **Dr. Tonelli Elisa, University of Milano-Bicocca**
Chemotherapy-induced ER-mitochondrial alterations in Schwann cells: a possible threat for lipid homeostasis in the peripheral nervous system?

09:40 - 09:50 **Dr. Invernizzi Chiara, University of Milano-Bicocca**
Role of the Sodium–Calcium Exchanger NCX2 in Oxaliplatin-Induced Peripheral Neurotoxicity

09:50 - 10:00 **Dr. Ravasio Mattia, Université catholique de Louvain**
Targeting the Insula with Temporal Interference Stimulation

10:00 - 10:10 **Dr. Porzano Edoardo, Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia**
Enhancing Visual Restoration in rd10 mice via P3HT Nanoparticles and Environmental Enrichment

10:10 - 10:20 **Dr. De Magistris Mattia, Sapienza University of Rome**
Brain – Meninges dialogue tunes mouse behavior

10:20 - 10:30 **Dr. Mazzarella Letizia, Sapienza University of Rome**
Natural Killer cells prime microglial synaptic pruning necessary for physiological memory formation through the release of interferon-gamma

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10:30 - 12:00 **Parallel Symposia I: "Innovative Gene Therapy Strategies for Neurodevelopmental and Neurodegenerative Disorders"** (Hall 1)

Chairs: Dr. Lisastella Morinelli & Dr. Lia Annamaria

10:30 - 10:50 **Dr. Iovino Ludovica, National Research Council (CNR), Pisa**
Challenges and Progress in the Development of Gene Therapy for Creatine Transporter Deficiency

10:50 - 11:10 **Dr. Morosi Francesca, IRCSS San Raffaele Hospital and Neuroscience Institute, CNR – Milan**
-Base editing–mediated Mecp2 gene correction as a novel gene therapy strategy for Rett syndrome

11:10 - 11:30 **Dr. Lia Annamaria, University of Padua**
RNA-based targeting of P2X7R to halt neuroinflammation and neuronal hyperexcitability in Alzheimer's disease

11:30 - 11:50 **Dr. Maffei Benito, University College London**
Closed-loop network suppression for the prevention of tau-associated Dementias

10:30 - 12:00 **Parallel Symposia II: "Neurobiological Mechanisms of Developmental Vulnerability: The Role of Prenatal Stress, Maternal Care, and Substance Exposure"** (Hall 2)

Chairs: Dr. Meanti Ramona & Dr. Borgonetti Vittoria

10:30 - 10:50 **Dr. Curti Lorenzo, University of Florence**
The Neurodevelopmental Impact Of Prenatal Ethanol Exposure: Linking Molecular Mechanisms to Functional and Behavioral Alterations

10:50 - 11:10 **Dr. Viscuso Simona, Sapienza University of Rome**
Maternal care and pup ultrasonic vocalizations as early markers of PTSD susceptibility in rats

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11:10 - 11:30 **Dr. Caria Francesca, University of Cagliari**
Stress-related neuroadaptations following adolescent exposure to the synthetic cannabinoid 5F-MDMB-PICA

11:30 - 11:50 **Dr. Sara Salviati, University of Siena**
Long-term impact of adolescent Alcohol Drinking on Pain Sensitivity and Endocannabinoid Signaling

12:00 - 12:45 **Light Lunch**

12:45 - 13:15 **Digital Poster Session & Round Table** (Main Hall)

Chairs: Luigi Balasco & Dr. Celentano Camilla

Dr. Taddei Anna Aurora, University of Perugia
Empagliflozin as a candidate for therapeutic repositioning in Lafora disease

Dr. De Carluccio Maria, Università Cattolica del Sacro Cuore di Roma
Voluntary intensive exercise: neuroprotective effects on striatal functions of parkinsonian α-syn mice model

Dr. Tedesco Michele, University of Trento
Zebrafish model of IMPG2-related Retinitis Pigmentosa reveals driver mechanisms behind photoreceptor degeneration

Dr. Gallo Maria Teresa, University of Milan
Perineuronal nets in flux: sex-specific long-term consequences of perinatal fluoxetine

Dr. Zanchi Giovanni, University of Milan
Stress granules: an unexplored nidus for α-synuclein aggregation in Parkinson's Disease

Dr. Mariani Fabrizio, Università degli studi di Roma "Tor Vergata", Rome, Italy - IRCCS San Raffaele Roma, Rome, Italy
Immune cell-driven synaptic dysfunction in multiple sclerosis: a chimeric experimental approach

13:15 - 14:00 **Award Ceremony & Concluding Remarks** (Main Hall)

CON IL PATROCINIO SCIENTIFICO DI

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PER LA RICERCA E LE CONOSCENZE
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BEST POSTER AWARD

MDPI

Abstract selected for Oral presentation

Session I: “Decoding Nervous System Disorders: mechanisms, biomarkers and therapeutic opportunities."

Sex-Specific Behavioral Deficits and Regional Signaling Dysregulation in a Novel Pten T167N Knock-In Mouse Model of Autism Spectrum Disorder and Macrocephaly

Dott.ssa Veronica Galli (1), Prof. Luigi Balasco (2)(5), Sig.ra Giulia Cinquetti (1), Sig. Alessio De Leonardis (1), Prof. Alessandro Gozzi (3), Prof.ssa Simona Casarosa (1)(4), Prof.ssa Marta Biagioli (1), Prof. Andrea Lunardi (1), Prof. Yuri Bozzi (5), Prof. Giovanni Provenzano (1)

(1) CIBIO - Cellular, Computational and Integrative Biology, University of Trento, Trento, Italia.
(2) Department of Life Sciences and Public Health, Catholic University of the Sacred Heart, Roma, Italia.
(3) Functional Neuroimaging Laboratory, Center for Neuroscience and Cognitive Systems, Istituto Italiano di Tecnologia, Rovereto, Italia.
(4) CISMed - Centro Interdipartimentale di Scienze Mediche, University of Trento, Trento, Italia.
(5) CIMeC - Center for Mind/Brain Sciences, University of Trento, Rovereto, Italia.

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition often characterized by heterogeneous genetic etiologies and clinical phenotypes, including macrocephaly. A key genetic contributor is the tumor suppressor gene Phosphatase and Tensin Homolog (PTEN), which regulates cell growth by inhibiting the canonical mTOR pathway. However, PTEN has also been implicated in non-canonical signaling pathways, including the phosphorylation of ERK, a key transcription regulator. PTEN mutations account for approximately 2% of all ASD cases and up to 20% of the ASD macrocephaly subgroup, highlighting a strong correlation between PTEN signaling dysregulation and brain overgrowth. We generated a novel knock-in mouse model carrying the de novo T167N mutation, originally identified in a female patient with ASD but no oncogenic history. In our model, the absence of homozygous (Pten T167N/T167N) offspring indicates embryonic lethality; therefore, all analyses were performed on heterozygous (T167N/+) mice. We performed a comprehensive behavioral battery in young adult heterozygous (Het) and wild-type (WT) littermates, alongside histological quantification of phosphorylated ERK (pERK) in key brain regions, including the prefrontal cortex (PFC), hippocampus, hypothalamus, amygdala, and piriform cortex. Macrocephaly was assessed via brain weight and volumetric measurements. Consistent with the clinical phenotype, T167N/+ mice exhibited significant macrocephaly in the absence of tumor formation. Behaviorally, a sex-specific phenotype emerged: Het females displayed increased anxiety-like behavior, while Het males showed reduced sociability and increased self-grooming. Immunohistochemical analyses revealed region-specific alterations in pERK signaling, suggesting a non-canonical involvement of the ERK pathway. Notably, we successfully optimized a pipeline for the isolation of intact, high-quality, and debris-free nuclei from the PFC, which will enable cell-type-specific and high-resolution transcriptomic analyses. Overall, these findings establish the T167N mutation as a potent driver of ASD-relevant endophenotypes and macrocephaly, providing new insights into PTEN-related neurodevelopmental mechanisms.

Progressive Sensory Dysfunction driven by altered Skin-Nerve Remodeling in Flvcr1 P221S mice

Dott.ssa Raluca Elena Abalai (1), Dott.ssa Diletta Isabella Zanin Venturini (1), Prof.ssa Letizia Marvaldi (2), Dott. Francesco Ferrini (3), Dott. Emanuela Tolosano (1), Dott. Deborah Chiabrando (1)

(1) Department of Molecular Biotechnology and Health Sciences, Molecular Biotechnology Center "Guido Tarone", University of Torino, Via Nizza 52, 10126, Torino, Italia.
(2) Department of Neuroscience "Rita Levi Montalcini", Neuroscience Institute Cavalieri Ottolenghi, University of Torino, Via Cherasco, 15, 10126, Torino, Italia.
(3) Department of Veterinary Morphophysiology and Rita Levi-Montalcini Center for Brain Repair, Largo Paolo Braccini, 2, 10095, Grugliasco (to), Italia.

Mutations in *FLVCR1* (Feline Leukemia Virus Subgroup C Cellular Receptor 1), a choline/ethanolamine transporter involved in membrane lipid synthesis and heme homeostasis, are associated with rare sensory and autonomic neuropathies, but underlying mechanisms remain poorly defined. We investigated the P221S variant identified in a patient with progressive loss of pain, thermal, and tactile perception, gait imbalance, and proprioceptive ataxia. To model this condition, we generated a CRISPR-Cas9 knock-in mouse carrying the homozygous *FLVCR1* P221S mutation.
To assess early cellular consequences, we first analyzed murine embryonic fibroblasts (MEFs). We observed reduced ATP (adenosine triphosphate) levels and ALAS1 (aminolevulinate synthase 1) activity, together with increased TBARS (thiobarbituric acid reactive substances) and membrane fluidity, consistent with impaired heme metabolism, oxidative stress, and altered membrane integrity. To further define neuron-specific vulnerability, dorsal root ganglia neurons were analyzed in vitro, revealing reduced soma size and axonal outgrowth, indicative of an intrinsic growth defect independent of the microenvironment. To determine whether these cellular alterations translate into functional sensory deficits, we next performed behavioral analyses. From 7 months of age, mutant mice exhibited increased latency in response to both noxious heat and cold stimuli, indicating impaired thermal sensitivity and dysfunction of peripheral sensory pathways. Ex vivo analysis of skin innervation revealed dynamic changes in intraepidermal nerve fiber density, with a significant increase at 4 months followed by a marked reduction at 15 months, suggesting a biphasic remodeling of peripheral innervation characterized by early dysregulation and subsequent degeneration.
Together, these findings support a model in which metabolic dysfunction and intrinsic neuronal impairment converge with dynamic neuron–skin interactions to drive progressive sensory dysfunction in *FLVCR1* P221S mice. Further experiments will be required to directly test the contribution of this crosstalk.

Repetitive Transcranial Magnetic Stimulation Improves Motor and Non-Motor Symptoms and Modulates Inflammatory Markers in Parkinson's Disease

Dott.ssa Jasmine Gambelli (1)(2), Dott. Alessandro De Biase (1)(2), Dott. Riccardo Di Iorio (2), Dott.ssa Giulia Di Lazzaro (1)(2), Dott.ssa Emanuela Cardile (3), Dott. Vincenzo Rizzo (3), Prof.ssa Annarita Bentivoglio (2)(1), Prof. Paolo Calabresi (2)(1)

(1) Università Cattolica del Sacro Cuore, Roma, Italia.
(2) Fondazione Policlinico Agostino Gemelli, Roma, Italia.
(3) Azienda Ospedaliera Universitaria Policlinico Gaetano Martino, Messina, Italia.

Background: Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra and the presence of protein inclusions known as Lewy bodies. Another hallmark of PD is the upregulation of inflammatory markers, which, through a cascade of signalling events, ultimately contribute to neurotoxicity. Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique used in a broad range of neurological disorders, including PD. rTMS has been shown to modulate the expression of inflammatory cytokines, thereby playing an important role in the regulation of neuroinflammation. Intermittent Theta Burst stimulation (iTBS) is an excitatory protocol of rTMS which has shown benefits in animal models of PD. Here, we present a clinical study involving 120 patients.
Methods: Patients were randomized into two groups: an iTBS treatment group (n = 60) and a sham stimulation group serving as a placebo control (n = 60). Every patient received 10 sessions of bilateral motor cortex iTBS for 5 consecutive days for 2 weeks. All participants were assessed at baseline and after intervention with Movement disorder society Unified Parkinson disease rating scale (MDS-UPDRS), Nonmotor Symptoms Scale (NMSS). In parallel, blood samples were collected to evaluate the peripheral inflammatory profile.
Results: iTBS resulted in a significant improvement in UPDRS-III scores, reflecting an amelioration of motor symptoms, as well as reductions in non-motor symptoms with NMSS. Furthermore, iTBS led to a decrease in neurofilament light chain levels, a structural protein released upon neuronal damage and used as a biomarker of neuroaxonal injury. Additionally, iTBS modulated interleukin-10 expression, further supporting the neuromodulatory role of this approach.
Conclusions: Our findings suggest that iTBS may represent a promising adjuvant treatment for PD.
Acknowledgements: This work was supported by the Italian Ministry of Health under the Piano Nazionale di Ripresa e Resilienza (PNRR), PNRR-MAD-2022-12375804.

Optimized isolation and profiling of brain-derived extracellular vesicles in a 5XFAD mouse model of Alzheimer's disease

Dott. ssa Monica Piemontese (1)(2), Dott.ssa Antonietta Vilella (1)

(1) University of Modena and Reggio Emilia, Via Giuseppe Campi 287, Modena, Italia.
(2) University of Camerino, Via Gentile da Varano III, Camerino, Italia.

Alzheimer's disease (AD) is the leading cause of dementia worldwide, with a steadily increasing prevalence driven by population aging. Pathological alterations are known to begin years before clinical onset, with early synaptic dysfunction representing a critical determinant of cognitive decline. However, the mechanisms underlying the propagation of neuronal damage across brain networks remain incompletely understood. Extracellular vesicles (EVs) have emerged as key mediators of intercellular communication within the central nervous system. These nanosized particles transport bioactive cargoes—including proteins, lipids, and nucleic acids—that can influence recipient cell function. In AD, EVs have been shown to carry pathogenic molecules such as amyloid-β, tau, and pro-inflammatory factors, contributing to the spread of neurodegeneration. Their ability to cross the blood–brain barrier and be detected in peripheral biofluids further highlights their potential as accessible biomarkers and modulators of disease progression.
To identify and validate EV-derived biomarkers of AD at the preclinical level, we optimized a novel protocol for isolating vesicles from mouse brain regions, ensuring sufficient yield for downstream analyses. Four isolation methods were systematically compared, and EVs were quantified by Nanoparticle Tracking Analysis. The expression of membrane-associated EV markers was then assessed using an untargeted proteomic approach. Finally, integrated proteomic and miRNome analyses were performed in the cortex and hippocampus of wild-type and 5XFAD mice, a transgenic AD model, across different stages of disease progression (2, 4, and 8 months). This approach enabled the identification of differentially expressed miRNAs associated with early pathological changes and region-specific molecular alterations.
Overall, this study provides a standardized workflow for region-specific EV isolation and characterization, offering new insights into their molecular cargo and supporting their potential role in early AD pathogenesis and biomarker discovery, with possible implications for future diagnostic and therapeutic strategies.

Differential Priming Approaches Shape the Molecular Cargo and Functional Activity of MSC-Derived Extracellular Vesicles

Dott. Luca Fontana (1), Dott.ssa Eva Costanzi (1), Dott.ssa Giulia Morello (2), Dott.ssa Francesca Giroldo (1), Dott.ssa Federica Oltolini (1), Dott.ssa Lisa Pagani (3), Dott.ssa Elena Lonati (1), Dott.Ssa Clizia Chinello (3), Prof. Fulvio Magni (3), Prof.ssa Alessandra Bulbarelli (1), Prof. Mario Buffelli (4), Prof.ssa Elena Tamagno (2), Dott.ssa Silvia Coco (1)

(1) University of Milano-Bicocca, Via Cadore, 48, Monza, Mb, Italia.
(2) University of Torino, Torino, To, Italia.
(3) National Council of Research, Monza, Mb, Italia.
(4) University of Verona, Verona, Vr, Italia.

Alzheimer's disease (AD) represents the leading neurodegenerative condition globally, marked by chronic and progressive memory loss and cognitive decline. Due to the central neuroinflammatory state typical of the disease pathogenesis, Extracellular Vesicles (EVs) secreted by Mesenchymal Stem Cells (MSCs) have gained interest as potential therapeutic agents, although the molecular mechanisms underlying their anti-inflammatory properties are still to be understood. In this context, several preconditioning approaches have been demonstrated to influence the biological activity of MSC-EVs.
In the present study, MSCs were subjected to two widely used priming protocols: oxygen-glucose deprivation (OGD) and stimulation with pro-inflammatory cytokines (TNF-α and IFN-γ). The main objective was to determine whether different priming conditions could generate functionally distinct EV populations characterized by specific molecular profiles. Proteomic analyses revealed differences in the protein landscape depending on the priming strategy: OGD-primed EVs were enriched in proteins related to catabolic activities, whereas cytokine-stimulated MSCs produced EVs with higher expression of extracellular matrix-associated proteins.
To investigate functional outcomes, primed MSC-EVs were administered intranasally to 5xFAD mice, leading to the attenuation of astrocytic and microglial activation, as evidenced by GFAP and Iba-1 immunostaining. *In vitro*, MSC-EVs were tested in OGD-pretreated SH-SY5Y cells, with preliminary data suggesting their involvement in regulating proteins linked to neuronal stress responses and apoptotic signaling. These investigations are currently being expanded to primary astrocyte and microglial cultures to further characterize the modulatory effects of primed MSC-EVs on inflammatory cell populations.
Overall, these findings suggest that different priming strategies can influence the molecular cargo and functional properties of MSC-EVs, supporting their potential modulatory role over neuroinflammatory and metabolic stress pathways in AD.

Modeling Alzheimer’s disease co-pathology using patient derived induced neurons

Dott.ssa Federica Gorla (1), Dott.ssa Ramona Stringhi (1), Prof. Andrea Pilotto (2)(3), Dott.ssa Caterina Martinuzzo (2)(3), Dott.ssa Chiara Tolassi (2)(3), Prof. Alessandro Padovani (2)(3), Prof.ssa Monica Di Luca (1), Dott.ssa Silvia Pelucchi (1), Prof.ssa Elena Marcello (1)

(1) Department of Pharmacological and Biomolecular Sciences "Rodolfo Paoletti," Università degli Studi di Milano, Via Giuseppe Balzaretti 9, Milano, Italia.
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Dementia diagnosis and treatment are challenged by marked clinical heterogeneity and the frequent co-existence of multiple pathologies. Alzheimer’s disease (AD), defined by amyloid-β and tau aggregation, often overlaps with α-synucleinopathies, such as Dementia with Lewy Bodies (DLB). Notably, up to 30% of AD patients exhibit concomitant α-synuclein pathology, complicating diagnosis and potentially reducing treatment efficacy (Pilotto A, et al., Alzheimer's Dement. 2023). Despite its clinical relevance, the biological mechanisms driving the onset and progression of these co pathologies remain poorly understood, largely due to the lack of experimental models that faithfully recapitulate their complexity. Here, we took advantage of patient-specific induced neuron (iN) model to investigate α-synuclein co-pathology in sporadic AD (Mertens J, et al., Cell Stem Cell. 2015). Fibroblasts obtained from individuals with AD, AD with α-synuclein pathology, DLB (as a model of primary α-synucleinopathies), and age-matched healthy controls obtained in collaboration with University of Brescia, are directly converted into glutamatergic iNs. The iNs system preserves key aspects of donor-specific cellular identity, including aging. Neuronal populations are purified by PSA NCAM-based fluorescence-activated cell sorting (FACS), enabling the analysis of homogeneous and well-characterized neuronal cultures. Here, we present the initial characterization of purified iNs derived from the different groups. This includes imaging-based approaches to assess neuronal purity following sorting, immunostaining for glutamatergic markers, and RNA-based profiling to validate neuronal identity. This platform enables the investigation of molecular and cellular pathways underlying selective neuronal vulnerability associated with aging and neurodegeneration, with a particular focus on mechanisms influenced by α-synuclein co-aggregation. By comparing disease groups, we aim to identify both distinct and shared biological signatures associated with AD, α-synucleinopathies, and their co-occurrence.

Nav1.6 is a key effector of PRRT2-linked cerebellar dysfunction

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Proline-rich transmembrane protein 2 (PRRT2) is a neuron-specific protein that modulates neuronal function by interacting with presynaptic proteins and voltage-gated Na^+ (Nav) channels (Valente et al., 2016, 2018). Loss-of-function mutations in PRRT2 are linked to paroxysmal kinesigenic dyskinesia, and its high expression in cerebellar granule cells (GCs) implicates cerebellar dysfunction in the dyskinetic phenotype observed in patients and PRRT2 knockout (KO) mice (Michetti et al., 2017). Primary GCs from PRRT2 KO mice show increased Nav channel expression and increased cell excitability (Binda et al., 2021). In this context, Nav1.2 and Nav1.6, the main Nav subtypes in GC functional maturation, show distinct patterns of membrane targeting during development. Specifically, Nav1.2 is expressed earlier and localizes to the axon initial segment (AIS) before Nav1.6, which accumulates at later stages (Osorio et al., 2005). Our biochemical analyses revealed a selective upregulation of Nav1.6 in PRRT2 KO GCs, accompanied by AIS elongation and increased channel clustering, indicating structural AIS remodeling and enhanced Nav1.6 functional impact. To assess subtype-specific contributions, we combined RNA interference with whole-cell patch-clamp recordings. Nav1.6 knockdown significantly reduced Na^+ currents, with a stronger effect in KO than in WT neurons, supporting its selective upregulation in PRRT2-deficient cells. Consistently, current-clamp recordings showed reduced action potential amplitude and firing frequency, indicating that Nav1.6 upregulation drives the altered excitability phenotype. In contrast, Nav1.2 silencing produced comparable reductions in Na^+ currents in both KO and WT GCs, indicating that PRRT2 preferentially regulates the less abundant developmental subtype, Nav1.6, while having little effect on Nav1.2, which is already highly expressed and stably enriched at the AIS from early stages of differentiation. Our findings show that PRRT2 maintains proper GC activity by restraining Nav1.6-dependent excitability, whose upregulation drives hyperexcitability in KO cells, highlighting its predominant role during early GC functional differentiation.

Sigma-1R: a key modulator of Ca2+ homeostasis and inter-organelle communication in preclinical models of Amyotrophic Lateral Sclerosis

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Sigma-1 receptor (Sigma-1R) is a molecular chaperone enriched at the mitochondria-associated membranes (MAMs), where it regulates endoplasmic reticulum (ER) Ca^{2+} channels and is involved in the functional crosstalk between intracellular organelles. Pharmacological activation of Sigma-1R by small molecules such as Pridopidine has shown neuroprotective potential and is currently under clinical evaluation for Amyotrophic Lateral Sclerosis (ALS). With the aim to investigate the role of Sigma-1R in organelle crosstalk we used two different preclinical ALS models: NSC-34 motor neuron–like cells exposed to L-BMAA (beta-methylamino-L-alanine), a widely used ALS/PDC model, and $SOD1^{G93A}$ transgenic mice at different stages of disease. Immunofluorescence analysis in control NSC-34 cells revealed that Sigma-1R, expressed on ER, co-localized with lysosomes, indicating a potential role in ER–lysosome communication. Moreover, we detected a significant reduction of Sigma-1R expression in spinal cord, brainstem and cortex of symptomatic $SOD1^{G93A}$ mice, three regions critically affected in ALS, as well as in motor neurons exposed to the neurotoxin. In parallel, the expression of the lysosomal Ca^{2+} channel two-pore channel 2 (TPC2) was also downregulated in the same experimental conditions. These reductions were associated with enhanced ER stress responses and impaired mitochondrial function, suggestive of altered Ca^{2+} homeostasis. Notably, pharmacological activation of Sigma-1R with Pridopidine partially restored cellular homeostasis *in vitro*. Collectively, these findings indicated alterations of Sigma-1R, ER and lysosomal Ca^{2+} signalling as common features of ALS preclinical models, supporting a crucial role for disrupted inter-organelle Ca^{2+} communication in ALS pathogenesis and highlighting Sigma-1R as a promising therapeutic target.

Session II: "Molecular mechanisms and therapeutic targets in Disorders of the Nervous System."

Synucleinopathies predictors and where to find them

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Background: α-Synucleinopathies have been recently classified as multisystem disorders, giving the broad spectrum of peripheral and autonomic symptoms that features these pathologies. Diagnosis of synucleinopathies relies on clinical evaluations supported by neuroimaging, but is often inaccurate, and identifying at-risk groups is crucial for early prevention and treatment. This study examines peripheral α-Synuclein aggregation to understand its role in disease progression and to find non invasive biomarkers for distinguishing synucleinopathies, even in early stages.
Methods: Volar forearm, paraffin embedded skin biopsies were collected from idiopathic (iPD) Parkinson’s disease patients, multiple system atrophy (MSA) patients and idiopathic REM sleep behaviour disease (iRBD) patients, as well as healthy controls. The autonomic synaptic compartment and peripheral nerve fibers were analysed through immunofluorescence and Proximity Ligation Assay for the detection of α-Synuclein aggregates.
Results: Autonomic synapses of both PD and MSA patients are featured by an increased burden of α-Synuclein oligomers, compared to healthy controls. Interestingly, in MSA patients these inclusions could be found within Schwann cells surrounding peripheral nerve fibers, endorsing the hypothesis that α-synuclein oligomers could constitute a reliable diagnostic biomarker. Observations in iRBD patients, integrated with various other clinical and molecular markers, aim to develop a strategy to predict phenoconversion from the preclinical to the manifest stage of the disease.
Conclusions: Despite preliminary, this work reinforces the idea that skin biopsies can be an important tool for the study and the early identification of patients affected by synucleinopathies.
This work was supported by Fondazione Pezzoli per la Malattia di Parkinson (5xmille funding) and FRRB under the frame of ERA PerMed 2022 program.

A Treatable TEFM-Related Mitochondrial Disorder: Transcriptomic Evidence of CoQ10-Induced Metabolic Remodeling

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Background. Primary mitochondrial disorders are heterogeneous neurogenetic diseases caused by impaired energy metabolism. TEFM variants impair mitochondrial transcription and oxidative phosphorylation. We report a novel, treatable TEFM-related phenotype with complete remission after CoQ10 and riboflavin, expanding the clinical and mechanistic spectrum of the disease.
Methods. Transcriptomic and biochemical analyses of patient-derived myoblasts and skeletal muscle biopsies from two siblings with TEFM variants were integrated with long-term clinical follow-up spanning 32 years. Myoblast analyses were performed across four groups: untreated patient cells, CoQ10-treated patient cells, untreated and treated controls.
Results. Transcriptomic profiling, integrating differential expression and pathway enrichment analyses across experimental groups with validated public datasets (Hallmark, Reactome, KEGG, and GO), identified a distinct treatment-associated gene signature. Untreated myoblasts showed a transcriptional profile consistent with TEFM-related alterations, with marked downregulation of pathways involved in muscle contraction, function, and regeneration, notably including class I and II myosin heavy chain (MyHC) genes (MYH2, MYH4, and MYH7). The data on mitochondrial function documented a significant disruption of homeostasis with the activation of compensatory metabolic pathways. Following CoQ10 treatment, myoblasts exhibited restoration of mitochondrial function, oxidative phosphorylation, and muscle regenerative pathways, along with enrichment of fatty acid metabolism and β-oxidation. This profile suggests a shift toward oxidative energy utilization and mitochondrial recovery through activation of the MEF2C–PPARG–PGC-1α axis. Muscle biopsy analysis further confirmed the beneficial transcriptional and protein-level changes, with marked downregulation of pathways linked to mitochondrial myopathy and dysfunction, alongside increased expression of the principal fast- and slow-contracting MyHC isoforms.
Conclusions. Our findings identify a previously unrecognized therapeutic opportunity in TEFM-related mitochondrial dysfunction, showing that metabolic treatment can restore oxidative programs and mitochondrial biogenesis despite the persistent genetic defect. The sustained clinical benefit likely reflects long-term bioenergetic stabilization and preservation of muscle integrity, supporting TEFM associated disease as a potentially treatable mitochondrial myopathy.

The 5-HT7R as a therapeutic target in synaptopathies

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Synaptopathies are defined as disorders caused by functional or structural alterations of synapses, a common pathological mechanism in both neurodegenerative and neurodevelopmental diseases conditions. Disruption of local synaptic translation and altered dendritic spine maturation has emerged as a convergent pathogenic mechanism across different synaptopathies, highlighting the importance of identifying common modulatory targets capable of restoring synaptic homeostasis. The serotonin receptor 7 (5-HT7R) is a Gs-coupled receptor widely expressed in cortical and hippocampal circuits, involved in the regulation of synaptic plasticity. Activation of 5-HT7R has been shown to ameliorate behavioral and synaptic deficits in neurodevelopmental disorders mice model.
Based on this evidence, we investigated the effects of 5-HT7R activation on synaptic alterations of two different mouse models of synaptopathies. Using BTBR mice, a well-established ASD model displaying impaired social interaction and repetitive behaviors, we observed that pharmacological activation of 5-HT7R corrects abnormalities in dendritic spine morphology and synaptic protein synthesis. Similarly, we examined the effects of 5-HT7R activation in an Angelman syndrome mouse model, characterized by loss of UBE3A expression and severe impairments in synaptic plasticity. Activation of 5-HT7R rescues hippocampal long-term potentiation (LTP) and improves cognitive performance. These effects are associated with modulation of local translational machinery and cytoskeletal dynamics, promoting dendritic spine maturation.
Collectively, these findings obtained across different mouse models support the view that alteration of synaptic protein synthesis is a common mechanism of synaptopathies. Moreover, 5-HT7R emerges as a promising molecular target to restore synaptic functions for the treatment of these pathologies of the nervous system.

Investigation of the supraspinal pain pathway in a rat model of paclitaxel induced peripheral neuropathy

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Chemotherapy-induced peripheral neuropathy (CIPN) is a prevalent dose-limiting toxicity of many common anticancer drugs, inducing paresthesia, numbness, dysesthesia and neuropathic pain. Central nervous system comorbidities are also reported. Most of the CIPN studies have focused on peripheral nervous system, but it is reasonable to consider the central pathways of pain transmission as another important target for chemotherapy-induced neurotoxicity. To investigate the role of supraspinal brain areas involved in neuropathic pain development and chronicization, we evaluated central nervous system changes and possible related symptoms in rats exposed to neurotoxic chemotherapy.
Rats were treated with paclitaxel 10 mg/kg i.v. once a week for 4 weeks, followed by 4 weeks of follow-up. Animals were tested for nerve conduction abnormalities, neuropathic pain onset, cognitive skills, and mood alterations at the end of the treatment and follow-up period. A cohort of rats was implanted with multi-electrode arrays (MEA) in brain areas of interest for pain development. Western Blot analysis for a brain plasticity marker was performed. Histological evaluations of the peripheral nerves were executed to validate the neuropathic model.
Rats exposed to paclitaxel displayed painful sensory axonopathy which was confirmed through nerve conduction studies and histological evaluation. Paclitaxel-induced neurotoxicity affected the rats' short term-memory, evaluated with the T-maze test, and induced stress-related comfort eating, evaluated with the sucrose preference test. Electrophysiological analysis of spontaneous and pain-evoked neuronal activity enlightened the consequences of peripheral neuropathy as neuronal activity alterations in supraspinal areas involved in emotional and somatosensory pain processing and modulation, such as prefrontal cortex, thalamus and periaqueductal grey. Changes of expression of the p-CAMKIIα marker were observed.
These results can contribute to shed light on new pathogenetic mechanisms and potential novel therapeutic approaches for painful CIPN.
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Modulation of IL-1β signaling rescues behavioral and synaptic deficits in an α-synuclein model of Parkinson's Disease

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Parkinson's disease (PD) is characterized by degeneration of dopaminergic neurons and accumulation of misfolded alpha-synuclein (α-syn). Recently it has been hypothesized that altered neuroinflammatory responses associated with cognitive dysfunction occur at the very early phases of the disease. However, the mechanisms underlying synaptic alterations associated with these early events have not been explored. In our mouse PD model, unilateral intrastriatal injection of human-α syn pre-formed fibrils (PFF) induced significant cognitive and social deficits in both sexes three months post-injection, at a very early symptomatic phase. Electrophysiological recordings revealed a complete loss of long-term potentiation in hippocampal CA1. Despite no significant increase in microglial cell density within CA1 subregions, PFF-injected mice showed a trend toward higher microglial cell numbers. Notably, confocal 3D reconstruction identified pronounced morpho-functional changes in microglial somata in males, whereas in females only a trend was observed, indicating gender-sensitive morphological activation in the absence of overt microgliosis. This effect was accompanied by increased expression of CD68 and inflammasome-related markers (NLRP3, IL-1β, IL-18), consistent with an enhanced inflammatory response. To investigate IL-1β signaling, which plays a key role in the modulation of synaptic plasticity, mice were treated with Anakinra, an IL-1β receptor antagonist. Treatment significantly improved behavioral performance, restored synaptic plasticity, and reduced neuroinflammatory markers in PFF-injected mice. These findings identify IL-1β mediated neuroinflammation as a key contributor to hippocampal dysfunction in PD and support the concept that targeting this pathway represents a potential strategy to alleviate non-motor symptoms and modify disease progression.

Identification of microRNAs enhancing direct cell reprogramming into dopaminergic neurons

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by progressive dopaminergic neuron (DAN) loss not addressed by current symptomatic treatments [1]. Direct reprogramming of somatic cells into DANs represents a promising strategy for cell replacement therapy. Fibroblasts can be converted into DANs by overexpressing ASCL1, NURR1, and LMX1A (ANL) [2]. However, to be clinically relevant, efficiency must be improved, and additional factors, such as microRNAs (miRNAs), may help [3]. This study aimed to identify miRNAs involved in fibroblast-to-DAN conversion. Mouse embryonic fibroblasts (MEFs) were reprogrammed using the ANL cocktail, and time-course transcriptomic analysis identified nine miRNA clusters with distinct expression profiles. Among miRNAs upregulated during differentiation, miR-218, miR-204, and miR-375 were selected based on their roles in neuronal differentiation. Thus, MEFs were reprogrammed with ANL alone or in combination with each miRNA, and neuronal differentiation was assessed after 16 days.
All tested miRNAs increased β3-tubulin expression when combined with ANL, indicating enhanced neuronal induction. However, miR-218 and miR-204 showed the greatest effect in promoting dopaminergic features. Both miRNAs upregulated tyrosine hydroxylase expression at both gene and protein levels, and increased the gene expression of additional dopaminergic markers, including *Vmat2* and *Ddc*, compared to ANL alone. Notably, both miRNAs also improved neuronal maturation, as shown by increased neurite length and branching complexity. Given their effect on ANL-induced reprogramming efficiency, co-expression of miR-218 and miR-204 with ANL was also tested to evaluate potential synergistic effects; however, no further improvement was observed compared to individual miRNAs.
Overall, our findings identify miRNAs regulated during fibroblast-to-DAN conversion and highlight miR-218 and miR-204 as enhancers of ANL-mediated reprogramming, supporting miRNA modulation as a strategy to improve dopaminergic neuron generation for PD therapies.

[1] Poewe et al., 2017. doi: 10.1038/nrdp.2017.13. [2] Caiazzo et al., 2011. doi: 10.1038/nature10284. [3] Pascale et al., 2022. doi: 10.3390/cells11060940.

Early IL-17A blockade following maternal immune activation prevents autism-like brain and behavioral abnormalities in mice

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Autism spectrum disorder (ASD) is an early-onset neurodevelopmental condition characterized by impairments in social communication and interaction, accompanied by restricted, repetitive behaviors or interests. Increasing evidence implicates immune dysregulation in the pathogenesis of ASD. Notably, preclinical studies indicate that maternal infection, especially early in pregnancy, and the consequent maternal immune activation (MIA), induce ASD-like neurobehavioral abnormalities in offspring, driven by interleukin-17A (IL-17A) released by T helper 17 cells. We assessed the therapeutic potential of IL-17A antibody administration shortly after MIA induction to mitigate ASD-like phenotypes in both male and female mouse offspring. MIA was induced by administering polyinosinic:polycytidylic acid [Poly(I:C)], a double-stranded RNA analog, to pregnant mice on gestational day 12.5, followed twenty-four hours later by treatment with an IL-17A antibody. Offspring of both sexes underwent behavioral testing across three developmental cohorts, from early neonatal stages to late adolescence, using batteries targeting ASD core symptoms and comorbidities. Furthermore, to gain insight into early MIA-induced molecular changes and the potential efficacy of IL-17A antibody treatment in preventing these effects, hippocampal synaptic and neuroinflammatory markers were analyzed. Prenatal administration of IL-17A antibody significantly attenuated early motor abnormalities and reduced repetitive behaviors selectively observed in MIA male offspring, while mitigating social deficits emerging in late adolescence in both sexes. Importantly, IL-17A antibody administration prevented early MIA-induced alterations in key players of synaptic plasticity and neuroinflammation in both sexes. These findings indicate that early and precisely timed IL-17A blockade represents a promising therapeutic strategy to prevent ASD-like brain and behavioral abnormalities associated with MIA.

A multitarget lipid–polyphenol strategy rescues diabesity-associated neuropathy by rebalancing adipokines and rewiring neuroinflammation-microRNA networks

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Background: Diabetic neuropathy (DN), a major complication of diabesity, arises from the interplay between metabolic dysfunction and chronic inflammation, leading to altered pain processing and progressive nerve damage. Adipokine imbalance and microRNA dysregulation are emerging as key modulators of DN pathogenesis. Palmitoylethanolamide (PEA), an endogenous lipid mediator, exerts metallic and neuroprotective effects, while polyphenols modulate oxidative and inflammatory pathways. Here, we investigated whether a multitarget lipid–polyphenol approach can simultaneously modulate these pathways and counteract DN.
Methods: Male C57BL/6J mice were fed a high-fat diet (HFD) for 19 weeks and treated during the last 7 weeks with a formulation containing PEA, rutin, and hydroxytyrosol. Glucose tolerance (OGTT), insulin sensitivity (ITT), and pain-related behaviors (Von Frey, Randall–Selitto) were assessed. Molecular and biochemical analyses were performed by ELISA, Western blot, and RT-qPCR. Intraepidermal nerve fiber (IENF) density and DN-related miRNAs were evaluated.
Results: Treatment markedly improved glucose homeostasis and insulin sensitivity in HFD mice. Strikingly, it reversed mechanical allodynia, restoring pain thresholds in the Von Frey test without affecting general nociception. This functional recovery was paralleled by a robust reduction of neuroinflammation in key pain-processing regions, including thalamus, prefrontal cortex, and spinal cord. Notably, these effects were associated with a rebalancing of adipokine signaling, with normalization of leptin and adiponectin levels both systemically and in central and peripheral tissues. Importantly, treatment preserved peripheral nerve integrity, as demonstrated by increased IENF density (PGP9.5, TUJ1). At the molecular level, it modulated DN-associated miRNAs in the spinal cord, including miR-146a and miR-21, linking metabolic inflammation to neurodegenerative processes.
Conclusions: By concurrently targeting metabolic dysfunction, adipokine imbalance, and neuroinflammation–miRNA crosstalk, this approach effectively counteracts DN features in obese mice. These findings support a multitarget strategy to preserve nerve integrity and restore pain thresholds in diabesity-associated neuropathy, with potential translational relevance.

Session III “Integrative Neuroscience: From Molecules to Neural Networks"

Nano-optoporation as a therapeutic strategy for the treatment of glioblastoma

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults, with a median survival of less than 15 months despite multimodal treatment. Resistance to surgery, radiotherapy, and chemotherapy is driven by its highly infiltrative nature, the presence of tumor stem-like cells, and an immunosuppressive, acidic tumor microenvironment. These features highlight the urgent need for therapeutic strategies achieving high specificity with minimal invasiveness. In this context, our recent work has focused on BV-1, a novel photosensitizing membrane-active compound developed for light-triggered tumor ablation. BV-1 spontaneously partitions into cell membranes and, upon illumination, generates reactive oxygen species that induce membrane optoporation and rapid cell death. In vitro experiments in GBM cells (CSC-204D) demonstrated dose dependent cytotoxicity following light activation. In vivo studies in an orthotopic GBM murine model further showed that the local activation of BV-1 by optical stimulation induced tumor volume reduction and necrosis, as confirmed by MRI and histological analyses. These findings support BV-1 as a promising phototherapeutic strategy for GBM.
To further advance this concept, we are developing pHlux, a genetically encoded system that generates light directly within the tumor microenvironment. By sensing extracellular acidosis, a hallmark of GBM metabolism, pHlux converts local biochemical signals into autonomous bioluminescent emission that activates BV-1 without the need for external light delivery. In addition to the results presented here, this closed-loop strategy could enable self-targeted, spatially restricted phototherapy, overcoming one of the main limitations of conventional optogenetic and photodynamic approaches in brain tumors.

Pupillometry and Brain-wide c-fos mapping reveal multimodal networks underlying emotional contagion in mice

Dott. Matteo Caldarelli (1), Dott. Stefano Zucca (2), Dott.ssa Aurelia Viglione (1), Dott.ssa Alessandra Stella (2), Sig.ra Rida Nisar (1), Dott.ssa Giulia Sagona (3), Sig.ra Ester Maria Papini (1), Dott. Fabio Carrara (4), Prof.ssa Serena Bovetti (2), Dott. Raffaele Mario Mazziotti (3)(5), Dott. Tommaso Pizzorusso (1)(3)

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Emotional contagion (ECo), the automatic sharing of affective states between individuals, is a fundamental component of empathy. In this study, we demonstrate that pupillometry provides a high-sensitivity, objective tool for quantifying ECo in mice. While both pupil size and locomotor activity track direct emotional responses (DER) to aversive tail shocks, pupillary dilation, but not locomotion, specifically tracks vicarious emotional responses (VER) in observer mice. Psychometric modeling of these dynamics revealed that VER thresholds are significantly higher than DER thresholds. Importantly, sensitivity to direct and vicarious distress did not correlate within individuals, suggesting that the underlying mechanisms for these two responses can be dissociated. Our results highlight ECo as a multisensory process where vision plays a critical modulating role. Observer pupillary responses could be triggered by video playback of a mouse in distress, but this effect was significantly attenuated when the video frames were phase-scrambled or vertically inverted. This indicates that the response is not driven merely by physical properties like motion or contrast, but requires the holistic processing of conspecific body configurations. To map the underlying circuits, we performed brain-wide c-Fos mapping using light-sheet fluorescence microscopy, identifying 88 regions significantly recruited during ECo. We found that every brain region activated in the demonstrator was also recruited in the observer, alongside 44 regions activated exclusively in the observer. Network analysis identified hubs such as the prelimbic cortex and agranular insula, while inter-individual analysis revealed significant correlations within dyads for both physiological thresholds and regional neural activity. These findings provide a quantitative, systems-level framework for studying the shared neural states that define emotional contagion in health and disease.

Chemotherapy-induced ER-mitochondrial alterations in Schwann cells: a possible threat for lipid homeostasis in the peripheral nervous system?

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Anticancer treatment with the proteasome inhibitor bortezomib (BTZ) frequently leads to painful peripheral neuropathies, whose pathogenesis is reportedly linked to mitochondrial dysfunction. Mitochondrial homeostasis is highly dependent on the interactions that these organelles establish with the endoplasmic reticulum (ER) at mitochondria-ER contact sites (MERCS), central hubs for the coordination of pivotal physiological processes such as lipid transfer and homeostasis. Here, we propose MERCS as key targets of BTZ-induced toxicity, particularly in Schwann cells, primary drivers of lipid metabolism in the peripheral nervous system (PNS).
Accordingly, we tested the MERCS-related effects of BTZ on the immortalized Schwann cell line MSC80, employing different imaging approaches (confocal, transmission electron microscopy, Nanolive holotomography), lipidomic analysis and western blotting. We found that BTZ induces a drastic reduction in the number of MERCS and an overall increase in ER-mitochondrial distance, accompanied by a significant alteration of the mitochondrial network and distribution. In line with these observations, we demonstrated that BTZ reshapes the lipidomic profile of MSC80, impairing organelle-specific lipid handling and leading to aberrant neutral lipid accumulation and storage into lipid droplets.
Furtherly, we generated a rat model of BTZ-induced peripheral neuropathy and, after a prior characterization via neurophysiological and behavioral tests, we observed that BTZ leads to MERCS alterations and abnormal lipid droplets accumulation in Schwann cells from explanted caudal nerves, as well. This study provides evidence that BTZ impairs MERCS ultrastructure and functionality in Schwann cells both *in vitro* and *in vivo*, specifically by affecting their ability to maintain a proper intracellular lipid homeostasis. Further studies will be needed to shed light on the functional implications of these molecular alterations, particularly considering the pivotal role played by Schwann cells in the architecture and metabolism of the PNS.

Role of the Sodium–Calcium Exchanger NCX2 in Oxaliplatin Induced Peripheral Neurotoxicity

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Oxaliplatin (OHP) is a cornerstone of colorectal cancer therapy, but its clinical use is limited by OHP induced peripheral neurotoxicity (OIPN), which manifests as both acute and chronic syndromes. Acute OIPN is characterized by transient axonal hyperexcitability during each treatment cycle, whereas chronic OIPN features persistent axonal damage that can lead to sensory loss and neuropathic pain, substantially impairing survivors' quality of life. A mechanistic link between acute and chronic OIPN may involve the Na^+/Ca^{2+} exchanger 2 (NCX2). Increased intracellular Na^+ during the acute phase may activate reverse-mode NCX2, promoting Ca^{2+} influx and thereby contributing to later axonal damage. Here, we investigated the role of NCX2 in OIPN using an *in vitro* model.
Primary cultures of mouse dorsal root ganglion neurons were exposed to OHP to induce neurotoxicity. NCX2 expression was quantified by Western blot, and its cellular localization was assessed by immunofluorescence. To test a potential neuroprotective approach, NCX2 was selectively downregulated using siRNA. Neurite length and cell viability were measured as neuroprotection readouts. OHP exposure altered NCX2 protein expression relative to controls. Immunofluorescence suggested a redistribution of NCX2 within sensory neurons and in satellite cells surrounding degenerating neurons. Importantly, siRNA-mediated NCX2 silencing significantly reduced OHP-induced neurotoxicity.
Overall, these data support a key contribution of NCX2 to OIPN pathogenesis and suggest that modulating NCX2 may represent a promising therapeutic strategy. More broadly, since the same pathogenic cascade was described in other peripheral neuropathies, NCX2-related mechanisms may also inform the pathogenesis and treatment of other peripheral neuropathies.

Targeting the Insula with Temporal Interference Stimulation

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Temporal Interference Stimulation (TIS) is an emerging non-invasive brain stimulation method that enables the modulation of neural activity in deep brain structures while minimizing exposure of superficial tissues. Because the insula is critically involved in pain perception, we aim to validate TIS for the modulation of neural activity in this region. The project includes both computational and experimental components.
First, we used Finite Element Modeling (FEM) to simulate electric fields for multiple electrode montages and to compute TIS envelopes. We extracted ROI-specific metrics to assess the exposure, selectivity relative to the overlying cortex, and unintended stimulation of neighboring regions associated with the insula. We repeated this process using structural MRI data from 20 healthy participants, and for each we identified the best TIS configuration using a ranking system. From these individual montages, we derived the optimized universal montage for group-level testing. In a following step, we will conduct a concurrent TIS-fMRI study to test whether the optimized TIS montage induces significant modulation in the BOLD activity and functional connectivity in the insula. This experiment will include both measurements of brain activity during resting-state and while experiencing tonic heat pain to quantify the effects of TIS at rest and during sustained nociceptive processing.
In summary, this project aims to validate the use of TIS to target the human insula. The results will demonstrate the potential of this technique for modulating deep brain regions, establishing the foundation for future TIS studies to probe the involvement of the insula in pain perception.

Enhancing Visual Restoration in rd10 mice via P3HT Nanoparticles and Environmental Enrichment

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While poly(3-hexylthiophene) (P3HT) nanoparticles are a promising strategy to rescue visual responses in retinal degeneration, maximizing post-intervention neural rewiring remains challenging. This study investigates the synergy between a subretinal treatment with P3HT nanoparticles and Environmental Enrichment (EE) to boost neural plasticity in the *rd10* mouse model. Following the subretinal microinjection of P3HT nanoparticles, *rd10* mice underwent a one-month EE protocol designed to promote sensory, motor, and cognitive stimulation. Visual recovery was assessed via behavioral assays, including Optomotor Response, Light-Dark box, fear-conditioning, Visually Evoked Potentials (VEPs). Preliminary observations suggest a positive trend in visually guided behaviors when combining the treatment with P3HT nanoparticles with EE. Mice exposed to EE showed improved performance in OMR scores, suggesting enhanced visuomotor acuity, and stronger responses in fear-conditioning paradigms, consistent with more effective processing of learned visual cues. In parallel, VEP recordings revealed a tendency toward increased cortical responsiveness to light stimulation, with larger and more reliable evoked responses in the combined treatment group. Ongoing immunohistological analyses aim to map the nanomaterials within the retinal layers and correlate their presence with synaptic reorganization. Ultimately, this study demonstrates that coupling a subretinal prosthetic strategy using conjugated polymer nanoparticles with multisensory stimulation can effectively optimize neurorehabilitation in retinal dystrophy caused by photoreceptor degeneration.

Brain – Meninges dialogue tunes mouse behavior

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The mechanisms underlying bidirectional communication between the central nervous system (CNS) and the immune system under physiological conditions remain partially understood. The meningeal compartment hosts both innate and adaptive immune cells, providing continuous immune surveillance of the CNS within a highly dynamic microenvironment. Recent evidence indicates that meningeal Natural Killer (NK) cells, particularly in the dura mater, communicate with the brain and contribute to the modulation of neural functions and behavior in mice. Building on this evidence, we investigate the bidirectional dialogue among the CNS and meningeal immunity. At this aim we took advantage of the environmental enrichment (EE) paradigm, an experimental model that, through prolonged physical, social, and sensory stimulation, promotes coordinated remodeling of neural circuits and immune responses, enabling the investigation of how environmental signals shape CNS–meningeal crosstalk. C57BL/6 mice were housed for 5 weeks in either a standard environment (SE) or an (EE). At first, we showed that the environmental stimuli impact the immune landscape in the meninges reshaping the composition of meningeal immune populations. Then, meningeal NK cells were selectively depleted using a local protocol that achieved near-complete targeting within the meningeal compartment while sparing peripheral NK cells. Four experimental groups were generated: SE control, SE anti-NK1.1, EE control, and EE anti-NK1.1. Following the 5-week exposure, mice underwent a behavioral test battery assessing anxiety-like behavior, short-term memory, and sociability. Notably, the absence of meningeal NK cells modulates the behavioral effects of EE. Together, these findings are expected to clarify how neuronal activity shapes meningeal immunity, and vice versa, under physiological conditions.

Natural Killer cells prime microglial synaptic pruning necessary for physiological memory formation through the release of interferon-gamma

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Proper microglial activity is necessary for synaptic pruning and the establishment of memory. To exert its function, microglia receive a wide plethora of molecular signals that are poorly understood. It is known that microglia are sensitive to interferon-gamma. To evaluate the possible role of the cytokine on microglial cells, we first specifically suppressed the interferon-gamma receptor 1 (IFN γR1) expression in microglia using a dendrimer-conjugated siRNA. This manipulation led to a deregulation of genes involved in synaptic pruning, impaired performance in spatial memory tasks, and increased density of dendritic spines with an increased percentage of immature spines. Since STAT1 is a critical downstream effector of IFN-γ signaling, we confirmed the effects of the silencing, using STAT1 knockout mice that showed behavioral, morphological, and transcriptional alterations similar to those upon microglial IFN-γR1 silencing, proving the functional relevance of this pathway. Similarly, data obtained by mice treated with XMG1.2, an interferon-gamma blocking antibody, confirmed the essential role of IFN-γ in the regulation of microglial activity. Finally, to assess the possible source of IFN- γ in the central nervous system, we depleted NK cell from mice. Microglia from NK-depleted mice expressed greater levels of inflammatory genes, reduced pruning-related gene expression, more ramified morphology, and fewer microglia–neuron contacts, resulting in impaired memory performance. Collectively, these data support the existence of a NK cell-microglia communication axis that is pivotal for the physiological microglial function.

Symposium selected for Parallel Symposia Sessions

Parallel Symposia I: "Innovative Gene Therapy Strategies for Neurodevelopmental and Neurodegenerative Disorders"

Iovino Ludovica (1), Morosi Francesca (2), Lia Annamaria (3), Maffei Benito (4)
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Gene therapy is rapidly transforming the therapeutic landscape for brain diseases, offering the ability to directly target pathogenic mechanisms at molecular, cellular, and circuit levels. This symposium will provide a multidisciplinary and translational overview of cutting-edge gene therapy approaches across diverse brain disorders. Dr. Iovino will present promising advancements in developing a novel gene therapy cassette for Creatine Transporter Deficiency, highlighting safety as a key factor for future clinical translation. Dr. Morosi will discuss a novel adenine base-editing strategy to precisely correct recurrent pathogenic mutations in the MECP2 gene, restoring physiological gene regulation while avoiding pathogenic overexpression. The session will then focus on neurodegenerative disease models. Dr. Lia will present a novel RNA based strategy to silence the P2X7 receptor in the early stages of Alzheimer's disease, illustrating its effects on neuronal hyperactivity and neuroinflammation. The symposium will conclude with Dr. Maffei's contribution on the importance of therapeutic timing, presenting an early, network based intervention in an ageing model of tauopathy for the prevention of tau-associated dementias. By integrating molecular mechanisms, circuit-level outcomes, and translational considerations, this symposium highlights how innovative gene therapy strategies can be effectively developed and applied even to the brain, a historically challenging organ to target. It aims to foster cross-disciplinary discussion, stimulate new ideas, and accelerate the translation of gene-based therapies toward safe, precise, and impactful treatments for disorders of the nervous system.

Parallel Symposia II: "Neurobiological Mechanisms of Developmental Vulnerability: The Role of Prenatal Stress, Maternal Care, and Substance Exposure"

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The developing brain is highly sensitive to environmental perturbations, including stress and substances exposure. Insults occurring during critical neurodevelopmental windows—prenatal life, the early postnatal period, and adolescence—can induce long-lasting alterations in synaptic organization, circuit connectivity, stress reactivity, and behavioral regulation, thereby programming vulnerability to psychiatric disorders in adulthood.
This symposium integrates molecular, circuit-level, behavioral, and neuroimmune perspectives to elucidate how early-life stress and substance exposure converge in shaping adult phenotypes, outlining a developmental continuum linking early perturbations to long-term dysregulation of stress-responsive systems.
Lorenzo Curti will examine how prenatal alcohol exposure disrupts synaptic organization and cortical connectivity using molecular, imaging, electrophysiological, and behavioral approaches, linking early synaptic alterations to circuit-level dysfunction underlying social deficits.
Simona Viscuso will explore how PTSD vulnerability may be influenced by maternal caregiving and early emotional reactivity. In a rat model of susceptibility, alterations in maternal behavior are associated with early changes in offspring vocalizations and stress-related responses, suggesting early markers of intergenerational vulnerability.
Francesca Caria will focus on adolescence, investigating whether self-administration of the synthetic cannabinoid 5F-MDMB-PICA induces persistent alterations in stress reactivity and prefrontal cortical function, leading to aggression, social deficits, and anhedonia in adulthood.

Abstracts selected for Digital Poster Session & Round Table

Empagliflozin as a candidate for therapeutic repositioning in Lafora disease

Dott.ssa Anna Aurora Taddei (1), Dott.ssa Laura Bellingacci (1)(2), Dott.ssa Valentina Imperatore (1), Prof. Alessandro Tozzi (2), Prof.ssa Lucilla Parnetti (3), Dott. Andrea Mancini (3), Prof.ssa Miriam Sciaccaluga (4)(5), Prof.ssa Cinzia Costa (3)(6)

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Background: Lafora disease (LD) is a rare, autosomal recessive and fatal form of progressive myoclonus epilepsy caused by mutations in Epm2a and Epm2b, encoding laforin and malin, key regulators of glycogen metabolism. Their dysfunction leads to abnormal glycogen accumulation (Lafora bodies, LBs) in brain and peripheral tissues, which drive neurodegeneration and clinical manifestations. Currently, no disease-modifying therapies are available. Given the central role of glycogen metabolism, targeting glucose-related pathways may represent a promising therapeutic strategy. Empagliflozin, a selective SGLT2 inhibitor approved for type 2 diabetes, reduces systemic glucose availability and modulates cellular metabolism, potentially addressing glycogen accumulation in LD.
Aim: The present study evaluates the therapeutic potential of empagliflozin in knock-in (KI) Epm2a^{R240X} mouse model of LD, assessing its effects on disease-associated alterations, focusing on neuronal hyperexcitability and network dysfunction.
Methods: Ex vivo electrophysiological recordings of the dentate gyrus were performed in hippocampal slices. Dose-response curve and membrane properties were evaluated to rule out potential toxic effects associated with the applied drug concentration. Epileptic-like activity was induced using a pro-epileptogenic artificial solution, and synaptic plasticity was assessed by eliciting long-term potentiation (LTP) through a high-frequency stimulation protocol (HFS).
Results: Intrinsic membrane properties showed no significant alteration in slices treated with the used drug concentration, chosen from the dose-response curve. The hyperexcitability present in the Epm2a^{R240X} slices was restored to control levels in the presence of empagliflozin. The drug was also able to re-establish normal level of LTP compared to the Epm2a^{R240X} group, which shows a pathological increase of synaptic plasticity.
Conclusions: Our data support the potential repositioning of empagliflozin as a therapeutic candidate for Lafora disease, warranting further investigation alongside future treatment strategies in preclinical and clinical settings.

Voluntary intensive exercise: neuroprotective effects on striatal functions of parkinsonian α-syn mice model

Dott.ssa Maria De Carluccio (1)(2), Dott.ssa Federica Campanelli (2), Dott.ssa Gioia Marino (3), Dott.ssa Giuseppina Natale (2), Dott.ssa Federica Servillo (1)(2), Prof.ssa Maria Teresa Viscomi (3)(4), Prof.ssa Veronica Ghiglieri (3)(2), Dott. Paolo Calabresi (1)(3)

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Parkinson's disease (PD) is a debilitating neurodegenerative disorder affecting approximately 1-2% of the adult population. The progressive loss of dopaminergic neurons in the substantia nigra pars compacta is considered the hallmark feature of PD. Over the past two decades, epidemiological studies have demonstrated that voluntary exercise reduces the risk of developing PD and slows disease progression after diagnosis, emerging as a key non-pharmacological intervention.
The aim of this study was to investigate how early-life exposure to exercise induces stable neurobiological changes that confer long-term neuroprotection in a murine model of α-synuclein driven pathology. Electrophysiological recordings showed that voluntary exercise prevents the impairment of long-term potentiation (LTP) from corticostriatal synapses induced by α-synuclein preformed fibrils (α-syn PFFs) and restores the spontaneous glutamatergic activity of striatal spiny projection neurons (SPNs). Furthermore, we found that exercise-induced LTP depends on dopamine D1 receptors (D1R) and GLUN2A-GLUN2B subunits of NMDA receptor. Interestingly, endocannabinoid CB1 receptor emerged as a novel player in the exercise-induced LTP differing from physiological LTP. Morphological analyses revealed that exercise preserves nigrostriatal dopaminergic terminals, while behavioral tests indicated that it preserves motor performance, improving both strength and balance. Additionally, exercise preferentially modulates astrogliosis in the motor cortex rather than microgliosis in the motor cortex and dorsolateral striatum.
These findings highlight the potential of early exercise as a strategy to induce long-lasting neuroprotective mechanisms in PD.

Zebrafish model of IMPG2-related Retinitis Pigmentosa reveals driver mechanisms behind photoreceptor degeneration

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Inherited retinal dystrophies, such as retinitis pigmentosa (RP), are pathological conditions affecting the retina. They are characterized by progressive photoreceptor degeneration, which ultimately leads to vision loss. One of the genes involved in RP is the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene, which encodes a proteoglycan essential for retinal homeostasis of the interphotoreceptor matrix (IPM). Its dysregulation has been suggested to cause various retinal dystrophies, including RP.
To investigate the physiological and pathological roles of IMPG2, we generated mutant lines for the two zebrafish IMPG2 paralogues, impg2a and impg2b, using CRISPR/Cas9 technology. Photoreceptor degeneration, showing a rod-cone RP-like pattern, is evident in the two single-mutant lines and the double mutant line, beginning in early developmental stages and progressing with age. All mutant lines also show mitochondrial dysfunction, which supports the hypothesis of a metabolic disruption exacerbating – or possibly causing - the neurodegeneration, inflammatory markers upregulation, and microglial activation, suggesting that neuroinflammation may further contribute to retinal damage.
These findings establish zebrafish as a valuable model for investigating IMPG2-related retinal diseases. Understanding the interplay between cellular stress, inflammation, and photoreceptor degeneration in the IPM is crucial for developing therapeutic strategies to preserve or restore vision.

Perineuronal nets in flux: sex-specific long-term consequences of perinatal fluoxetine

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Brain development relies on experience-dependent plasticity within temporally restricted windows known as sensitive periods (SPs). Their timing is regulated by “trigger” and “brake” genes, by the maturation of parvalbumin-expressing (PV+) interneurons and the formation of perineuronal nets (PNNs), extracellular matrix structures that stabilize synaptic circuits. PNN remodeling is largely mediated by matrix metalloproteinase-9 (MMP9). Disruptions in these processes have been linked to psychiatric disorders. We investigated whether perinatal fluoxetine (FLX) alters SP dynamics and PNN degradation in a sex-specific manner, impacting adult behavior.
Male and female rats were exposed to FLX during gestation (prenatal-FLX) or lactation (postnatal FLX). Behavioral assessments conducted during adolescence and adulthood evaluated multiple behavioral domains. Immunohistochemistry was used to quantify PV+ interneurons, PNNs, and MMP9- positive cells, while Western blot and qRT-PCR assessed PNN components, “trigger” and “brake” gene expression, and MMP9 levels in the dorsal hippocampus. Adult prenatal-FLX males exhibited an anhedonic-like phenotype, whereas adult postnatal-FLX females showed cognitive impairments, revealing sex- and timing-specific effects. Both groups displayed reduced PV+/PNN co-labeling in the dorsal dentate gyrus and altered expression of “trigger” and “brake” genes that suggest an anticipation of SP in prenatal-FLX males and a delayed SP opening in postnatal-FLX females. In the dorsal hippocampus of postnatal-FLX females, brevican and hyaluronan synthase 3, two PNN components, were reduced, accompanied by increased MMP9 expression and MMP9-positive cell density, indicating enhanced enzymatic PNN degradation. In contrast, prenatal-FLX males exhibited a distinct profile, with different changes in PNN components and MMP9, highlighting a sex-specific PNN remodeling pattern.
These findings demonstrate that perinatal FLX exposure induces long-term, sex- and timing dependent biobehavioral outcomes. Despite the absence of behavioral changes in adolescence, FLX exposure disrupts SP dynamics. Moreover, postnatal FLX selectively increases MMP9 expression in females, likely promoting enhanced PNN degradation, contributing to the cognitive deficits observed in adulthood.

Stress granules: an unexplored nidus for α synuclein aggregation in Parkinson's disease

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Stress granules (SGs) are membraneless organelles constituted primarily of untranslated messenger ribonucleoproteins, whose assembly occurs via liquid–liquid phase separation (LLPS) in response to various cellular stressors and that play a critical role in maintaining proteostasis. Increasing evidence indicates that chronic stress can disrupt the tightly regulated dynamics of SGs assembly and disassembly, leading to their persistence. These pathological SGs potentially serve as nucleation sites for aberrant protein aggregation in several neurodegenerative disorders (NDDs). Focusing on Parkinson's disease (PD), current knowledge on the dysregulation of SGs assembly and disassembly in relation to α-Synuclein (α-Syn) aggregation is only based on cellular models, with no evidence from human brain tissue. It is well established that, under specific conditions, α-Syn can form condensates, promoting amyloid fibril formation via LLPS, and that α-Syn interacts with several RNA binding proteins (RBPs) involved in SGs formation. Based on this we aim to determine whether: *i)* α-Syn pathology and SGs are associated in PD *post mortem* human brains and *ii)* α-Syn aggregation correlates with SG formation under conditions of acute and chronic stress in a neuronal cell model. Combined immunohistochemical and FISH analysis on *post-mortem* PD brains highlighted the presence of mRNA accumulation colocalizing with both RBPs-positive granules and α-Syn in dopaminergic neurons. Moreover, RBPs-positive granules also accumulated alongside α-Syn oligomers. This suggests that SGs may act as an intermediate for α-Syn aggregation in PD brains. Simultaneously, SH-SY5Y cells exposed to both acute and chronic oxidative stress revealed a local redistribution and accumulation of α-Syn within G3BP1 positive granules. This could reflect an early step in stress-induced protein clustering, potentially contributing to the conditions that favour its aggregation.
Future work will aim to explore the impact of both α-Syn aggregation on the homeostasis of SGs in PD pathology, providing further insight into the molecular mechanisms of the disease.

Immune cell-driven synaptic dysfunction in multiple sclerosis: a chimeric experimental approach

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Multiple sclerosis (MS) is a chronic inflammatory disease affecting the central nervous system (CNS) and characterized by both demyelinating and neurodegenerative features. Clinical and preclinical data have revealed that lymphocyte infiltration, together with microglial and astroglial activation, are key events in the onset and progression of the disease. Recent advances in MS and in its mouse model, experimental autoimmune encephalomyelitis (EAE), have clearly shown that neuroinflammation disrupts synaptic transmission, shifting it toward increased hyperexcitability and ultimately resulting in excitotoxic neurodegeneration. Soluble inflammatory mediators released by infiltrating lymphocytes and by microglia/macrophages are major drivers of inflammatory synaptopathy. Remarkably, inflammatory synaptopathy represents an attractive therapeutic target in MS, as early synaptic alterations are potentially reversible. The development of experimental strategies based on chimeric ex vivo models has revealed that T lymphocytes from patients with MS (heterologous chimeric model) or derived from the spleen of EAE mice (homologous chimeric model) exert a direct detrimental effect on synaptic function when placed in contact with murine brain slices, mimicking the excitotoxic damage described in MS models. Notably, we demonstrated that T cells obtained during an MS relapse alter glutamatergic transmission, an effect also observed in patients with non-relapsing secondary progressive MS (SPMS). Given the central role of B cells in progressive MS, we investigated for the first time whether B cells exert direct synaptotoxic effects and examined whether they modulate T cell–mediated synaptopathy in patients with PPMS. Furthermore, this translational model enables the identification of key molecular mediators of synaptopathy, including pro-inflammatory cytokines such as TNF and IL-1β, and represents a valuable tool to evaluate the neuroprotective effects of therapeutic strategies, including both pharmacological treatments (e.g., sphingosine receptor modulators and anti-CD20 therapies) and non-pharmacological interventions (e.g., physical exercise and dietary modulation). Importantly, it can be applied to other neuroinflammatory disorders, making it a valuable tool to study neuro-immune interactions and explore new therapies.

Abstracts selected for Posters

Poster Session I

POSTER ID: 1.01 - Novel rs199645736G>A mutation in the human SYN3 gene: pathological effects on neuronal homeostasis and autophagy

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Parkinson's Disease (PD) is characterized by nigrostriatal dopaminergic neuron loss and accumulation of fibrillary aggregated α-synuclein (α-syn) in Lewy bodies (LB). Recently, synapsin III (syn III) has emerged as a new key α-syn interactant modulating the fibrillary aggregation of α-syn and it has been also found to participate in α-syn pathology in PD and dementia with LB (DLB) (Zaltieri et al., 2015; Longhena et al., 2018; Faustini et al 2018; Faustini et al., 2022). In addition, network proteomic studies support that Syn III increase serve as discriminant factor between PD with dementia or DLB and Alzheimer's disease (Shantaraman et al., 2024). Our team has recently identified a novel mutation of the human SYN3 gene (rs199645736G>A) in a patient suffering from an early onset parkinsonism. In order to gain insights into the possible pathogenic role of this novel mutation, we expressed rs199645736G>A mutant and wild type (wt) Syn III in SK-N-SH neuroblastoma cells in basal condition or subjected to dopaminergic-like differentiation. We observed that the rs199645736G>A mutation altered the physiological expression of other synapsin isoforms and induced a reduction in total α-syn protein levels. Moreover, cells expressing mutant Syn III showed an altered morphology characterized by a reduction in soma area and number of processes compared to cells expressing wt syn III. Finally, cells expressing mutant syn III displayed an increase in p62 expression and aggregation, as well as a decrease in total LAMP1, supportive of autophagy engulfment and lysosomal depletion. These findings indicate that the rs199645736G>A mutation impairs physiological functions of syn III affecting neuronal homeostasis, maturation and autophagy, suggesting that it may induce Parkinson-like pathology through different mechanisms than wt syn III.

POSTER ID: 1.02 - Molecular And Functional Characterization of CCDC32: a tDARK Gene Associated With A Rare Syndromic Intellectual Disability.

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Coiled-coil domain-containing protein 32 (CCDC32), also known as C15orf57, is a small 185 amino acid protein whose molecular function is still unknown. Homozygous loss-of-function mutations cause a severe congenital cardio-facial-neurodevelopmental syndrome (CFNDS) characterized by intellectual disability and developmental abnormalities that resembles ciliopathy-like syndromes. Genome wide co-essentiality and mass spectrometry analyses in non-neuronal cells have linked CCDC32 to the Adaptor Protein complex 2 (AP2), suggesting a role in clathrin-mediated endocytosis and vesicular trafficking. Thus, CCDC32 may serve as an interface between primary cilium-related pathways and the endocytic processes that influence neuronal morphology and connectivity. Our preliminary data support its neuronal role: quantitative RT-qPCR in rats shows that CCDC32 is strongly enriched in cortex and hippocampus compared to other organs. High-resolution imaging show protein localization at the base of dendritic spines, along axons, within dendrites, and in the soma, thus the specific localization and role of CCDC32 in neurons remains to be elucidated. We are currently developing a CRISPR-Cas9-mediated knockout strategy to investigate how the loss of this protein may affect neuronal organization and function, by combining functional readouts of endocytic pathways with analyses of synapse morphology, number, and nanoarchitecture. In parallel, we will define the molecular interactome of CCDC32 through IP-MS, to identify partners and pathways that may link CCDC32 to endocytic control. In addition to advancing our understanding of a rare genetic disorder, this project may have the potential to reveal fundamental neurobiological mechanisms with broader implications for brain health and disease.

POSTER ID: 1.03 - Effects of early astaxanthin therapy via lipid nanoparticles on hippocampal neurogenesis and dendritic maturation in a model of Down syndrome

Dott.ssa Francesca Flotta (1), Dott.ssa Laura Angelozzi (2), Dott.ssa Beatrice Uguagliati (1), Dott. Marco Emili (1), Dott.ssa Debora Santonocito (3), Dott.ssa Carla Russo (2), Prof. Carmelo Puglia (3), Prof.ssa Sandra Guidi (1), Prof.ssa Fiorenza Stagni (2)

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Down syndrome (DS) is a genetic condition caused by the triplication of chromosome 21, in which intellectual disability (ID) represents the most invalidating aspect. ID in DS can be attributed to impaired neurogenesis, defective dendritic maturation, and increased cerebral oxidative stress (OS). These pathological features are recapitulated in the Ts65Dn mouse model of DS. Astaxanthin (AST), a lipophilic carotenoid with strong antioxidant and neuroprotective properties, has been shown to improve neurogenesis both *in vitro,* in neural progenitor cells (NPCs) and in healthy adult mice. AST modulates oxidative stress-related pathways (OS), which are dysregulated in DS. Despite its great therapeutic potential, AST shows high chemical instability and low bioavailability; to stabilize the molecule, AST was inserted into stealth solid lipid nanoparticles (AST-SSLNs). Our study aimed to evaluate in the Ts65Dn model the ability of AST-SSLNs to counteract neurogenesis and neuronal alterations in the hippocampal dentate gyrus (DG), a region severely impaired in DS. Euploid and Ts65Dn pups received daily subcutaneous injections of AST-SSLNs or unloaded SSLNs (vehicle) from postnatal day 3 to 15 (P3-P15). In Ts65Dn mice, treatment with AST-SSLNs notably increased the reduced total number of BrdU-positive cells and increased granule neuron density and the total number of granule neurons of the DG, restoring the proliferation potency of hippocampal NPCs. To evaluate dendritic maturation, we quantified spine density on Golgi-stained dendritic segments of DG granule neurons and reconstructed the dendritic tree of these neurons. Neonatal treatment with AST SSLNs in Ts65Dn mice increased the reduced spine density and increased both the reduced total dendritic length and total number of segments, suggesting a restoration of the dendritic architecture of granule neurons. Taken together, these positive effects of treatment on neurogenesis and dendritic maturation support the potential use of AST as a promising early-life therapeutic strategy to counteract neurodevelopmental alterations in DS.

POSTER ID: 1.04 - Obstacle shapes oculomotor behavior during spatial navigation: a machine learning approach

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Spatial navigation relies on visuomotor transformations that are highly sensitive to obstacle interference, which can significantly alter saccadic latency and the timing of motor execution. This study investigated whether ocular behavior recorded exclusively during the reaction time window could predict the trial outcome, with broader implications for the oculomotor assessment of populations with visuomotor impairments, such as individuals with Parkinson's disease. Twenty right-handed participants completed a joystick-controlled center-out task toward eight peripheral targets (Psychophysics Toolbox, MATLAB), with eye movements recorded via EyeLink eyetracker. Three conditions were tested: no obstacles (1), obstacles appearing simultaneously with the target (2), and obstacles appearing only upon movement onset (3). Statistical analyses employed linear mixed effects models, ANOVA, and Kruskal–Wallis tests (Bonferroni-corrected) revealing significant oculomotor modulation across conditions and between trials in which participants hit an obstacle (Hit) versus avoided it (No Hit). Two machine learning classifiers, a Support Vector Machine (SVM) and a Random Forest (RF), were trained on oculomotor features to classify: obstacle onset conditions 2 vs 3 and Hit vs No Hit trials. When classifying 2 vs 3 condition, performance was above the chance level across both models (accuracy ≈ 60 - 63%), suggesting that obstacle could influence oculomotor behavior primarly after movement initiation. For Hit vs. No Hit classification, both models performed above chance (RF: accuracy = 63%, F1 = 0.72; SVM: accuracy = 57%, F1 = 0.65). Balanced accuracy, adopted to account for class imbalance, revealed a more conservative picture (RF = 54%, SVM = 57%). These findings carry potential clinical relevance, particularly for neurodegenerative conditions such as Parkinson's disease, where both visuomotor integration and obstacle avoidance are known to be compromised. The present methodology could therefore inform the development of sensor-based assessment tools capable of monitoring visuomotor decline in patients.

POSTER ID: 1.05 - When self-action is harder to compensate: active sensing along the autistic-traits continuum

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Perception during active sensing depends on the brain's ability to distinguish sensory changes arising from the external world from those generated by one's own actions, including eye and body movements. To achieve perceptual stability, the brain must predict and compensate for the sensory consequences of its own actions yet remain flexible enough to adapt when sensorimotor contingencies change. A central question is whether all observers solve this problem in the same way. Here, we argue that variation in autistic characteristics provides a useful window onto this issue. Rather than indicating a general perceptual alteration, our data point to a distinct mode of sensorimotor inference. During rapid eye movements, we found that efference-copy–based signals are used less effectively to stabilize perception: when stimuli are briefly flashed on otherwise stable displays, spatial updating across saccades is weaker; when motion unfolds continuously, observers with more autistic characteristics become broadly insensitive to intra-saccadic motion, although comparable motion remains detectable during fixation. During dynamic visuomotor interaction, prior-informed expectations continue to shape behavior even when current sensory evidence no longer matches those expectations. We propose that altered compensation for self-generated sensory consequences may help explain why dynamic sensory scenes can become overwhelming and why adaptation becomes more rigid along the autistic-traits continuum.

POSTER ID: 1.06 - The combination of the PPARγ agonist pioglitazone and the GLP1R agonist liraglutide act synergistically to attenuate alcohol consumption in genetically selected alcohol preferring rats

Dott.ssa Sofia Christina Gkolfinopoulou (1)(2), Dott.ssa Mathoula Olga Kyratzi (1)(2), Dott. Davide Tronconi (1)(2), Dott.ssa Esi Domi (1), Dott. Li Hongwu (3), Dott.ssa Marisa Roberto (4), Dott. Roberto Ciccocioppo (1)

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Preclinical evidence shows that activating the GLP-1 receptor with liraglutide reduces alcohol intake and modulates central GABAergic transmission, while activating the PPARγ receptor with pioglitazone decreases alcohol consumption. Since both GLP-1R and PPARγ act as insulin sensitizers, they may share mechanisms linked to glucose regulation. Here, we tested whether combining agents targeting these receptors produces additive effects in reducing alcohol drinking in Marchigian Sardinian alcohol-preferring (msP) rats.
Male (N=18) msP rats, were trained to 10% alcohol operant self-administration. After stable baseline of responding was established, we tested the effect of pioglitazone (0, 10 and 30 mg/kg, os), liraglutide (0, 0.1 and 0.3 mg/kg, sc) and their combination (pioglitazone 10 mg/kg os plus liraglutide 0.1mg/kg, sc). Subsequently, the effect of chronic administration of the two compounds alone or in combination was evaluated in the two-bottle choice test. Rats were given free choice between water and 10% ethanol for 24 hours a day and drug effect was tested at 2,8 and 24 hours.
Results of the acute treatment show decrease of alcohol consumption when 30 mg/kg of pioglitazone (given o.s.) or 0.1mg/kg of liraglutide (given s.c.) were given. When the two drugs (pioglitazone 10 mg/kg, os; liraglutide 0.1 mg/kg s.c)) were given together the inhibitory of alcohol drinking was significantly more pronounced. In the chronic study coadministration of pioglitazone 10 mg/kg o.s., and liraglutide 0.05 mg/kg s.c, also showed enhanced effect compared to single drug administration.
Our findings show that activation of either PPARγ or GLP-1R reduces alcohol motivation, and their combined activation produces an enhanced effect. Further studies are warranted to clarify underlying mechanisms and potential sex-specific effects.
Grand support: NIH-NIAAA grant AA017447 (MR and RC); PRIN-2022 20227HRFPJ (to RC), PRIN-2022 2022H77XB7; NGEU+MUR Award ID: project MNESYS (PE0000006)- Project AMSUD 2024

POSTER ID: 1.07 - Development of an ex vivo innervated human skin model on high-density MEA platforms

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Background – The development of a human neuro-cutaneous interface is highly relevant for dermatological research, both for pharmacological and cosmetic screening and for the study of disease-related pathophysiological mechanisms in a dermatological context. Recent studies have demonstrated the feasibility of re-innervated skin models; however, ex vivo platforms that preserve the native tissue architecture while enabling high-resolution functional readouts are still needed (Bellantoni et al., 2026; Lebonvallet et al., 2021; Schutte et al., 2021; Nguyen et al., 2023).
Objective – Development of an ex vivo innervated skin model to investigate neurocutaneous crosstalk underlying irritation, itch, and sensitive skin, enabling predictive in vitro testing for cosmetic and drug safety and efficacy, as well as translational applications in dermatological research.
Experimental plan – Full-thickness skin biopsies (6–8 mm), obtained from abdominoplasty procedures, are maintained in air–liquid interface culture to preserve the three-dimensional organization of the tissue. Initial innervation is achieved using neonatal rat dorsal root ganglia (DRG; P1–P3), selected for their plasticity. The explants are placed in direct contact with neurons to promote neurite outgrowth and axonal integration. Structural validation has so far been performed by β-III-tubulin immunohistochemistry, and additional structural analyses are planned, including Ki-67, TUNEL assay, confocal microscopy, and 3D reconstruction. Functional validation will be carried out using HD-MEA recordings (MaxOne) to assess spontaneous and evoked activity in innervated models compared with non-innervated ex vivo controls. In a subsequent phase, the system will be implemented with human iPSC-derived sensory neurons (Rancan et al., 2025).
Conclusions – The development of a neuro-cutaneous platform based on human skin explants would enable the study of skin–neuron interactions within a physiologically relevant context. Its versatility would support applications ranging from cosmetic and drug screening to dermatological research, making it a robust tool for advancing both fundamental and translational studies in skin biology and neuroscience.

POSTER ID: 1.08 - A Human 3D Corticospinal Connectoid Platform to Model Development and Disease

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Voluntary movement depends on the corticospinal tract, a highly organized pathway that transmits motor commands from the cerebral cortex to the spinal cord through long projecting axons. Developmental abnormalities, neurodegeneration, and traumatic injury can disrupt this circuitry, leading to impaired motor function. To investigate this system, we developed a human 3D in vitro model of the corticospinal tract. We first generated brain and spinal cord organoids from healthy human induced pluripotent stem cells. Brain organoids progressively transitioned from neural progenitor states toward neuronal differentiation and were enriched in neurons with distinct cortical identities. Spinal cord organoids were patterned toward a motor neuron fate and displayed strong expression of mature motor neuronal markers. To model corticospinal connectivity, we used a microfluidic device consisting of two chambers connected by a central channel, kindly provided by Prof. Yoshiho Ikeuchi (Institute of Industrial Science, University of Tokyo) and produced as described by Osaki et al. (DOI: 10.3791/61544). Brain and spinal cord organoids were placed in opposite compartments, allowing axons to extend through the channel toward the opposing tissue. This strategy generated corticospinal connectoids characterized by a robust axonal bundle spanning the two organoids. Imaging analysis of this bundle showed strong neurofilament and HOMER1B/C expression, supporting both axonal identity and synaptic organization. We anticipate that this platform will be applicable to the study of amyotrophic lateral sclerosis and spinal cord injury, enabling the investigation of disease-associated phenotypes in a human context. Overall, this system provides a versatile platform to study human corticospinal development and disease, and to support target discovery and therapeutic testing.

POSTER ID: 1.09 - Neural Time Course of Size Adaptation

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Perceptual adaptation provides a powerful tool to investigate how recent sensory history shapes visual representations. In the domain of object size, adaptation alters perceived size and has been linked to activity in early visual cortex, yet the temporal emergence of this recalibration remains unclear. In addition, recent findings in numerosity perception suggest that adaptation effects depend on the similarity between adaptor and test stimuli in their low-level features (e.g., color, luminance, shape), pointing to a potential role of object-based processes in these phenomena. Whether such principles extend to size adaptation is currently unknown. Here, we combined psychophysics and electroencephalography (EEG) to examine both the temporal dynamics of size adaptation and its sensitivity to feature similarity. Participants first performed a 2AFC size discrimination task between two circular stimuli presented on either side of a central fixation point (baseline condition), with one stimulus fixed at 5° diameter and the other varying around this value. The same task was then performed after exposure to a large adapting circular stimulus for 4 seconds. Across blocks, the color of the adaptor and test stimuli either matched (congruent condition) or differed (incongruent condition).
Psychophysical results showed that adaptation to large stimuli led to a compression of perceived size, with stronger effects observed in congruent compared to incongruent conditions. EEG results revealed that the earliest reliable neural effect of adaptation emerged around ~100 ms, independent of congruency. In contrast, modulation by feature color became evident only at later stages (~200–300 ms and beyond), with enhanced responses for congruent relative to incongruent conditions. These findings indicate a temporal dissociation between early adaptation effects and later congruency-dependent modulation, suggesting that perceptual recalibration is not limited to early sensory encoding but involves later integrative, potentially object-based processes.

POSTER ID: 1.10 - Thalamo-cortical circuits regulate sensory plasticity: insights from ultra-high field fMRI in adult humans

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Short-term monocular deprivation in human adults reveals a latent capacity for plasticity, transiently enhancing responses to the deprived eye. Here, we asked whether this short-term deprivation reshaped intrinsic visual networks beyond the primary visual cortex (V1), revealing a potential thalamic mechanism supporting short-term plasticity.
Using ultra-high-field 7T fMRI, we measured anatomical, functional, and effective connectivity in 22 normally sighted adults before and after two hours of monocular deprivation. Analyses focused on interactions between V1 and two thalamic nuclei: the lateral geniculate nucleus (LGN) and the pulvinar, a higher-order nucleus involved in modulatory feedback loops.
Monocular deprivation selectively reduced resting-state functional connectivity between V1 and the pulvinar, while anatomical connectivity remained unchanged. Dynamic causal modeling revealed that this effect was direction-specific, reflecting a selective decrease in pulvinar-to-V1 effective connectivity, with preserved cortical feedback. In contrast, LGN–V1 connectivity was unaffected by deprivation, suggesting unaltered bottom-up drive to V1.
Given the evidence that pulvino-cortical projections can exert inhibitory control and that short-term monocular deprivation reduces GABA concentration in V1, these findings support a model in which adult plasticity is enabled by transient disinhibition of primary visual cortex V1, mediated through the downregulation of pulvinar feedback. Our results identify a form of inhibitory plasticity within a thalamo-cortical network as a key mechanism for adaptive visual behavior, positioning the pulvinar as a gatekeeper of cortical plasticity.

POSTER ID: 1.11 - DNA hydroxymethylation of NCX1 heart promoter by GATA6/TET3 epigenetic complex participates in neuroprotection induced by hypoxic preconditioning

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The Na+/Ca2+ exchanger 1 (NCX1) is a key regulator of calcium and sodium homeostasis and contributes to neuroprotection induced by ischemic preconditioning (PC). In neurons, NCX1 expression is controlled by two promoters, NCX1-Br (brain-specific) and NCX1-Ht (heart-specific). While DNA methylation represses NCX1-Ht activity, hydroxymethylation mediated by ten-eleven translocation (TET) enzymes is associated with transcriptional activation. In this study, we investigated the epigenetic mechanisms regulating NCX1 expression in neuronal cells. In ascorbic acid–treated SH-SY5Y cells, TET3 selectively increased 5-hydroxymethylcytosine (5hmC) levels at the NCX1-Ht promoter, leading to enhanced NCX1 mRNA and protein expression. This effect was not observed at the NCX1-Br promoter. Mechanistically, TET3 physically interacted with the transcription factor GATA6, whereas no interaction was detected with REST. Importantly, mutagenesis of GATA binding sites within the NCX1-Ht promoter abolished both GATA6 and TET3 recruitment, demonstrating the requirement of GATA6 for TET3-mediated epigenetic activation.
Furthermore, cortical neurons exposed to hypoxic preconditioning followed by oxygen-glucose deprivation/reoxygenation (OGD/Rx) showed increased GATA6 and TET3 protein levels together with enhanced DNA hydroxymethylation at the NCX1-Ht promoter. These changes were absent in neurons subjected to OGD/Rx alone.
Overall, our findings identify a novel neuroprotective mechanism in which the GATA6/TET3 complex epigenetically activates NCX1 transcription during ischemic preconditioning. Targeting this pathway may represent a promising therapeutic strategy for stroke and ischemic brain injury.

POSTER ID: 1.12 - Neuroglial Network Remodeling in Glioblastoma: Coordinated Alterations in Gap Junction and Purinergic Signaling

Dott. Luca Riccio (1), Dott. Ciro De Luca (1), Dott. Giovanni Cirillo (1), Dott. Michele Papa (1), Dott. Assunta Virtuoso (1)

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Glioblastoma (GBM) progression is increasingly driven by dynamic interactions within the neuroglial microenvironment rather than tumor-intrinsic properties alone. Here, we tested the hypothesis that GBM actively disrupts and functionally rewires neuroglial connectivity to sustain tumor growth and invasion.

We investigated the coordinated remodeling of glial interactions by focusing on Connexin43 (Cx43), the principal astrocytic gap junction protein, and the purinergic receptor P2X4R, a key regulator of microglial inflammatory signaling. Using a syngeneic organotypic brain slice model with intracortical injection of GL261 murine glioma cells, we performed confocal immunofluorescence and Western blot analyses at acute, 3-day, and 7-day time points.

We found a time-dependent reduction of peritumoral Cx43 expression, indicating loss of astrocytic gap junction coupling and disruption of network homeostasis. In parallel, changes in P2X4R expression/distribution were observed in association with NFκB pathway modulation, consistent with microglial inflammatory remodeling. These concurrent changes were accompanied by reactive astrogliosis and increased tumor proliferative activity, suggesting coordinated remodeling of neuroglial interactions within the tumor microenvironment.

Collectively, our findings identify coupled alterations in astrocytic connectivity and microglial purinergic signaling as a network-level feature of GBM progression. Targeting this coordinated glial remodeling may provide a strategy to interfere with tumor–glia crosstalk and reshape the neuroglial microenvironment.

POSTER ID: 1.13 - The GLP-1 receptor agonist semaglutide dose-dependently reduces voluntary alcohol consumption in Marchigian Sardinian alcohol-preferring rats

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Glucagon-like peptide-1 (GLP-1) receptor agonists have been shown to reduce alcohol consumption and other addiction-like behaviors. Here, we examined the effects of semaglutide, a long-acting GLP-1 analogue, on alcohol intake and anxiety-related behaviors in Marchigian Sardinian alcohol-preferring (msP) rats, a genetic model characterized by excessive alcohol intake and pronounced anxious and depressive-like phenotypes. The two-bottle choice paradigm was used in male and female rats to assess voluntary ethanol consumption under an intermittent access schedule. Rats were treated with semaglutide (0.001, 0.01 and 0.1 mg/kg, s.c.) and vehicle using within- and between-subject designs. Following a period of prolonged alcohol abstinence, general locomotion, anxiety and stress responses were assessed in alcohol-withdrawal animals, and an independent cohort of alcohol-naïve rats, without prior alcohol exposure, was evaluated in behavioral assays following administration of the highest dose of semaglutide (0.1 mg/kg). Semaglutide dose-dependently reduced alcohol intake and produced anxiolytic-like effects during withdrawal in the open field test, without affecting locomotion. In naïve msP rats, semaglutide reduced locomotor activity in both open field and elevated plus maze test and lowered blood glucose levels. In conclusion, semaglutide decreased voluntary alcohol consumption and modulated anxiety-like behaviors in a genetically vulnerable rat model of AUD. Our findings support the use of the GLP-1 receptor agonist semaglutide as promising pharmacotherapy for the treatment of AUD and its associated psychiatric symptoms.

POSTER ID: 1.14 - Modulation of PIWI proteins expression in the brain of mice subjected to middle cerebral artery occlusion: effects of remote ischemic post-conditioning

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P-element-induced wimpy testis (PIWI) proteins play specific functions in transcriptional regulation beyond the germline. In the brain, they modulate neuronal polarisation and cortical development under physiological conditions. Although altered expression of PIWI proteins has been reported in patients with chronic neurodegenerative disorders, little is known about their role in acute brain injury. Here, we characterise the expression dynamics of the major murine PIWI proteins (PIWIL1, PIWIL2 and PIWIL4) in mice subjected to cerebral ischemia and their involvement in neuroprotection induced by remote ischemic post-conditioning (RIC).
Ipsilateral (I) and contralateral (C) brains were collected from adult male C57BL6J mice subjected to 1h transient middle cerebral artery occlusion (MCAo) and sacrificed 48h after reperfusion. While PIWIL1 transcript was unaffected by the ischemic stimulus, real-time PCR revealed a significant upregulation of PIWIL2 and PIWIL4 in the ipsilateral ischemic cortex (1.5 and 5.9-fold, respectively, $P<0.0001$ vs C). Immunofluorescence analysis revealed that PIWIL2 was uniquely expressed in neurons, whereas PIWIL4 protein was also present in astrocytes and myeloid cells.
Interestingly, RIC induced by 10-min occlusion of the femoral artery (FAo) attenuated the elevation of both PIWIL2 and PIWIL4 in the ipsilateral hemisphere (0.7 and 0.6-fold, respectively, $P<0.01$ vs MCAo I). Moreover, RIC caused a selective upregulation of PIWIL4 (3.4-fold, $P<0.01$ vs C) in hippocampal neurons of the CA3 and dentate gyrus of the ischemic hemisphere, coinciding with upregulation of TGF-β1 and TREM2 (3.9 and 2.8-fold, respectively, $P<0.001$ vs C), two factors implicated in neuronal differentiation and repair-associated plasticity. Consistently, PIWIL4 co-localised with SOX2/Ki67-positive cells in the ipsilateral subventricular zone, supporting its potential involvement in ischemia-induced neurogenesis.
In conclusion, this study demonstrates the involvement of PIWIL4 in ischemic brain injury and supports its role in neuroprotective and neuroregenerative transcriptional programs triggered by remote ischemic conditioning.

POSTER ID: 1.15 - Inflammasome driven neuronal cell death in Huntington's Disease: modulation by the NLRP3 inhibitor MCC950

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Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by progressive motor, cognitive, and psychiatric decline caused by the expression of mutant huntingtin (mHTT). Beyond classical mechanisms of neurodegeneration, increasing evidence indicates that chronic neuronal inflammation significantly contributes to disease progression. In this context, the NLRP3 inflammasome has emerged as a potential therapeutic target, although its role in neurons remains poorly defined. In this study, we evaluated the effects of pharmacological inhibition of the NLRP3 inflammasome using MCC950 in mouse striatal progenitor cell models (STHdh) expressing either wild type or mutant human huntingtin. Pro-inflammatory stimulation activated the NLRP3 pathway, leading to increased caspase-1 activation and upregulation of gasdermin D, particularly in mutant cells, indicating enhanced susceptibility to inflammatory pyroptotic cell death. Treatment with MCC950 effectively reduced caspase-1 activation and gasdermin D expression, demonstrating selective inhibition of NLRP3-dependent pyroptotic signalling in cells expressing mHTT. In parallel, preliminary results indicate that MCC950 partially restores mitochondrial metabolic function under inflammatory conditions, promoting improved oxidative metabolism, increased ATP production, and reduced mitochondrial dysfunction in mutant cells. Despite these protective effects on inflammasome activation and mitochondrial function, MCC950 did not rescue cell viability, suggesting the involvement of additional inflammatory cell death pathways beyond canonical pyroptosis. Consistent with this observation, increased caspase-3/7 activity in HTT-mutant cells indicates concurrent activation of apoptotic mechanisms, supporting the possibility that PANoptosis, a coordinated inflammatory cell death program integrating pyroptosis, apoptosis, and necroptosis, may be triggered in neurons under inflammatory conditions. Preliminary data obtained from differentiated neuronal models revealed a distinct response to NLRP3 inhibition, with MCC950 partially restoring both caspase-1 activation and cell viability following inflammatory stimulation. Overall, these results identify MCC950 as a modulator of neuronal inflammasome activation in HD and highlight the complexity of inflammation-driven neuronal cell death, underscoring the need for combinatorial therapeutic strategies targeting multiple death pathways.

POSTER ID: 1.16 - Driving Behavioral Rescue and Molecular Modulation through Neuronal Stimulation in Rett Syndrome

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Rett syndrome (RTT) is an X-linked *MECP2*-related neurodevelopmental disorder and the leading cause of severe intellectual disability in girls worldwide. MeCP2 functions as a master regulator of gene expression, and its deficiency leads to numerous pathological features, including synaptic alterations and a consequent reduction in neuronal connectivity and plasticity; as a consequence, patients manifest critical motor and cognitive disfunction along with a spectrum of respiratory and cardiac abnormalities. Since a feedforward cycle between gene transcription and neuronal maturation has been postulated, we hypothesized that impaired transcription in RTT disrupts this loop and thereby tested the therapeutic value of stimulating neuronal activity through a clinical grade positive allosteric modulator of AMPA receptors. Beneficial effects were initially proved in mouse context. *In vitro,* the drug rescued multiple RTT-related phenotypes exhibited by *Mecp2* knockout primary neurons. Therapeutic efficacy was then assessed *in vivo* in RTT mice. We found that a short, early treatment administered during the peak of brain plasticity induces robust and long-lasting effects, delaying disease progression and rescuing both motor performance and spatial memory. Benefits were prolonged by extending the treatment with an intermittent regimen of administration, and confirmed by e*x vivo* molecular and electrophysiological analyses carried out starting from the prefrontal cortex of treated mice. As a further step toward the bedside, we are currently assessing the efficacy of Ampakine in RTT human neurons. Overall, our findings emphasize the importance of early intervention to restore neuronal circuits and suggest a new frontier in Rett syndrome care.

POSTER ID: 1.17 - Therapeutic Effects of Melatonin on Cerebellar Dysfunctions and Behavioral Deficits in a Murine Model of ASD

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Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition often characterized by an imbalance between excitatory and inhibitory (E/I) neurotransmission and disrupted neuroplasticity. While traditionally associated with motor coordination, the cerebellum is now recognized as a pivotal hub for social cognition and emotional regulation, making its dysfunction a core feature of ASD pathophysiology. Melatonin, a potent endogenous antioxidant and neuromodulator, has demonstrated neuroprotective potential in various neurodevelopmental models. However, its specific capacity to stabilize cerebellar circuitry and restore Purkinje cell functional markers in the context of ASD remains to be fully elucidated.
Objectives: This study investigates the potential neuromodulatory effects of melatonin on cerebellar alterations in the BTBR T^{+}Itpr3tf/J mouse model, focusing on behavioral recovery and the restoration of neuroplasticity.
Methods: BTBR mice were chronically treated with melatonin to evaluate its therapeutic impact. A multimodal approach was employed, including behavioral assays for social interaction and stereotypies. Molecular and structural changes were quantified using Western Blot, qPCR, and Immunohistochemistry (IHC) to evaluate markers of neuroplasticity and excitatory/inhibitory balance.
Results: Data confirm significant repetitive behaviors and cerebellar molecular dysregulation in BTBR mice, characterized by a marked reduction in GAD67 and Calbindin levels. Melatonin administration is able to modulate these deficits, suggesting a protective effect on Purkinje cell integrity and a trend toward restoring the E/I balance. Behavioral assessments indicate a reduction in stereotypical self-grooming, while molecular analysis points to a stabilization of neuroplasticity markers such as BDNF.
Conclusions: Our findings suggest that melatonin exerts a beneficial impact on the BTBR cerebellum by addressing core molecular and behavioral pathologies. By restoring inhibitory markers and protecting Purkinje cells, melatonin represents a promising pharmacological strategy to ameliorate cerebellar-mediated symptoms in autism.

POSTER ID: 1.18 - Glyceraldehyde-Induced metabolic defects in cortical astrocytes: implication for Alzheimer's disease pathogenesis and therapy

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A growing body of evidence suggests that reduced metabolic activity in astrocytes may compromise their normal supportive role for neurons and trigger pathophysiological pathways that contribute to the progression of Alzheimer's disease (AD). Due to the complexity of AD pathophysiology, it is crucial to study the disease not only within those contexts traditionally viewed from a neuron-centric perspective. In this study, we settled up a new model of AD by exposing primary rat cortical astrocytes to glyceraldehyde (GA), an inhibitor of glycolytic pathway able to induce a significant hypometabolism and recapitulate several AD pathomechanisms. Accordingly, GA-induced hypometabolism produced (a) astrocytosis, as revealed by the increase in GFAP and Glutamine Synthase (GS) immunosignals, (b) mitochondrial dysfunction, detected as reduced ATP level, mitochondrial ROS hyperproduction and Ca^{2+} dyshomeostasis at both cytosolic and mitochondrial level (c) inflammation, measured as NF-κB activation, TNFα release, AGEs hyperproduction/RAGE hyperexpression and increase in S100b immunosignal, and, finally (d) autophagy impairment, characterized by the p62 and LC3II protein accumulation. By virtue of glutamate ability to stimulate cell metabolism, we examined the effect of the neurotransmitter supplementation on cell damage and those correlated mechanisms in the proposed AD model. Of interest, metabolic, inflammatory and autophagy defects were mitigated when astrocytes were exposed to glutamate as metabolic boosting substrate. The protective effect of glutamate was counteracted by the pharmacological inhibition of astrocytic glutamate transporters, thus highlighting the relevance of glutamate intracellular action. Collectively, these results highlight the importance of considering astrocyte targeted therapies as potential strategy in AD.

POSTER ID: 1.19 - High-density multielectrode array recordings uncover early retinal ganglion cell dysfunction in EAE

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Introduction: One of the main challenges in finding new therapeutic strategies for progressive multiple sclerosis is detecting neuronal dysfunction before irreversible degeneration is established. The retina offers a particularly informative model, as retinal ganglion cell axons are unmyelinated within the retinal tissue and can therefore reveal early inflammatory effects independently of demyelination. In this study, we asked whether experimental autoimmune encephalomyelitis (EAE) induces early alterations in retinal ganglion cell axonal signalling before conventional measures of retinal damage become abnormal.
Methods: EAE was induced in female C57BL/6J mice through MOG immunization. Mice receiving mock immunization, by only complete Freund's adjuvant (CFA) without antigen, served as controls. Animals were assessed at baseline and during presymptomatic (6–11 days post-immunization, dpi), acute (14–19 dpi), and chronic phases (21–30 dpi). Full-field electroretinography (f-ERG) was used to examine photoreceptor and bipolar cell function in vivo, and visual behavior was tested with the optomotor response. To probe retinal ganglion cells more specifically, retinal explants were analyzed ex vivo with high-density multielectrode arrays (HD-MEA), enabling direct measurement of action potential propagation along intraretinal axons at the same time-points.
Results: f-ERG responses remained stable during the early stages of disease, and optomotor deficits were not evident until the chronic phase. In contrast, high-density multielectrode array analysis detected an early decrease in conduction velocity in retinal ganglion cell axons during the symptomatic phase. Because these axons are not myelinated within the retina, this impairment likely reflects inflammation-related disruption of axonal function rather than classical demyelination.
Conclusions: These results suggest that retinal ganglion cell axonal conduction is affected early in EAE, whereas conventional retinal and behavioral measures remain initially preserved. HD-MEA based axonal tracking may thus serve as a useful electrophysiological biomarker of early neuroinflammatory damage and a potential tool for evaluating neuroprotective interventions.

POSTER ID: 1.20 - Immune evasion of Epstein-Barr virus in multiple sclerosis brain: more than latency

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Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) resulting from complex interactions between susceptibility genes, lifestyles and environmental triggers. Among the latter, Epstein-Barr virus (EBV) infection is widely considered a prerequisite for developing the disease, but the pathological mechanisms linking EBV to the disease progression are still unclear. Studies in post-mortem brain tissue from donors with progressive MS revealed the presence of both virus-infected B-cells and EBV-specific cytotoxic T-cells in the inflammatory infiltrates. These findings indicate a chronic yet ineffective antiviral immune response in advanced disease stages, suggesting that EBV may exploit intracerebral immune evasion strategies. One of these strategies could consist in the use of the inhibitory immune checkpoint protein programmed cell death PD-1 and its ligand (PD-L1). Because PD-L1 is inducible by some EBV latency antigens in EBV-associated malignancies, the aim of this study, performed using multiple immunostainings on highly inflamed brain tissue from donors with progressive MS, has been to verify whether EBV uses PD-1/PD-L1 axis to escape the immune surveillance and establish a persistent infection in MS brain. The results obtained demonstrated that in the immune infiltrates invading MS brain, EBV induces the upregulation of PD-L1 on infected B cells, not only during the latency, but also during the early lytic phases of the virus life cycle. Moreover, PD-1-expressing T cells establish close contact with PD-L1+EBV-infected B cells, mainly in the inflamed meninges. This mechanism, sustaining intracerebral viral persistence, could play an essential role in driving chronic brain inflammation, through a continuous antigenic stimulation. By dissecting the local interplay between EBV-infected B cells and the antiviral immune response, it may be possible to identify novel therapeutic strategies that disrupt EBV-mediated immune evasion and restore effective antiviral immunity in progressive MS.

POSTER ID: 1.21 - Parkinson's Disease midbrain organoids reveal Tau isoforms imbalance associated with altered alternative splicing

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The establishment and maintenance of neuronal identity critically rely on tightly controlled alternative splicing programs. Disruption of these processes has emerged as a key contributor to neurodegenerative diseases, including Parkinson's disease (PD). The RNA-binding protein PTBP1 is a major regulator of splicing during brain development, yet its role in PD remains poorly understood. Tau pathology, characterized by an imbalance between 3R and 4R isoforms has been also found in PD, including LRRK2 G2019S-associated cases. However, the splicing regulatory network underlying Tau isoform imbalance in dopaminergic neurons still needs to be elucidated.
Patient-derived human midbrain organoids (hmOs) carrying the LRRK2 G2019S mutation were generated to investigate whether changes in PTBP1 mRNA expression are associated with alterations in Tau splicing in a disease-relevant context. Organoids were analyzed by qPCR and Western Blot at early and intermediate stages of maturation (20 and 40 days) to capture dynamic changes during neuronal differentiation and maturation. Immunofluorescence staining of organoids was performed to assess neuronal markers expression.
A significant upregulation of PTBP1 in PD-derived hmOs at 40 days was observed, concomitant with a shift in Tau isoform expression toward increased inclusion of exon 10 and exon 6, indicative of a pathological enrichment of 4R-Tau. The expression of MAP2, FOXA2, and TH via immunofluorescence demonstrates a midbrain dopaminergic identity, supporting the relevance of the model as a midbrain-like system.
These findings suggest that overexpression of splicing regulators such as PTBP1 may alter Tau isoform balance in dopaminergic neurons, potentially contributing to neuronal vulnerability in PD. Elucidating the link between RNA splicing regulation and neuronal dysfunction may provide new insights into the molecular mechanisms underlying neurodegeneration.

POSTER ID: 1.22 - CDKL5 Deficiency Disrupts Enteric Nervous System Organization and Gut Motility in a Mouse Model of CDD

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CDKL5 Deficiency Disorder (CDD) is a severe neurodevelopmental condition characterized by early onset epilepsy, intellectual disability, and autistic features. In addition to central nervous system (CNS) dysfunction, patients frequently present gastrointestinal disturbances, including altered intestinal motility, although the underlying mechanisms remain poorly understood. Using the Cdkl5 knockout (KO) mouse model, we investigated whether CDKL5 deficiency impacts the enteric nervous system (ENS) and gut function. Functional analyses revealed that both hemizygous male Cdkl5 KO mice and heterozygous females display increased intestinal motility, indicating dysregulated gastrointestinal transit. To assess whether these alterations were associated with epithelial barrier defects, intestinal permeability was evaluated using FITC-dextran in male Cdkl5 KO mice and corresponding controls. No significant differences were observed between genotypes, indicating preserved barrier integrity. To explore the cellular basis of enteric dysfunction, we analyzed CDKL5 expression and ENS organization. RNA analysis and in situ hybridization demonstrated that CDKL5 is expressed in enteric neurons. Consistently, reduced phosphorylation levels of the CDKL5 substrate EB2 were detected in the intestine of KO mice. Morphological analysis of the myenteric plexus showed no changes in ganglion size, but a trend toward reduced ganglionic density. Although the total number of cells per ganglion was unchanged, ganglion composition was altered, with a reduction in neuronal cells (HuC+) and a relative increase in enteric glial cells (S100+). Moreover, AIF1 immunostaining revealed increased density of immune cells within myenteric ganglia, suggesting activation of neuroimmune components in the ENS. Overall, these findings identify CDKL5 as a regulator of ENS organization and function. CDKL5 loss leads to altered enteric cellular composition and increased intestinal motility in the absence of barrier dysfunction, supporting a primary neuronal and neuroimmune contribution to gastrointestinal abnormalities in CDD.

POSTER ID: 1.23 - Cross-Correction–Based Delivery of TATk-CDKL5 via Hematopoietic Stem Cells Promotes Functional Recovery in CDD

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CDKL5 Deficiency Disorder (CDD) is a severe X-linked neurodevelopmental condition characterized by early-onset epilepsy, cognitive impairment, and autistic-like features. Currently, no disease-modifying therapies are available, and conventional gene therapy approaches are limited by inefficient and heterogeneous protein distribution within the central nervous system (CNS). To overcome these limitations, we developed a hematopoietic stem cell–based gene therapy (HSC-GT) strategy that exploits a cross-correction mechanism to achieve widespread CDKL5 delivery. In this approach, hematopoietic stem cells (HSCs) are engineered to express a secretable, cell-penetrating TATk-CDKL5 protein, enabling its release and uptake by neighboring cells and thereby amplifying therapeutic distribution beyond the transduced population. Following optimization of conditioning and transplantation protocols, engineered HSCs efficiently engrafted in the brain of Cdkl5 knockout (KO) mice, where they differentiated into long-lived microglia-like cells acting as a stable source of therapeutic protein. This cross-correction–based approach led to detectable CDKL5 mRNA signals in disease-relevant brain regions, including cortex and hippocampus. Functionally, HSC-treated Cdkl5 KO mice exhibited significant improvements in disease-relevant phenotypes, including reduced repetitive behaviors, improved motor performance, and a robust rescue of associative memory deficits, without adverse effects associated with conditioning or transplantation. Notably, transplantation of unmodified HSCs did not produce behavioral improvements, supporting the specificity of CDKL5-dependent rescue. At the cellular level, treatment promoted normalization of dendritic spine maturation in cortical neurons, shifting spine composition toward a more mature profile. Importantly, safety and tolerability assessment revealed no adverse neurological effects in mice receiving unmodified HSCs and no functional improvement, supporting the specificity of CDKL5-dependent rescue. Overall, these findings demonstrate that cross-correction–mediated delivery of TATk-CDKL5 via HSCs enables widespread CNS distribution of the therapeutic protein and promotes functional and synaptic recovery in a preclinical model of CDD, supporting its translational potential as a disease-modifying strategy.

POSTER ID: 1.24 - Asymmetric Parietal Cortical Atrophy in a Patient with RAB39B-Associated Parkinsonism: A Novel Truncating Variant

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Introduction: Loss-of-function variants in RAB39B (Ras Analogue in Brain 39B) cause X-linked Intellectual disability and early-onset Parkinsonism; however, whether the RAB39B mutation can lead to late Parkinsonism remains unclear. Recently, we identified a 57-year old male with lifelong mild cognitive impairment who developed atypical RAB39B like Parkinsonism late in life. Here, we ask if a novel variant of RAB39B underlies these late Parkinsonism symptoms.
Methods: We conducted neurological exams, performed MRI/CT imaging over five years, and sequenced the patient's genome.
Results: This patient had right-predominant tremor, mild bradykinesia, craniofacial dysmorphisms, and initially responded to levodopa. Brain MRI demonstrated bilateral paramagnetic deposits in the substantia nigra and globus pallidus, with diffuse asymmetric cortical atrophy affecting the left parietal lobe. CT imaging excluded basal ganglia calcifications. Over a five-year follow-up, motor fluctuations, autonomic dysfunction, mood disturbances, dysphagia, and sleep disorders developed, with stable cognitive function. Asymmetric parietal atrophy without progression persisted. Genetic analysis identified a novel truncating RAB39B variant.
Conclusion: Our study identifies a novel RAB39B variant linked to late-onset Parkinsonism with stable asymmetric parietal atrophy and paramagnetic deposits. Static cortical changes suggest a neurodevelopmental substrate rather than progressive neurodegeneration.

POSTER ID: 1.25 - Isoform-selective modulation of dopamine D2L receptor expression using antisense oligonucleotides

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Dopamine D2 receptor signaling plays a central role in the pathophysiology of several neuropsychiatric disorders. However, currently available antipsychotic drugs target dopamine D2 receptor isoforms non-selectively, often resulting in limited therapeutic efficacy and significant adverse effects. The dopamine D2 receptor exists as two main splice isoforms, the long (D2L) and short (D2S) variants, which play distinct biological roles and synaptic functions. This highlights the need for isoform-selective therapeutic strategies that can more precisely modulate dopaminergic signaling. In this study, we performed an *in vitro* evaluation of antisense oligonucleotides (ASOs) designed to selectively target the D2L isoform. To quantitatively assess D2L synthesis, we employed a plasmid-based reporter system in which the D2L coding sequence was fused to a NanoLuc luciferase reporter. HEK cells transfected with this construct were treated with a panel of D2L selective ASO candidates, and luciferase activity was measured as a functional readout of D2L expression. Several ASO candidates produced a significant and reproducible reduction in luciferase activity, demonstrating effective and isoform-selective suppression of D2L synthesis *in vitro*. These results provide a clear proof-of-concept for the selective modulation of D2 receptor isoforms using RNA-based approaches. Ongoing and future studies will extend this work to *in vivo* mouse models to assess the pharmacodynamic effects, safety profile, and therapeutic potential of D2L-selective ASOs as a novel antipsychotic approach.

POSTER ID: 1.26 - Modeling TDP43 proteinopathy in ALS using patient-derived cortico-spinal organoids

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the progressive dysfunction and loss of cortical and spinal motor neurons. Despite genetic heterogeneity, a number of familial cases is driven by *TARDBP* mutations. When mutated, TDP43 - which physiologically shuttles from the nucleus to the cytoplasm - becomes hyperphosphorylated and accumulates into insoluble cytoplasmatic aggregates. The lack of reliable models that accurately recapitulate ALS onset and progression remain a significant barrier to developing effective medical treatments. To overcome this limitation, we utilize a 3D modeling approach by deriving cortical and spinal organoids from patient-iPSC lines carrying heterozygous point mutations in the *TARDBP* C-terminal domain, alongside their isogenic control lines. Temporal analysis over a 90-day differentiation period revealed increased nuclear-to-cytoplasmic TDP43 mislocalization in mutant organoids as early as DIV (day *in vitro*) 30, compared to isogenic controls. Furthermore, we detected significant pTDP43 inclusions in both the cytoplasm and the nucleus, providing evidence of protein aggregation during early and late developmental stages. These results confirm that our 3D model effectively captures the early-stage pathological behavior and protein dynamics that characterize TDP43 proteinopathy. Consequently, we aim to employ cortical and spinal organoids to recapitulate the cortico-spinal tract within an *in vitro* device, exploring the region-specific vulnerability of our ALS patient lines. The complete validation of these models provides a robust *in vitro* platform to investigate ALS traits across both the brain and the spinal cord.

POSTER ID: 1.27 - The interplay between alpha-synuclein, inflammation and BBB in human Brain-chip model of Parkinson's Disease.

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons. Most clinically defined PD patients have pathological misfolded aggregates of α-synuclein spread throughout the nervous system causing a widely spectrum of clinical symptomology that afflicts their daily activities. Simultaneously, the progression of PD pathology is worsened due to oxidative stress occurring and the increase of inflammation processes. Emerging evidence strongly correlates blood-brain barrier (BBB) alterations with inflammatory events. However, it remains unclear if BBB disruption precedes inflammation and α-synuclein accumulation or, on the contrary, occurs because of misfolded protein accumulation and related inflammatory system activation. Besides, impairment of BBB integrity, could expose neuronal tissue to detrimental substances circulating in the blood, thereby accelerating the onset or worsening the progression of the disease. Hence, to better understand the mechanisms related to inflammation, α-synuclein and BBB integrity, we firstly collected and characterized PD patients' serum through Luminex assay to detect pro-and anti-inflammatory interleukins, metalloproteinases and interferons. The results suggested the activation of neuroinflammatory pathways in PD patients. Therefore, we evaluated the direct effect of PD serum on 2D-neuronal cells differentiated from PC12 cell line, assessing the cellular viability and the electrophysiological activity through a multiple-electrode array system (MEAs). To further evaluate the human biological samples' effect in a more complex *in-vitro* model, we quantified its α-synuclein content to treat human endothelial cells, microglia, pericytes and astrocytes composing the BBB in innovative Organ-on-the-chip system, which allowed us to mimic the cross- exchanges between brain tissue and the blood circulating system. These preliminary data could provide new insights on BBB alterations in the progression of PD. However, additional studies are required to better define the affected mechanisms.

POSTER ID: 1.28 - Dual Modulation Of Pathogenic And Neuroprotective Pathways In ALS Using A Chimeric Sineup-Based RNA Construct

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Amyotrophic lateral sclerosis (ALS) is a fatal, progressive neurodegenerative disease characterized by the selective loss of motor neurons, for which effective disease-modifying therapies remain limited. Pathogenic variants in the Superoxide Dismutase 1 (SOD1) gene associated with toxic gain-of function mechanisms account for approximately 15-20% of familial ALS cases and 2-4% of sporadic cases. In parallel, glial cell line-derived neurotrophic factor (GDNF) has been shown to promote motor neuron survival in both in vitro and in vivo models of neurodegeneration. In this project, we developed a novel chimeric RNA construct that combines two complementary therapeutic strategies: an artificial microRNA built upon a pri-miR155 scaffold designed to downregulate SOD1 expression and a SINEUP RNA engineered to enhance GDNF translation. The expression cassette was cloned into the 3'-UTR of a GFP expressed by a pAAV vector under a Pol-II promoter. As assessed by qPCR and western blot analyses, this construct effectively decreases SOD1 levels while concomitantly increasing GDNF expression in murine P19 and Neuro-2a cell lines. The next phase of the project will focus on in vivo evaluation of the construct, including its biodistribution, safety, and therapeutic efficacy in WT and ALS mouse models. By simultaneously reducing a key pathogenic driver and enhancing a neuroprotective factor, this approach aims to restore a more favorable cellular environment for motor neurons' survival. Overall, this work provides a proof-of-concept for chimeric RNA constructs that integrate gene silencing and translational enhancement within a single molecular framework, with potential applicability to ALS and other neurodegenerative disorders.

POSTER ID: 1.29 - Molecular and functional characterization of adult microglia in NRG1 TM HET mice, a mouse model of neurodevelopmental disorder

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Microglia are the tissue-resident immune cells of the central nervous system and contribute to a wide range of processes, from neurodevelopment to adult brain homeostasis, through surveillance, phagocytosis, and the release of soluble factors. Neuregulin-1 (Nrg1) is a key neurotrophin that plays critical roles in brain development. Through activation of ErbB tyrosine kinase receptors, Nrg1 regulates crucial processes, including neuronal differentiation, migration, synapse formation, myelination and neuron-glia communication. In addition, Nrg1 also modulates neuroinflammatory responses and exerts neuroprotective effects in the adult brain. Dysfunctional Nrg1 signaling has been implicated in neurodevelopmental disorders such as schizophrenia (SZ) and autism spectrum disorders (ASD), and experimental evidences indicate microglial involvement in these conditions, supporting the role of neuroinflammation as a contributing factor. To investigate the role of impaired Nrg1 signaling in the pathophysiology of these disorders, Neuregulin-1 transmembrane domain heterozygous mutant mice (*Nrg1 TM* HET) are widely used. In this context, to determine whether the mutation induces long-lasting alterations in microglial physiology that may influence their responsiveness to subsequent inflammatory stimuli, we characterized the molecular and functional properties of adult microglia derived from *Nrg1 TM* HET mice. We isolated microglia from the cerebral cortex of adult female and male *Nrg1 TM* HET and wild-type (WT) mice and assessed the expression of inflammatory and phagocytic markers, as well as their phagocytic activity. We found that LPS induced a comparable response across genotypes in both male and female mice, while basal phagocytic activity was increased in mutant mice of both sexes. Notably, in females this was accompanied by an upregulation of phagocytic markers, whereas in males these markers were unexpectedly downregulated. These findings suggest that Nrg1 signaling modulates microglial phenotype in a sex-specific manner, with potential implications for understanding the cellular mechanisms underlying differential neurodevelopmental trajectories described in the *Nrg1 TM* HET mouse model.

POSTER ID: 1.30 - Anti-Inflammatory Effects Of Allopregnanolone In LéS-Activated HMC3 Microglial Cells: Insights Into Molecular Mechanisms

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Catamenial epilepsy is a neuroendocrine condition affecting up to 70% of women with epilepsy, characterized by seizure clustering around specific phases of the menstrual cycle. Emerging evidence supports a critical role for neurosteroids in modulating neuronal excitability. In particular, allopregnanolone (ALLO) has GABAergic properties, and exerts anti-inflammatory effects in the CNS; however, the molecular mechanism underlying its action remain unclear.
This study aimed to evaluate the ability of ALLO to modulate inflammatory pathways in microglia. *In vitro* experiments were performed using human HMC3 microglial cells treated with lipopolysaccharide (LPS, 100 ng/mL) to induce a pro-inflammatory state. Cell viability was evaluated with MTT assay.
Cells were treated for 24 hours under four conditions: 1) vehicle control, 2) ALLO (10 μM), 3) LPS, 4) and LPS + ALLO combination. Then, qRT-PCR analysis and Western Blot analysis were performed to evaluated cytokine mRNA levels and protein expression levels of NLRP3 and mTOR pathways. In addition, confocal laser scanning microscopy was used to characterize the morphological changes of HMC3 microglial cells across the four experimental groups.
The results demonstrate that ALLO exerts anti-inflammatory effects, modulating NLRP3, NFkB, and mTOR pathways, which are key driver of neuroinflammation. Consistent with these findings, phagocytosis assays revealed that cells co-treated with LPS and ALLO exhibited a significantly reduced phagocytic activity, indicative of an attenuated cellular activation state and a broader suppression of the inflammatory response.
These findings demonstrate that ALLO effectively modulates the pro-inflammatory phenotype of HMC3 microglial cells by suppressing key pro-inflammatory signaling pathways. These immunomodulatory actions, together with the intrinsic GABAergic properties of ALLO, support its potential therapeutic application in patients with catamenial epilepsy.

POSTER ID: 1.31 - The Contribution of Oxidative Stress to Megalencephalic Leukoencephalopathy with Subcortical Cysts (MLC): Toward Innovative Therapeutic Approaches

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare inherited leukodystrophy caused by mutations in the astrocyte-specific membrane protein MLC1. Clinically, it is characterized by macrocephaly, brain edema, subcortical cysts, and white matter vacuolation, leading to motor dysfunction, seizures, and cognitive impairment. Previous studies from our group have shown that MLC1 protects astrocytes under osmotic and inflammatory stress. However, its physiological function and the molecular mechanisms underlying MLC-related brain damage remain unclear, limiting therapeutic development. Based on evidence that MLC1 is selectively activated by oxidative stimuli, such as hydrogen peroxide, in primary rat astrocytes and in U251 astrocytoma cells expressing wild type (WT) but not mutant MLC1, we investigated its role in astrocytic responses to oxidative stress. Functional analyses in U251 cells demonstrated that WT MLC1 expression reduces basal activation of Nrf2, the key regulator of antioxidant responses, along with its downstream signaling pathways, suggesting that MLC1 modulates oxidative stress responses. In line with this, cells expressing mutant MLC1 showed increased lipid peroxidation and decreased mitochondrial membrane potential compared to WT cells, supporting a protective role for MLC1. To validate these findings in a disease relevant model, we analyzed astrocytes derived from induced pluripotent stem cells (iPSCs) of MLC patients and healthy controls. Combined proteomic, biochemical, and imaging approaches revealed elevated oxidative damage and mitochondrial dysfunction in patient-derived astrocytes. Overall, our findings highlight oxidative stress as a key contributor to MLC pathogenesis and support the development of therapeutic strategies based on antioxidant approaches and drug repurposing aimed at correcting altered molecular pathways and restoring normal astrocyte function.

POSTER ID: 1.32 - Sex-dependent imbalance in synaptic architecture and signaling contributes to impaired dendritic spine maturation in a mouse model of ataxia

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Postnatal cerebellar development involves extensive structural and functional remodelling, a process fundamentally driven by coordinated interactions between neurons and glial cells. Current research suggests that the “crosstalk” between astrocytes, microglia and neurons is essential for regulating dendritic spine maturation, refining synapses and establishing balanced circuits. To investigate these mechanisms, we examined cerebellar development in the context of Niemann-Pick disease type C1 (NPC1), a lysosomal cholesterol storage disorder associated with progressive neurodegeneration, by integrating *in vivo* and *ex vivo* analyses.
Morphometric assessments of NPC1 models revealed significant reductions in total cerebellar volume and the internal granule layer. These structural alterations stem from defects in granule cell proliferation and migration, associated with diminished levels of Sonic Hedgehog (Shh) and Brain Derived Neurotrophic Factor (BDNF). At the synaptic level, granule neurons exhibited an increased number of immature dendritic spines, particularly in males, alongside an excitatory imbalance in glutamate levels. In addition to neuronal abnormalities, NPC1 models have also consistently reported glial dysfunction, including altered astrocytic and microglial homeostasis, which may further contribute to cerebellar circuit instability and neurodevelopmental impairment.
About synaptic pruning and phagocytosis, we analyzed markers such as C1qA, C1qB, MEGF10, CD47 and SIRPα to characterize the molecular environment of synaptic refinement. Finally, we compared their expression to identify potential sex differences between males and females.
As known, proper synaptic maturation depends on a high degree of cellular complexity, where the neuron-glia homeostatic synergy is essential for functional assembly. Ultimately, restoring this cellular balance may represent a key therapeutic strategy for addressing the neurodevelopmental hallmarks of NPC1.

POSTER ID: 1.33 - Human Neurons and Astrocytes exposed to hypoxia promote neurogenesis-related pathways in NPCs through extracellular miR-25/miR-106a

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Stroke is the second leading cause of death worldwide, and ischemic stroke is the most common type, accounting for 84% of cases. Ischemic injury may promote endogenous repair mechanisms, including neurogenesis and neuroplasticity, which are regulated by microRNAs (miRNAs) and guidance molecules. Ischemic-like hypoxia reshapes intercellular communication within the human neural niche, but the contribution of extracellular miRNA remains incompletely defined. We generated neurons and astrocytes from human induced pluripotent stem cell-derived neural progenitor cells (NPCs) and exposed them to hypoxia followed by reoxygenation. We quantified miR-25-3p and miR-106a in culture media and assessed whether conditioned media from hypoxic and reoxygenated cells modulate neurogenesis-related pathways in naïve NPCs. Under hypoxia/reoxygenation, both neurons and astrocytes showed a significant increase in extracellular miR-25-3p and miR-106a, indicating active release. NPCs exposed to miRNA-enriched conditioned media displayed selective regulation of specific targets and pathways: HMGB1 expression was significantly reduced, most notably after reoxygenation, whereas p57 (CDKN1C) levels were significantly increased, consistent with miRNA-mediated post transcriptional control of cell-cycle and differentiation programs. In parallel, BDNF levels were markedly increased in the culture media of NPCs exposed to reoxygenated conditioned media, indicating enhanced paracrine trophic signaling. These findings identify a hypoxia-induced extracellular miRNA-based communication mechanism whereby neurons and astrocytes influence NPC signaling through miR-25- and miR-106a-dependent regulation of neurogenesis related pathways.

POSTER ID: 1.34 - Circulating miRNA Biomarkers Linking NeuroCOVID and Cognitive Decline

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SARS-CoV-2 infection can lead to neurological deficits in a significant number of patients. NeuroCOVID seems to be associated with an increased risk of developing a neurodegenerative disorder or could accelerate its progression. The aim is to investigate a potential link between NeuroCOVID and cognitive decline in blood samples from patients to understand this phenomenon and develop new therapeutic strategies. 15 healthy subjects, 8 affected by NeuroCOVID and 7 with Mild Cognitive Impairment were selected. RNA was extracted and reverse transcribed from the patients' plasma. The concentration of miRNAs was assessed by RT-qPCR. In a previous study carried out by us, we identified three circulating miRNAs (miR-92a-3p, miR-320a and miR-320b) that regulate the MAPT gene differentially expressed in patients with frontotemporal dementia (FTD) compared to healthy controls and/or patients with Alzheimer's disease (AD). The analysis of these miRNAs in our populationshowed a significant increase in the expression of miR-320a and miR-320b in NeuroCOVID patients compared to controls. Additionally, miR-320b expression was increased in patients with MCI. No significant differences were observed for miR-92a-3p among the study groups. Conclusions: These findings indicate that the examined miRNAs, particularly miR-320a and miR-320b, are associated with both NeuroCOVID and MCI. Further investigation of these circulating biomarkers may help to identify shared molecular mechanisms underlying cognitive impairment in these conditions.

POSTER ID: 1.35 - Migration Of Extracellular Vesicles Derived From Adipose Mesenchymal Stem Cells Across An In Vitro Epithelial Barrier And Their Neuroprotective Effect On Injured Motoneurons

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Intranasal delivery is emerging as a non-invasive and promising strategy to directly target the central nervous system (CNS), as it allows therapeutic agents to bypass the blood–brain barrier, enabling repeated administration with minimal invasiveness. In this context, extracellular vesicles derived from adipose mesenchymal stem cells (ASC-EVs) have gained attention for their neuroprotective and immunomodulatory properties, particularly in the context of neurodegenerative diseases (NDs). Building on our previous *in vivo* evidence, this study aimed to develop an *in vitro* model of epithelial barrier to confirm ASC-EVs neuroprotective effect and investigate their migration mechanisms through the epithelium. An *in vitro* epithelial barrier model was established using RPMI 2650 nasal epithelial cells cultured on a transwell system and coupled with motor neuron-like NSC-34 cells, grown in the basolateral compartment. Motor neuron cells were treated with hydrogen peroxide to induce oxidative stress. This experimental model was suitable to evaluate the main ASC EVs migration mechanisms across the epithelial barrier, representing the first step of access to the CNS following intranasal administration, as well as their effects on motoneurons cells. Our results demonstrate that ASC-EVs efficiently cross the epithelial barrier and reach damaged motor neurons, where they are internalized and exert significant neuroprotective effects, by also preserving motoneurons mitochondrial membrane potential. Furthermore, ASC-EVs are able to cross the epithelium via both transcellular and paracellular route, with a predominant contribution for clathrin mediated endocytosis. These findings provide insight into ASC-EVs migration mechanisms across the epithelium, supporting the potential of intranasally delivered ASC-EVs as a non-invasive therapeutic approach for NDs.

POSTER ID: 1.36 - Prenatal microglial depletion alters neurodevelopmental trajectories following Early Immune Activation in mice

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Risk for neurodevelopmental disorders can be related to early immune activation (EIA), consisting of both a prenatal and postnatal immune challenge, and brain microglial functions. We previously showed that adolescent male mice have an increased vulnerability not only to the long-term behavioural (i.e. social novelty impairment) and inflammatory effects of the EIA condition but also to postweaning microglial depletion. Therefore, we further hypothesized that microglial depletion in earlier phases could better modulate offspring's neurodevelopmental trajectories.
To test this hypothesis, C57BL/6J pregnant mice were exposed to both polyinosinic:polycytidylic acid (Poly I:C) on gestational day (GD)12.5, and a diet containing the Colony Stimulating Factor-1 receptor inhibitor (PLX5622) to deplete maternal and fetal microglia, from GD11 to GD15; a Poly(I:C) only and a Saline group, fed with standard diet, were used as controls. On postnatal day (PND)9, offspring were subjected to a second immune challenge with lipopolysaccharide (LPS). In both male and female offspring, we evaluated on PND3, 6, and 9 body weight, spontaneous motor behavior, ultrasonic vocalizations (USVs), and on PND15 discrimination of nest olfactory cues (Homing test).
Our results showed that pups from Poly I:C + PLX dams showed higher body weight up to PND9, and early motor coordination deficits. On PND15 in males, exposure to EIA and concomitant prenatal microglia depletion induced deficits in discriminating familiar versus novel olfactory cues, while EIA condition alone decreased general locomotor activity levels and increased grooming response. By contrast, the combined treatment in females specifically affected only USV frequency.
In conclusion, prenatal microglial depletion and early immune activation affect motor coordination and induce detrimental effects (particularly in males) in recognition of familiar nest olfactory cues. Combined with our previous adolescent data, these results suggest that microglia depletion during developmental phases consistently targets male preference towards social cues.

POSTER ID: 1.37 - MiR-204 is required for photoreceptor rod differentiation and maturation

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Timely generation of distinct neural cell types in appropriate numbers and morphology is fundamental for the generation of a functional retina. Rod photoreceptors are a specialized type of neuronal cell found in the retina that is capable of visual phototransduction. They are composed of an inner segment that contains the nucleus and the cilium, which extends into the Outer Segment (OS). The OS that contains visual pigment-bound membrane disks and the synaptic terminal that forms synaptic connections to the bipolar cells, relaying visual input. The OS is a light-sensitive cilium that captures photons thanks to membranous discs packed with photopigment protein Rhodopsin. They undergo through daily renewal process, by phagocytosis of outer segment fragments (POS) from retinal pigment epithelium (RPE), to refresh membrane and protein. Importantly, the mechanisms by which OS is initially formed from the photoreceptor cilium and continually renewed is not well understood and are of longstanding interest. Our study focuses onthe role of miRNAs in controlling the molecular networks underlying the photoreceptor cell differentiation and maturation. MiRNAs are non-coding RNAs that, generally, inhibit the expression of target genes and have been involved, among other processes, in both cell identity and morphology acquisition. In vertebrates, miR-204 is initially expressed in multipotent retina progenitors and then becomes restricted to differentiated retinal cells, including photoreceptor cells. How miR-204 expression in the retina is controlled and what are its precise functions are still unclear. We tackle some of these knowledge gaps. By taking advantage of rods specific miR-204 transgenic and miR-204 KO Medaka-fish lines, we are characterising the specific role of miR-204 in building and maintaining the light-sensitive OS of vertebrate rod during their differentiation and maturation, addressing one of the most fundamental unsolved problems in vision.

POSTER ID: 1.38 - Effect of Early Life Adversity and chronic pain on anxiety, social behaviour and alcohol use

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Early life adversity (ELA) is a critical risk factor for the development of chronic pain and neuropsychiatric disorders, including anxiety, social dysfunction, and substance use disorders. This project aims to investigate the long-term effects of ELA on pain sensitivity, emotional behavior, and alcohol use propensity in a mouse model. Male and female mice are subjected to either social isolation or group housing beginning at postnatal day 21 to model ELA. At six weeks of age, all mice undergo sciatic nerve injury (SNI) to induce neuropathic pain. Pain sensitivity will be assessed at multiple time points—prior to injury, and at 1, 4, and 12 weeks post-SNI—to characterize the trajectory of pain development and maintenance.
Six weeks post-surgery, mice will undergo a battery of behavioral tests to evaluate anxiety-like behavior (Open Field Test, Light-Dark Box, Novelty Suppressed Feeding), social interaction (Direct Social Interaction Test, Three-Chamber Test), and alcohol consumption patterns using a two-bottle choice paradigm with escalating alcohol concentrations (4% to 20%). This design allows for the assessment of how early adverse experiences influence vulnerability to chronic pain and comorbid behavioral phenotypes, including anxiety, social deficits, and alcohol use disorder (AUD)-like behaviors.
The study is expected to provide insight into the sex-specific and long-term behavioral consequences of ELA in the context of chronic pain and alcohol consumption. These findings may inform future strategies for identifying individuals at heightened risk for developing pain-related psychopathologies and guide the development of targeted intervention.

POSTER ID: 1.39 - Subacute exposure to a mixture of three nanoplastic polymers triggers damage along the gut-brain axis

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Plastic is an escalating environmental risk, driven by uninterrupted production and its widespread distribution [1]. Nanoplastics (NPs), produced by biodegradation and mechanical fragmentation, raise particular concern for human health, with its capability in translocating across biological barrier [2,3]. While the current scientific literature focuses on a single polymer, this study aims to evaluate the effects of subacute exposure to three 50 nm different polymeric NPs (polystyrene, polypropylene, and polyethylene) along the gut-brain axis. In this experimental design, male and female C57Bl/6J mice were treated with NP mixture (in a 1:1:1 ratio) via oral gavage at a dose of 2.5 mg/kg for 7 consecutive days. Colon and hippocampal tissues were collected for Western blot and RT-qPCR analyses. Following the 7-day treatment period, parameters about intestinal and central biological barriers were modulated by NPs, as evidenced by changes in colonic tight junctions and blood-brain barrier integrity. In parallel, NPs trigger an enhanced inflammatory response in both the colon and the CNS. Interestingly, an initial sex difference was observed: colonic TNF-α mRNA expression increased predominantly in females, while IL-1β levels were elevated in males. This male-specific upregulation of IL-1β transcripts was mirrored centrally. Moreover, NPs induced a synaptic plasticity dysfunction, as indicated by decreased expression of PSD-95 and BDNF, with a more pronounced effect in male mice.
In conclusion, a subacute 7-day exposure to a mixture of three polymeric NPs, acts as an initial trigger for damage along the gut-brain axis, inducing both peripheral and central inflammation, as well as an impaired synaptic plasticity. Notably, our findings reveal early sex-specific differences, underscoring the need for further investigation into sex-dependent mechanisms underlying NP-induced neurotoxicity.

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POSTER ID: 1.40 - Nrf2 and neuroinflammation in Parkinson's disease patients and in an alpha-synuclein mouse model

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Objectives
Neuroinflammation plays a pivotal role in Parkinson's disease (PD) and represents a potential target for therapeutic intervention. We investigated the transcription factor Nrf2, a master regulator of antioxidant and anti-inflammatory pathways, as a potential index of PD pathophysiology and biomarker of disease progression in patients, and explored the impact of its pharmacological activation in a preclinical α-synuclein model.
Methods
Expression of Nrf2 and related antioxidant enzymes was evaluated in peripheral blood mononuclear cells (PBMCs) from PD patients at different disease stages and healthy controls (HC). Total and aggregated α-synuclein were measured by ELISA, and plasma inflammatory and neurodegenerative markers (NfL) by ELLATM technology. In parallel, C57BL/6 mice injected with α-synuclein preformed fibrils (α-syn-PFF) received dimethyl fumarate (DMF, 30 mg/kg), an Nrf2 activator. Motor and cognitive performance were assessed by rotarod and novel object recognition tests, and molecular endpoints by Western blot, RT-PCR and imaging.
Results
In patients, preliminary data showed a slight increase of Nrf2 in early PD and progressive rise of SOD1 with disease stage. HO1 levels were significantly reduced in early PD versus HC. Total α-synuclein showed no overall differences but was higher in females, whereas aggregated α-synuclein tended to increase in PD. Plasma NfL was elevated in PD, while inflammatory cytokines were unchanged. In mice, α-syn-PFF induced persistent motor and synaptic deficits. DMF treatment significantly improved motor coordination partially rescued dendritic spine loss, and increased Nrf2 mRNA expression.
Discussion
These findings suggest that Nrf2-related pathways are modulated during PD progression and may represent a pharmacological target. Ongoing patient recruitment and preclinical experiments will clarify their role.
This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), project ID 3433068 – Acronym: LINKING PARK.

POSTER ID: 1.41 - Dose- and Task-Dependent Effects of Neuropeptide S on Impulsivity, Attention, and Consummatory Behavior in Rats

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Neuropeptide S (NPS) is a neurotransmitter with both anxiolytic and pro-arousal properties. A genetic variant of the NPS receptor has been associated with maladaptive impulsivity and attention-deficit/hyperactivity disorder (ADHD); however, its functional role in impulsive and attentive behavior has not yet been evaluated. Here, we examined the effects of intracerebroventricular NPS (0, 0.5, 0.1 and 1 nmol) on impulsivity and attention using the Go/NoGo and two-choice serial reaction time task (2-CSRT), respectively, and on a continuous and intermittent sucrose self-administration (SA) to assess consummatory behavior in male and female Wistar rats (0.1, 1 and 2 nmol).
In the Go/NoGo task, rats were required to withhold lever pressing during NoGo cues and respond during Go cues to obtain sucrose. Intermediate and high NPS doses reduced premature responses during NoGo cues, whereas the highest dose also reduced Go responses. In the 2-CSRT, the rewarded lever switched randomly between left and right, and attention was indexed by correct responses; 0.1 and 1 nmol NPS improved attention. Finally, intermediate and high NPS doses reduced sucrose SA in a continuous trial, while in the intermittent trials only the highest dose reduced the sucrose SA.
These findings indicate that NPS produces dose- and task-dependent effects. Specifically, 0.1 and 1.0 nmol showed an anti-impulsive and pro-attentive profile without affecting overall consumption, whereas 2.0 nmol showed anti-consummatory effects (reduced both NoGo and Go responding). In sucrose self-administration tasks, NPS showed anti-consummatory effects.
Overall, anti-impulsive, pro-attentive and anti-consummatory profiles of NPS emerged independently and task-relatedly. NPS represents a target to develop ADHD therapies.

POSTER ID: 1.42 - Targeting tmCLIC1 electrophysiology to Improve Metformin Efficacy in Glioblastoma Cells

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Glioblastoma (GBM) is the most prevalent and lethal primary brain malignancy, characterized by poor survival outcomes and frequent recurrence. Central to GBM aggressiveness is a subset of tumor cells called cancer stem cells (CSCs), which drive tumor formation, sustain self-renewal, and promote resistance to conventional chemotherapy and radiation. Targeting CSCs therefore remains a pivotal therapeutic challenge.
Among the molecular factors governing CSC behavior, chloride intracellular channel 1 (CLIC1) has emerged as a critical regulator. This metamorphic protein exists in both soluble cytoplasmic and membrane-bound forms, with its membrane-associated chloride channel function being particularly prominent in GBM CSCs and essential for maintaining their self-renewal properties, positioning CLIC1 as a compelling therapeutic target.
Metformin, a widely used antidiabetic drug with growing anticancer interest, inhibits CLIC1 channel function by binding within the channel pore in an open-state-preferential manner. However, it produces meaningful antiproliferative effects on GBM CSCs only at elevated concentrations that are difficult to achieve in the central nervous system due to limited tissue penetration and blood–brain barrier constraints.
Strategies that enhance the metformin–CLIC1 interaction therefore hold considerable promise. Since CLIC1 open probability increases with membrane depolarization, manipulating membrane potential offers a rational approach to potentiating metformin's efficacy. Electromagnetic field stimulation and optogenetic techniques can generate repetitive membrane potential oscillations in GBM CSCs, increasing CLIC1 channel opening probability and thereby increasing metformin's inhibitory activity while reducing the required effective concentration. In vitro experiments combining metformin with repeated membrane depolarization demonstrate an average 30% decrease in CSC proliferation.
Collectively, these findings underscore a novel therapeutic strategy that integrates ion channel dynamics modulation with pharmacological intervention, offering a promising avenue to enhance metformin efficacy against GBM CSCs.

POSTER ID: 1.43 - Short-term plasticity of thalamo-cortical connectivity measured with Dynamic Causal Modelling of ultra-high field fMRI

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In adult humans, brief periods of monocular deprivation induce ocular dominance plasticity, transiently enhancing responses to stimuli in the deprived eye in the primary visual cortex V1 (Binda et al., 2018) and the ventral pulvinar (Kurzawski et al., 2022). The connectivity among these regions measured in resting state is also affected, with a selective reduction of the pulvinar-to-V1 pathway (Acquafredda et al., Science Advances 2025). Here we investigated whether and how the connectivity between V1 and the visual thalamus is modulated by visual stimulation.
We acquired ultra-high-field 7T fMRI EPI sequences from 25 normally sighted adults, pre- and post-2 hours of monocular deprivation. Participants were presented with monocular band-pass noise stimuli with five contrast levels. Dynamic Causal Modeling was used to analyse BOLD signals from three regions of interest: V1, lateral geniculate nucleus, and ventral pulvinar – all showing reliable visually evoked activity.
Short-term deprivation differentially modulated V1 connectivity with the two thalamic regions. During deprived eye stimulation, the connectivity between V1 and the pulvinar decreased post-deprivation (like in resting state), while the connectivity between V1 and LGN increased. The opposite was observed during stimulation of the non-deprived eye, resulting in a significant three-way interaction (time x eye x region of interest, $F(1,24) = 5.85$, $p = 0.024$).
We conclude that monocular deprivation produce changes that extend beyond local V1 processes and affect the connectivity between the cortex and the visual thalamus. Our results indicate that the pulvinar participates in regulating activity in the visual cortex and setting its short-term plasticity.

POSTER ID: 1.44 - GluA3 as a key regulator of dendritic structure and synaptic plasticity

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AMPA-type glutamate receptors (AMPARs), composed of GluA1–GluA4 subunits, mediate fast excitatory synaptic transmission in the brain. Among AMPARs' subunits, the physiopathological role of GluA3 remains poorly understood. To address this, we downregulated GluA3 using an shRNA-based approach in both in vitro and in vivo models.
Primary rat hippocampal neurons were transduced with either shRNA-GRIA3 or scrambled RNA viral vectors at DIV10, and analyses were performed at DIV20. GluA3 silencing significantly reduced dendritic arbor complexity and increased the proportion of thin spines, indicating impaired structural maturation. At the molecular level, GluA3 downregulation selectively decreased surface expression of GluA1-containing AMPARs, as well as GluN2A- and GluN2B-containing NMDARs. Consistently, live calcium imaging revealed a marked reduction in spontaneous NMDAR-mediated calcium transients, while glutamate release was not affected. Moreover, repeated glutamate uncaging-induced single-spine stimulation resulted in defective spine enlargement and abnormal calcium accumulation, likely due to impaired recruitment of GluA1-containing AMPARs to the postsynaptic membrane, as indicated by SEP-GluA1 fluorescence. RNA-seq analysis further identified dysregulation of key activity-dependent genes, including CAMK2A and MAPK1 (encoding ERK1), as well as pathways involved in protein trafficking and neuronal development. Rescue experiments demonstrated that re-expression of ERK1 was sufficient to restore dendritic arborization, thereby identifying ERK1 signaling as downstream effector of GluA3.
Notably, GluA3 silencing exhibited a clear time-dependent effect: early silencing (DIV3) caused more severe deficits, whereas late silencing (DIV14) had minimal impact. Considering the temporal specificity and the role of GluA3 in primary hippocampal neurons, we downregulated its expression in the CA1 region of the hippocampus in both adolescent and adult male mice. We detected alterations in dendritic complexity comparable to in vitro observations, further supporting a role for GluA3 as an upstream regulator of neuronal architecture. Ongoing behavioral studies are currently being conducted to determine whether GluA3 loss ultimately leads to cognitive deficits.

POSTER ID: 1.45 - Chronic Stress Reprograms Synaptic and Transcriptional Signatures of the mPFC-VTA Circuitry to Impair Cognition

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Cortical dysregulation of subcortical circuits is a key mechanism underlying mood and cognitive disorders. The medial prefrontal cortex (mPFC) exerts top-down control over the ventral tegmental area (VTA), a critical node of the mesocorticolimbic network regulating motivation and cognitive control. Previous work from our group showed that chronic stress induces structural and functional alterations in mPFC neurons projecting to the VTA. However, how these changes translate into downstream synaptic and transcriptional dysregulation within the VTA and impair cognition remains unclear. We first assessed circuit engagement using pathway-specific fiber photometry. Calcium imaging of mPFC-VTA neurons during a working memory task revealed time-locked transients preceding decision points, indicating recruitment of this pathway during cognitive processing. To identify the synaptic basis of this activity, we combined optogenetics with ex vivo electrophysiology. Optical stimulation of mPFC terminals in the VTA induced short-term facilitation of excitatory transmission onto dopaminergic neurons, relying on presynaptic calcium-interacting proteins. Stunningly, this plasticity is strongly impaired following chronic stress. To uncover the molecular substrate of this facilitation, and how stress reshapes these synapses, we performed a pathway-specific RNA sequencing of mPFC neurons projecting to the VTA. In females, chronic stress downregulated Calhm1, a gene encoding a Ca2+ homeostatic channel. CRISPR-mediated suppression of Calhm1 in naïve females recapitulated stress-induced synaptic deficits and impaired working memory and coping behavior. Together, these findings identify a stress-sensitive synaptic and transcriptional program at mPFC–VTA connections that supports cognitive function and highlights circuit-specific calcium regulation as a potential target for restoring cognition after stress.

POSTER ID: 1.46 - REST regulates NMDAR-dependent metaplasticity by tuning GluN2A/GluN2B balance

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NMDAR-dependent synaptic plasticity, including long-term potentiation (LTP) and long-term depression (LTD), represents a fundamental Hebbian mechanism underlying associative learning and memory [1,2]. The direction and magnitude of plasticity critically depend on NMDA receptor (NMDAR) subunit composition, particularly the balance between GluN2A- and GluN2B-containing receptors, which determines Ca^{2+} signaling dynamics and downstream synaptic responses [3]. While transcriptional activators of plasticity-related genes have been extensively characterized, the contribution of activity-dependent transcriptional repressors to the regulation of NMDAR-dependent plasticity remains incompletely understood.
The neuron-restrictive silencer factor (REST/NRSF) is a major epigenetic regulator that represses neuronal gene expression through chromatin remodeling and directly controls transcription of grin2b, encoding the GluN2B NMDAR subunit [4,5]. REST has also been implicated in the developmental GluN2B-to-GluN2A subunit switch, suggesting a potential role in regulating the molecular determinants of synaptic plasticity [5].
Here, we investigated the role of REST in NMDAR-dependent plasticity using chemically induced LTP and LTD combined with electrophysiological and pharmacological approaches. REST inhibition using decoy oligodeoxynucleotides selectively impaired LTP, as evidenced by a reduction in AMPAR-mediated mEPSC amplitude without affecting event frequency, indicating a postsynaptic deficit in potentiation mechanisms. In contrast, LTD expression remained unaffected.
Mechanistically, REST blockade markedly reduced evoked NMDA-EPSCs and synaptically driven Ca^{2+} responses, indicating altered synaptic NMDAR function. Pharmacological dissection using subunit-selective antagonists revealed increased sensitivity to the GluN2B-selective antagonist Ifenprodil together with reduced sensitivity to the GluN2A-selective antagonist TCN-201, consistent with a shift toward GluN2B-dominated synaptic receptor composition. In contrast, extrasynaptic NMDAR-mediated responses were largely preserved, indicating a selective effect on synaptic receptor populations.
Together, these findings identify REST as an epigenetic regulator of synaptic NMDAR composition that controls the GluN2A/GluN2B balance and selectively gates LTP expression. Our results support a model in which REST functions as a molecular mediator of metaplastic regulation by shaping the synaptic conditions that enable NMDAR-dependent plasticity.

POSTER ID: 1.47 - Neuroplastic effects of ketamine and psychedelics in primary neuronal cultures

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Background: Chronic stress is a key risk factor for major depressive disorder (MDD), a leading cause of disability worldwide. Despite available pharmacological therapies, treatment-resistant depression remains a clinical challenge. Ketamine and serotonergic psychedelics, produce fast and sustained antidepressant effects, possibly through modulation of synaptic plasticity. However, their cellular mechanisms remain incompletely understood.
Aim: To investigate structural, synaptic and functional alterations induced by ketamine, psilocybin and LSD in primary neurons subjected to chronic corticosterone exposure.
Methods: Primary cortical and hippocampal neurons from C57BL/6 mouse embryos (E16.5) were chronically exposed to corticosterone (CORT, 200 nM) or vehicle (VEH, DMSO) twice daily from DIV12 to DIV15. After the final CORT exposure, cultures were treated for 4 h with ketamine (1 µM),psilocybin (1 µM), or LSD (100 nM). Then, we characterized molecular, functional and synaptic changes induced by drug treatment through analysis of plasticity-related proteins, calcium dynamics and presynaptic markers by vGLUT1, vGAT and synaptophysin followed by puncta-based quantification.
Results: Chronic CORT reduced glutamatergic presynaptic density in primary cortical neurons compared with vehicle controls, as revealed by vGLUT1 and synaptophysin immunostaining. This alteration was largely reversed by LSD and psilocybin and only partially by ketamine. Acute ketamine also increased the levels of CaMKII, pSer880-GluA2 and GluA1 compared to CORT controls, supporting the involvement of plasticity-related pathways. In primary hippocampal neurons, calcium imaging revealed an enhanced cellular response in cultures treated with ketamine. Additional analyses are ongoing for psilocybin and LSD at molecular, structural and functional level.
Conclusions: These preliminary findings indicate that chronic CORT induces structural and synaptic deficits in primary neurons. Ketamine rapidly activates molecular and functional mechanisms associated with synaptic plasticity, while LSD and psilocybin restore glutamatergic presynaptic density. Ongoing studies will clarify whether these compounds produce shared or distinct plasticity-related mechanisms in this model.

POSTER ID: 1.48 - Neuroprotective effects of systemic administration of oxygen-rich extra virgin olive oil (Ozoile Food) in mice subjected to focal cerebral ischemia

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Ozoile Food is an oxygen-rich extra virgin olive oil (EVO) developed and patented according to a green technology by Erbagil, suitable for pharmaceutical and nutraceutical applications. Olive oil exhibits beneficial properties in diverse pathological conditions, supporting the therapeutic potential of Ozoile Food.
Here, we evaluate the effects of subchronic treatment with Ozoile Food in a mouse model of ischemic brain injury induced by transient middle cerebral artery occlusion (MCAo).
Subcutaneous daily administration of Ozoile Food (OzF, 2mL/Kg) for 7 consecutive days was well tolerated by adult male C57BL/6J mice, as it did not cause any sign of systemic toxicity, assessed by food and water intake, body weight and general behaviour, compared to saline (SAL)- or EVO-injected controls. Subchronic treatment with OzF before ischemia significantly reduced the number of animals with severe (De Simoni neuroscore >21) neurological deficits (SAL: 50%; OzF 17%) caused by 30min of MCAo followed by 24h of reperfusion. This effect was consistent with a significant reduction of brain infarct volume (SAL: 38.8822.54, OzF: 17.25 6.36 mm3, $P<0.05$, n=6-8) evaluated by cresyl violet staining after transient MCAo. Neuroprotection by OzF was associated with decreased plasma levels of reactive oxygen species ($P<0.01$ vs SAL, n=7) (d-ROMs test), with no effect on the plasma antioxidant barrier efficiency.
In conclusion, we have demonstrated that subchronic systemic administration of Ozoile Food is well tolerated and attenuates systemic oxidative stress providing neuroprotection in mice subjected to focal cerebral ischemia.

POSTER ID: 1.49 - Targeting NSC Metabolism to Promote Neurogenesis in Alzheimer's Disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by several factors including impaired glutamate neurotransmission, leading to sustained receptor activation and excitotoxic neuronal death. Neural stem cells (NSCs) are present in the adult brain and contribute to the continuous generation of neural cells throughout life. In AD, NSCs function is impaired, potentially due to metabolic dysregulation within the neurogenic niche. This research examines morphological and molecular differences between NSCs from healthy and AD mice and how modulation of mitochondrial impairment could impact during AD, to identify new druggable targets. To this aim, NSCs were isolated from both triple-transgenic (3xTg-AD) mice and age-matched wild- type (WT) controls. Neuron (N) and astrocyte (A) differentiation protocols were set up and morphological analyses were performed. Both WT and AD NSCs were strongly positive for Nestin and Sox2 (stem-related markers) and, under N or A differentiating protocols, both populations exhibited the ability to generate neurons and astrocytes that upregulated the expression of the lineage associated markers Neun/MAP2 and GS/GFAP, respectively. Preliminary behaviour tests, performed by using the novel object recognition test (NOR), may suggest a significant cognitive impairment in 3xTg-AD mice compared to WT at 5-6 months of age. Indeed, AD NSCs dissected from 3xTg-AD mice at 5-6 months, had a reduced proliferation and viability, along with an increase in glutamate transporters and glutamine synthetase, which indicated altered glutamate handling. The results suggest that early AD-related metabolic stress could lead to compensatory mechanisms in NSCs to sustain mitochondrial bioenergetics through glutamatergic pathways. Moreover, interventions that enhance metabolism may simultaneously limit the excitotoxicity burden, promote neurogenesis and contribute to neural repair in the early stages of disease progression. The connection between behavioural studies and molecular experiments on NSCs dissected from AD mice at different stages, may allow to study pathogenesis and design new pharmacological treatments.

POSTER ID: 1.50 - Studying the role of OPN-MMP9 pathway in 2D and 3D cellular models of familial ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by the selective loss of upper and lower motor neurons. The emerging OPN–MMP9 pathway, associated with the extracellular matrix, was first linked to neurodegeneration in the ALS mouse model SOD1^G93A. Bulk RNA-seq data from a previous study by our group showed OPN upregulation in sporadic ALS spinal cord organoids (SCOs) compared to controls. Here we investigate the OPN–MMP9 pathway in different ALS models, such as SH-SY5Y cells and SCOs, to assess its role in disease mechanisms and identify potential therapeutic targets. PBMCs, carrying mutations in TARDBP, SOD1, or C9ORF72, as well as PBMCs from healthy controls (CTRL) were reprogrammed into hiPSCs and differentiated into SCOs. SH-SY5Y cells were transfected with SOD1 (SH+SOD1) to model SOD1 ALS or treated with H_2O_2 (SH+H_2O_2) to induce oxidative stress. OPN expression was assessed by real-time qPCR. OPN transcription levels were significantly reduced in TARDBP, SOD1, and C9ORF72 SCOs compared to CTRL SCOs. Other components of the pathway (αV-integrin, β3-integrin, NIK, ERK, and p65) did not show significant differences between mutant and control SCOs, except for p50, which was upregulated in C9ORF72 and SOD1 SCOs. In contrast, OPN, αV-integrin, and β3-integrin were significantly upregulated in SH+H_2O_2 cells compared to SH. A similar increasing trend was observed in SH+SOD1 cells, although not significant. Overall, OPN transcription appears to differ across models. Its upregulation in SH+H_2O_2 cells is consistent with its known role as a pro-inflammatory cytokine, whereas its downregulation in mutant SCOs, including SOD1, suggests model-specific differences that warrant further investigation. These findings highlight potential limitations of SCO-based models in recapitulating ALS-related molecular pathways. Future work will focus on completing MMP9 transcriptional analysis across all models, assessing OPN–MMP9 protein levels by WB, and refining SCO differentiation protocols to improve model reliability.

POSTER ID: 1.51 - DNA methylation in 2D cultures and motor neuron organoids from sporadic ALS iPSCs

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease marked by the progressive loss of motor neurons (MNs) in cortex, brainstem, and spinal cord. Although its causes are not fully understood, both genetic and epigenetic factors are known to play a role. The majority of cases are classified as sporadic (sALS), with no clear familial history. The lack of reliable disease models has limited therapeutic advances, leaving only symptomatic treatments available. Organoids have recently emerged as promising *in vitro* systems that better mimic tissue organization. However, little is known about the epigenetic features of ALS-derived organoids. This study aimed to investigate DNA methylation patterns in both 2D cultures and MNs organoids (MNOs) obtained from healthy subjects and sALS patients, in order to identify differences related to 3D cellular organization. iPSCs from controls and sALS subjects were cultured and differentiated into MNOs. DNA methylation was analyzed in both 2D cultures and organoids at various stages of differentiation. Global methylation levels were measured using ELISA. In addition, the expression of key DNA methyltransferases (DNMT1, DNMT3A, DNMT3B) was assessed at the mRNA level by Real-Time qPCR and at the protein level by western blot. ELISA results showed lower global DNA methylation in sALS-derived organoids compared to 2D motor neurons. Gene expression analysis revealed that *DNMTs* regulation depends on the culture system: *DNMT1* and *DNMT3A* were increased in organoids, while *DNMT3B* was generally reduced. These findings indicate that the 3D environment influences DNA methylation machinery. However, protein levels of DNMTs did not mirror the observed methylation changes. Overall, MNOs display greater molecular alterations than 2D cultures, in line with previous transcriptomic data. These results support the use of organoid models as effective tools to study epigenetic mechanisms in ALS.

POSTER ID: 1.52 - Early Onset of Cerebellar and Peripheral Inflammation in Cntnap2 and Shank3b Mouse Models of Autism

Dott.ssa Martina Schiano Visconte (1), Prof. Luigi Balasco (2), Dott. Luca Pangrazzi (3), Prof.ssa Simona Casarosa (4)(5), Prof. Yuri Bozzi (1)(4)

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Autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized by social and communication deficits, restricted interests, and repetitive behaviors. ASDs are frequently associated with synaptopathies, suggesting impaired synaptic integrity and function. Although the neurobiological mechanisms underlying ASD remain incompletely understood, evidence indicates neuroinflammation and immune dysregulation, particularly involving microglia, in ASD pathophysiology. Previous studies indicate that dysfunctions in cerebellar microglial activation correlate with increased ROS and cytokine production in both Cntnap2 and Shank3b knockout mouse models. Treatments such as N acetylcysteine and Astaxanthin reduce neuroinflammation and improve social and motor behaviors in mutants, supporting a causal role for altered microglia within the cerebellum in ASD pathogenesis. Despite this evidence, it was still not clear when cerebellar inflammation is activated or whether its early onset directly influences the development of autistic phenotypes. To this aim, we identified early timepoints at which inflammation arises in Cntnap2$^{-/-}$and Shank3b$^{-/-}$ by quantifying the inflammatory cytokines in the cerebellum, hippocampus, and cortex, and then evaluated morphology and microglial density on sections of cerebellum of both *Cntnap2*$^{-/-}$ and *Shank3b*$^{-/-}$ and respective wild-type controls at early postnatal stages. We also determined when inflammation begins in peripheral immune organs such as the spleen and bone marrow in Cntnap2-/- and Shank3b-/-mice, and in their wild-type controls, during early postnatal development, to assess whether immune cells are present in the cerebellum of mutant mice and potentially contribute to inflammation in this organ. Overall, preliminary data indicate that peripheral and cerebellar inflammation and microglial activation appear early in *Cntnap2*, whereas a later profile is observed in Shank3b, supporting the idea of distinct mechanisms that converge toward similar autistic phenotypes. These results support the hypothesis that both cerebellar and peripheral inflammation play a crucial role in ASD pathogenesis and are essential for early detection and potential clinical treatment of individuals with ASD.

Poster Session II

POSTER ID: 2.01 - Contribution of Oligodendrocytes, Microglia, and Astrocytes to Myelin Debris Uptake in an Explant Model of Inflammatory Demyelination in Rats

Dott. Giovanna Vitolo (1), Dott. Mariarosaria Cammarota (1), Dott. Francesca Boscia (1)

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The internalization and degradation of myelin in glia contributes to the resolution of neuroinflammation and influences disease progression. The identification of a three-dimensional experimental model to study myelin processing under neuroinflammation will offer a novel approach for studying treatment strategies favouring inflammation resolution and neuroprotection. Here, by using a model of neuroinflammation in hippocampal explants, we show that myelin debris accumulated immediately after insult and declined at 3 days, a time point at which tentative repair processes were observed. $Olig2^+$ oligodendrocytes upregulated the LRP1 receptor and progressively increased MBP immunoreactivity both at peri-membrane sites and within the cytosol. Oligodendrocyte $NG2^+$ precursors increased in number and immunoreactivity one day after insult, and moderately internalized MBP particles. Three days after insult MBP was intensely coexpressed by microglia and, to a much lesser extent, by astrocytes. The engulfment of both MBP^+ debris and whole MBP^+ cells contributed to the greatest microglia response. In addition to improving our understanding of the spatial-temporal contribution of glial scarring to myelin uptake under neuroinflammation, our findings suggest that the exposure of hippocampal explants to LPS + IFN-γ-induced neuroinflammation may represent a valuable demyelination model for studying both the extrinsic and intrinsic myelin processing by glia under neuroinflammation.

POSTER ID: 2.02 - Exploring Glial involvement in a Mouse Model of NR2F1-Related Neurodevelopmental Disorder

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The *Nr2f1* gene encodes a transcription factor critical for neurodevelopment and neural plasticity, extensively studied for its functions in neural progenitor specification, cortical patterning, and neurogenesis. In contrast, little is known about Nr2f1 expression and function within glial lineages, despite emerging evidence suggesting its involvement in glial fate decisions and brain homeostasis. Pathogenic variants in NR2F1 cause Bosch–Boonstra–Schaaf Optic Atrophy Syndrome (BBSOAS), a rare neurodevelopmental disorder characterized by intellectual disability, visual impairment, hypotonia, and white matter abnormalities, including optic nerve atrophy and corpus callosum thinning. These white matter alterations, together with cognitive and motor deficits observed in patients, suggests that glial dysfunction, particularly involving oligodendrocytes, may contribute to disease pathophysiology. However, the specific contribution of Nr2f1 to oligodendrocyte lineage regulation remains poorly defined. Here, we investigated Nr2f1 expression and function in oligodendrocytes within the adult dentate gyrus (DG), a hippocampal region involved in cognitive functions, using Nr2f1 haploinsufficient mice. Immunofluorescence analyses revealed that Nr2f1 expression within the oligodendrocyte lineage is restricted to oligodendrocyte precursor cells (OPCs) and is downregulated in post-mitotic oligodendrocytes. Adult Nr2f1 heterozygous mice displayed a reduced oligodendrocyte population in the DG, suggesting a novel role for Nr2f1 in early stages of oligodendrocyte lineage progression. These findings extend the role of Nr2f1 beyond neurons and motivate a broader characterization of Nr2f1 expression across glial populations. Ongoing studies will determine whether the oligodendrocyte deficits observed in the DG are shared across other brain regions, including white matter tracts, providing new insights into glial contributions to BBSOAS pathophysiology.

POSTER ID: 2.03 - The involvement of group II metabotropic glutamate receptors in the modulation of endothelial properties at the blood-tumor barrier

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Blood-brain barrier (BBB) is a complex unit that maintains central nervous system homeostasis, thanks to endothelial cells (ECs) monolayer acting as a selective permeable barrier, supported by astrocytes, pericytes and microglia. Glioblastoma multiforme (GBM) is the most prevalent and aggressive primary brain tumor. BBB in contact with the GBM secretome may undergo changes in its properties leading to the blood-tumor barrier (BTB).
GBM releases high levels of glutamate, able to activate receptors like the metabotropic glutamate receptors (mGluR), promoting tumor malignancy. In GBM, group II mGluR (mGluR2 and mGluR3) activate pathways, like MAPK and PI3K, influencing tumor proliferation, while group II antagonists sensitize GBM to chemotherapeutic agents like temozolomide. Less is known regarding the role played by mGluRs in ECs at the BTB, although a previous study confirms their potential as pharmacological targets in ECs at the BBB.
In vitro human brain microvascular ECs were exposed to the human glioblastoma U87 secretome collected after 1 day *in vitro*. ECs were further stimulated with mGluR group II agonists LY379268 (1 µM) and LY2794193 (5 µM), mGluR2 negative allosteric modulator VU6001966 (3 µM) and the PI3K inhibitor LY294002 (10 µM).
Endothelial exposure to U87 secretome and group II mGluR agonists induced the overexpression of the adhesion molecule ICAM-1, increasing U87 adhesion. mGluR3 activation appeared to greater influence U87 cell adhesion, with a mechanism that seemed to involve the PI3K pathway.
Furthermore, BTB permeability, evaluated by dextran-FITC permeability assay, was increased in the presence of GBM secretome and by stimulating mGluR3; this effect was associated to claudin-5 cytosolic internalization.
In conclusion, our data suggests that U87 secretome seems to be pivotal in BTB properties modulation, and that modulating mGluR2 and mGluR3 on ECs may influence cancer adhesion and invasiveness, thus showing themselves as potential targets in addition to chemotherapy.

POSTER ID: 2.04 - Insights into O-GlcNAcylation dysregulation in Parkinson's Disease: from NF-kB/c-Rel dysfunction to peripheral biomarker discovery

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O-GlcNAcylation is a reversible, nutrient-sensitive post-translational modification (PTM) that support neuronal health and function in neurodegenerative diseases including Parkinson's disease (PD). We investigated on NF-κB/c-Rel as a putative target of altered O-GlcNAcylation in PD. In line with previous evidence that the defective NF-κB/c-Rel is associated with a PD-like phenotype in mice, we found a reduced NF-κB/c-Rel DNA-binding activity both in PBMCs and substantia nigra from PD patients. As this phenomenon occurred with unchanged NF-κB/c-Rel protein levels, we focused on OGlcNAcylation as a known PTM responsible for its DNA-binding activity. Western blot (WB) and immunoprecipitation assays confirmed reduced NF-κB/c-Rel O-GlcNAcylation levels in PBMCs isolated from fed PD patients. In parallel, we detected a defect in global protein O-GlcNAcylation in patientderived PBMCs and whole blood. Upon exposure to high glucose and O-GlcNAcase (OGA) inhibitor, cultured PBMCs from healthy controls (HC) exhibited a significant increase in total O-GlcNAcylation, whereas PBMCs from PD failed to mount this adaptive response, as assessed by WB and ELISA. Moreover, similar alteration was detected in whole blood collected under fasting and postprandial conditions. Dot blot analysis revealed reduced O-GlcNAc levels in PD postprandial samples compared to HC. To investigate whether this reduced O-GlcNAcylation stems from altered regulation of OGlcNAc cycling enzymes, we quantified the expression of O-GlcNAc transferase (OGT) and OGA by RT-qPCR. Notably, the OGT-to-OGA mRNA ratio was significantly decreased in PD PBMCs. These f indings suggest that defective O-GlcNAc process represents a systemic hallmark of PD and a source of accessible peripheral biomarkers for PD diagnosis.

POSTER ID: 2.05 - High-fat diet-induced gut dysbiosis drives synaptic impairment and clinical progression in an experimental model of multiple sclerosis

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Emerging evidence highlights the gut microbiota as a central regulator of immune–metabolic interactions in multiple sclerosis (MS). In this context, high-fat diet (HFD)-induced gut dysbiosis is a potential upstream driver of neuroinflammation and synaptic dysfunction contributing to disease severity, yet the mechanisms linking the microbiota, immune system and synapses remain poorly defined.
We investigate whether HFD-driven microbiota remodeling contributes to immuno-metabolic shifts underlying synaptotoxicity in MOG35–55 experimental autoimmune encephalomyelitis (EAE) mouse model. HFD-fed EAE mice (HFD-EAE) exhibited exacerbated clinical symptoms, compared to standard diet controls (SD-EAE), characterized by early anxiety-like behavior preceding more severe motor deficits. Notably, despite higher caloric intake, HFD mice displayed greater weight loss following EAE induction, suggesting profound metabolic dysregulation.
Focusing on the gut–immune–CNS axis, we performed 16S rDNA sequencing on stool samples from HFD-EAE and SD-EAE mice. HFD induced significant alterations in microbial ecology, characterized by shifts in beta diversity, resulting in a clear segregation of microbial communities, alongside alpha diversity changes marked by an increased Firmicutes/Bacteroidetes (F/B) ratio and Lactobacillus depletion. Importantly, F/B ratio positively correlated with elevated striatal expression of proinflammatory and T-cell-associated markers (Il1b, Tnf, Cd3e) supporting a link between dysbiosis and neuroinflammatory signaling. This gut dysbiosis was associated with enhanced infiltration of gutderived $CD4^+$ effector T cells into the striatum, promoting glutamatergic synaptotoxicity. Consistently, striatal metabolomic analyses revealed a HFD-driven metabolic reprogramming toward glycolysis and glutaminolysis, leading to increased glutamate levels.
By implementing a biphasic pre/probiotic supplementation with Saccharomyces boulardii, followed by Lactobacillus and Bifidobacterium, we successfully reduced T-cell migration and mitigated glutamatergic synaptotoxicity and motor deficits.
These findings identify HFD-induced gut dysbiosis as a key driver of T cell–mediated neuroinflammation, supporting microbiota-targeted interventions as promising adjuvant strategies to

improve both central synaptic and clinical outcomes, currently under evaluation in a randomized placebo-controlled trial in patients with MS.

POSTER ID: 2.06 - Loss of Ezrin triggers mitochondrial dysfunction and oxidative stress, associated with neuronal cell death

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Mitochondria, often referred as the powerhouses of the cell, play a key role in cellular respiration, essential to produce energy. Cellular respiration consists of three stages: the glycolysis, the citric acid cycle, and the oxidative phosphorylation. While the glycolysis occurs in the cytoplasm and produces a small amount of ATP, the citric acid cycle and the oxidative phosphorylation produces the majority of ATP in the mitochondria. Here, we report a new model of mitochondrial dysfunction related to the cytoskeletal protein Ezrin. The study of mitochondrial alteration, highlighted in *in vitro* and *in vivo* models of Ezrin knockout, has allowed to link the absence of this protein to an excess of cell death and decrease of neuronal survival. Here, we document a new mechanism of mitochondrial function in response to Ezrin modulation. Loss of Ezrin was deficient in mitochondria respiration and ATP production. In conclusion, our data identify a pivotal mechanism of mitochondria regulation involving Ezrin, essential for cell survival and neuronal homeostasis *in vitro* and in medaka fish model.

POSTER ID: 2.07 - Head-to-head comparison of Human Painless NGF with Wild-Type NGF in the 5xFAD Alzheimer's Disease Model

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Alzheimer's disease (AD) is characterized by β-amyloid (Aβ) and tau accumulation, neuroinflammation and degeneration of basal forebrain cholinergic neurons (BFCNs). The survival and function of BFCNs are regulated by the nerve growth factor (NGF) that binds its two receptors: TrkA and p75NTR. For this reason, NGF has been suggested as a potential therapeutic agent for AD. However, its clinical use is limited by poor brain delivery and nociceptive side effects. To overcome these limits, we combined an intranasal delivery – to bypass the blood brain barrier, with the development of human painless NGF (hNGFp), which lacks p75NTR binding and exhibits a 10-fold reduced pain-triggering activity while preserving TrkA-mediated trophic effects.
This study compares the neuroprotective efficacy of hNGFp and wild-type human NGF (hNGFwt) in an AD-relevant model.
Both neurotrophins were tested in vivo in 5xFAD mice, and in vitro, on primary microglia cells exposed to Aβ to confirm their action on microglia specifically. Behavioral tests, histological and biochemical analysis were performed to assess memory deficit, neurodegeneration, Aβ deposition and neuroinflammation.
While both treatments improved spatial memory, only hNGFp restored context-dependent object recognition memory. The sprouting of cholinergic neurons was similar for the two molecules, but only hNGFp significantly decreased Aβ deposition, inducing a reduction in the expression of γ and βsecretases. Furthermore, only hNGFp rescued the observed alterations in microglial morphology. hNGFwt increased the expression of pro-inflammatory cytokines and failed to modulate the cytokine panel linked to the neuroprotective effects of hNGFp. In vitro hNGFp and hNGFwt modulated distinct cytokines panels, indicating distinct immunomodulatory effects. Biodistribution analysis revealed that hNGFwt accumulated in the hippocampus but not in the cerebral cortex, whereas hNGFp levels were high in cortical regions.
Collectively, these findings identify hNGFp as a more effective neuroprotective agent than hNGFwt, supporting its therapeutic potential for chronic neurodegenerative diseases.

POSTER ID: 2.08 - Combined targeting of fatty acid amide hydrolase and melatonin receptors promotes neuroprotection and stimulates inflammation resolution in rats

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Background: Devising novel strategies to therapeutically favour inflammation resolution and provide neuroprotection is an unmet clinical need. Enhancing endocannabinoid tone by inhibiting the catabolic enzyme fatty acid amide hydrolase (FAAH), or stimulating melatonin receptors has therapeutic potential to treat neuropathological states in which neuroinflammation plays a central role.
Experimental approach: A rodent hippocampal explant model of inflammatory injury was used to assess the effects of UCM1341, a dual-acting compound with FAAH inhibitory action and agonist activity at melatonin receptors, against neuroinflammatory damage. FAAH activity was measured by a radiometric assay, and N-acylethanolamine levels were assessed by HPLC–MS/MS methods. FAAH distribution, evolution of inflammation and the contribution of UCM1341 to the expression of proteins controlling macrophage behaviour were investigated by biochemical and confocal analyses.
Results: UCM1341 exhibited greater neuroprotection against neuroinflammatory degeneration, compared with the reference compounds URB597 (FAAH inhibitor) and melatonin. During neuroinflammation, UCM1341 augmented the levels of anandamide and N-oleoylethanolamine, but not N-palmitoylethanolamine, up-regulated PPAR-α levels, attenuated demyelination and prevented the release of TNF-α. UCM1341 modulated inflammatory responses by contributing to microglia/macrophage polarization, stimulating formation of lipid-laden macrophages and regulating expression of proteins controlling cholesterol metabolism and efflux. The neuroprotective effects of UCM1341 were prevented by PPARα, TRPV1 and melatonin receptor antagonists.
Conclusion: UCM1341, by enhancing endocannabinoid and melatoninergic signalling, benefits neuroprotection and stimulates inflammation resolution pathways. Our findings provide an encouraging prospect of therapeutically targeting endocannabinoid and melatoninergic systems in inflammatory demyelinating states in the CNS.

POSTER ID: 2.09 - The role of prelamin A in Glioblastoma Stem Cells as a foundation for novel therapeutic approaches

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Glioblastoma (GBM) is the most common high-grade brain cancer. Despite timely and invasive interventions, the prognosis is very poor due to therapy resistance and tumor recurrence, highlighting the need for innovative therapeutic approaches. In glioblastoma cells, the nuclear lamina structure is profoundly altered, and recent evidence indicates that pharmacologically induced accumulation of prelamin A, the precursor of lamin A, reduces the aggressiveness and stemness of glioma stem cells (GSCs), a group of cancer cells thought to drive therapy resistance. Notably, GBM cells accumulating prelamin A show decreased expression of N-cadherin, a key factor involved in epithelial-to-mesenchymal transition (EMT). In addition, recent findings have identified a novel form of connexin, GJA1-20k (Cx20), implicated in cancer progression and potentially acting as a transcriptional regulator through binding to the N-cadherin promoter. Based on these observations, this study investigates the role of prelamin A in GSCs as a foundation for novel therapeutic approaches, focusing on its potential impact on the intranuclear translocation of Cx20 and the consequent regulation of N-cadherin transcription. In particular, prelamin A accumulation was induced using a farnesyltransferase inhibitor in glioblastoma cell lines, patient-derived GSCs and human astrocytres as control, cultured both as monolayers and as 3D spheroids. Our preliminary results suggest that drug-induced accumulation of prelamin A promotes redistribution of Cx20 toward a perinuclear localization in both glioblastoma cells and patient-derived GSCs, resembling the pattern observed in control astrocytes. These findings suggest a link between nuclear lamina defects, prelamin A accumulation, and the Cx20/N-cadherin transcriptional pathway involved in EMT and tumor progression, potentially opening new therapeutic avenues targeting GSCs.

POSTER ID: 2.10 - Peripheral TSPO Expression Reflects ALS Progression and Mitochondrial Adaptation in Patients and SOD1-G93A Muscle

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INTRODUCTION: Emerging evidence supports an active role of skeletal muscle in the pathogenesis of amyotrophic lateral sclerosis (ALS), involving inflammatory processes and mitochondrial dysfunction within muscle fibers. The translocator protein (TSPO) is an 18-kDa transmembrane protein primarily located on the outer mitochondrial membrane. It regulates critical mitochondrial functions, including cholesterol transport (steroidogenesis), bioenergetics (ATP production), reactive oxygen species (ROS) homeostasis, and apoptosis. TSPO acts as a biomarker for neuroinflammation and mitochondrial dysfunction. AIMS This study aimed to define the role of TSPO in ALS skeletal muscle and to assess its potential as a progression biomarker in ALS patients.
METHODS: TSPO expression was assessed by qPCR in both affected and unaffected skeletal muscles of SOD1G93A mice at early and late disease stages. These findings were validated by immunohistochemistry and confocal microscopy. Furthermore, mitochondrial biogenesis (NRF1) and dynamics (DRP1, MFN2) markers were evaluated in the skeletal muscles of SOD1-G93A mice and correlated with TSPO expression. In addition, TSPO was evaluated in human muscle biopsies and serum (ELISA) from ALS patients and healthy controls.
RESULTS: qPCR revealed a significant TSPO upregulation in SOD1-G93A gastrocnemius from early stages, while soleus and triceps expression increased at late stages. Immunohistochemistry and confocal microscopy confirmed high colocalization of TSPO with mitochondrial markers, suggesting that TSPO regulates mitochondrial activity from early disease stages. Consistently, increased TSPO expression was observed in muscle biopsies from ALS patients. Interestingly, circulating TSPO levels were increased in the serum of ALS patients, particularly in early-stage compared to late-stage patients. Notably, this increase was positively correlated with cumulative electromyography denervation scores.
CONCLUSION: TSPO upregulation in ALS skeletal muscle and serum supports its potential as a non-invasive biomarker for monitoring of muscle pathology in ALS. Furthermore, its association with mitochondrial markers suggests a role in biogenesis and remodeling during disease progression.

POSTER ID: 2.11 - Modulation of autophagy in a mouse model of cuprizone-induced demyelination

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Multiple sclerosis (MS) is a chronic immune-mediated disorder affecting the central nervous system (CNS), characterized by inflammation, demyelination, and neuronal loss. Although inflammatory activity drives lesion formation, MS is now recognized as a disease involving both inflammatory and neurodegenerative mechanisms (Haki et al., 2024).
Optic neuritis is the first clinical manifestation of MS in about 40% of patients and it occurs in up to 70% over the course of the disease (Tong et al., 2025).
Autophagy is a key homeostatic process involved in degradation and recycling of cellular components, essential for neuronal integrity (Li et al., 2022). Alterations in this process have been associated with several neuroinflammatory and neurodegenerative conditions; however, its contribution to MS pathology remains unclear (Misrielal et al., 2020).
The present study aims to evaluate the regulation of autophagy in the optic nerve and corpus callosum in a cuprizone-induced mouse model of MS.
Demyelination was induced by feeding 8-week-old male C57BL/6J mice a diet containing 0.2% (w/w) cuprizone for 8 weeks. Protein expression levels were analyzed by western blotting and immunofluorescence. Proteomic profiling was performed using mass spectrometry.
Cuprizone treatment induced a marked reduction in myelin basic protein (MBP) in both the corpus callosum and optic nerve, together with increased glial fibrillary acidic protein (GFAP). In the corpus callosum, levels of autophagy markers (i.e. p62 and LC3-I/II) were increase. PINK1 and Parkin expression were significantly upregulated, while optineurin remained unchanged. In the optic nerve, Parkin was increased, with no changes observed in retinal tissue. Proteomic analysis of the corpus callosum identified 5,987 proteins, of which 139 were significantly modulated by cuprizone treatment.
These preliminary findings indicate dysregulation of autophagy and mitophagy in the corpus callosum and optic nerve during cuprizone-induced demyelination and support further investigation of these pathways as modulators of neurodegeneration in MS.

POSTER ID: 2.12 - M2–β-Arrestin1 Axis in Glioblastoma cells: implication in Cell Proliferation and Migration

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Glioblastoma multiforme (GBM) is the most aggressive primary malignant brain tumours. Despite advances, GBM treatment has remained difficult since 2005, with the introduction of Temozolomide (TMZ) as first-line therapy. Although the current treatment efforts - including surgical resection followed by radio/chemotherapy using TMZ - more than half of patients do not respond and the median survival still short. In this context, the great challenge is to identify new therapeutic target to counteract GBM progression with reduced side effects on healthy cells. Over the past years, our group has demonstrated that the M2 cholinergic receptors (M2R), caused a negative modulation of cell proliferation and survival in GBM. In the present work, we focused on the differential modulation mediated by the orthosteric agonist APE and the dualsteric ligand N8: starting from the characterization of the drug-receptor complex and extending to intracellular outcomes in GBM cell lines. The concept of “biased agonism” has emerged in the last decade as a key paradigm in Gprotein-coupled receptors GPCR research, describing the ability of dualsteric ligands to selectively engage specific signaling pathways downstream of GPCR activation. M2R belongs to the GPCRs family and interestingly, the β-Arrestin1 - beyond its canonical role in GPCRs desensitization and recycling - also mediates “biased signalling” through pathways distinct from the G-protein-dependent canonical one. Here, by stable and transient transfection methodologies, we describe the M2R dynamics and internalization, as well as differences in intracellular pathways upon receptor stimulation with either APE or N8. Remarkably, in a GBM cell line model, we demonstrated that βArrestin1 not only exert its canonical role in receptor internalization across both treatments but is also essential downstream of M2R selective activation by the dualsteric N8 in mediating PI3K/Akt/mTOR signaling as well as cell migration.

POSTER ID: 2.13 - Functional adaptations of magnocellular red nucleus neurons following spinal cord injury: an electrophysiological study

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Background: Spinal cord lesions elicit a complex of neuronal responses in upstream brain regions, as a consequence of traumatic axon injury and loss of projection targets. The magnocellular part of the red nucleus (RNm) contains glutamatergic neurons that project to the spinal cord and participate in motor control. Recent research has highlighted emerging roles for the RNm in regulating goaldirected behavior by integrating behavioral valence and action plans, in addition to serving a motoreffector function. Currently, however, little is known about how RNm neurons respond to axotomy and how their functional properties change over time.
Methods: Ex vivo whole-cell patch clamp recordings of RNm neurons were performed on horizontal midbrain slices from mice that underwent spinal cord injury (SCI) at 7, 28, and 60 days after surgery. Intrinsic membrane properties were studied, together with action potential kinetics and spontaneous excitatory neurotransmission. Recorded cells were marked with biocytin, and immunofluorescence stainings were performed to confirm the identity of RNm cells.
Results: Persistent alterations of intrinsic membrane properties were observed in rubrospinal neurons from SCI mice. At all studied time points, the injection of negative current steps elicited greater membrane hyperpolarization than in controls. In parallel, a significant increase in neuronal excitability was assessed at the later time points following the lesion. Action potential kinetics were preserved across time.
Conclusions: Axotomy determines long-lasting electrophysiological anomalies in rubrospinal glutamatergic cells. Electrophysiological modifications accompanying response to axotomy in RNm cells might influence how this region integrates into broader brain networks following spinal lesions.

POSTER ID: 2.14 - Cebranopadol: a novel dual NOP/MOP receptor agonist to treat fentanyl use disorder and fentanyl induced respiratory depression in a rat model of addiction

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In the recent years, Fentanyl Use Disorder (FUD) has become a critical public health issue. Opioidinduced respiratory depression is the main reason of opioid overdose deaths. There is scientific evidence that the co-activation of the mu-opioid receptor (MOP) and nociceptin/orphanin FQ opioid peptide receptor (NOP) can decrease classical opioid adverse effects. Here we aim to evaluate the therapeutic potential of the long-acting pan-opioid agonist Cebranopadol in the treatment of FUD. We used male Wistar rats to explore the therapeutic potential of Cebranopadol on FUD after acute and chronic treatment. We also investigated the possibility of Cebranopadol inducing withdrawal symptoms compared to Naloxone a MOP antagonist and Buprenorphine a MOP/NOP partial agonist. Next, we investigated the effects of Cebranopadol on Fentanyl induced respiratory depression using whole-body plethysmography. Rats received Cebranopadol (0, 25 and 50 µg/kg) and Fentanyl (0, 50 µg/kg) intravenously in a within-subjects design during the plethysmography test. We evaluated the effect of acute Cebranopadol oral gavage (0, 12, 5, 25, 50 µg/kg) on Fentanyl long access selfadministration. We noticed that Cebranopadol efficaciously reduced the number of rewards after acute treatment at all doses. Then, using the two lower doses of Cebranopadol we performed a 7 day long chronic treatment and observed a reduction of the rewards obtained until tolerance appeared on the fifth day. Also, even though both Naloxone and Buprenorphine induced withdrawal symptoms, Cebranopadol (25 and 50 mg/kg o.s.) did not differ from the vehicle. Our results showed that intravenous supratherapeutic doses of Cebranopadol did not affect parameters associated with respiratory depression and when co-administered with Fentanyl, significantly attenuated it. These f indings support that Cebranopadol has a unique safety profile; protects from fentanyl induced respiratory depression and is capable to reduce operant self-administration of fentanyl in a rat model of addiction that mimics the clinical escalation of fentanyl abuse.

POSTER ID: 2.15 - Premature differentiation of human Neuronal Progenitor Cells derived from Angelman Syndrome iPSCs and possible involvement of 5-HT7R

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Angelman syndrome (AS) is a severe neurodevelopmental disorder caused by the loss of function of the maternally inherited UBE3A gene, showing high comorbidity with Autism Spectrum Disorders. Previous studies from our group demonstrated that stimulation of the serotonin receptor 7 (5-HT7R) rescues behavioural deficits, synaptic protein synthesis and dendritic spine alterations in AS animal models. In this study, we aimed to characterize the efficiency of neuronal differentiation of AS neuronal progenitor cells (NPCs) at early stages of neuronal induction and to investigate the role of 5-HT7R. We generated human cerebral organoids from induced pluripotent stem cells (iPSCs) of AS patients and isogenic control, and we dissociated them to isolate NPCs. NPCs were differentiated into young neurons for 8 and 14 days (NPCs-8D and NPCs-14D) to investigate early phenotypic alterations. Our results showed that the AS NPCs-14D positive for the proliferative marker Ki67 were less numerous than in control cells, while cells positive for the neuronal marker MAP2 were more numerous than controls. These results indicate that AS cells display decreased proliferation and premature differentiation. This trend was already present at 8 days of differentiation.
As a following step, we evaluated the expression levels of 5-HT7R in AS NPCs-8D and the effect of the chronic stimulation of the receptor with the selective agonist LP-211. We found a significant decrease of 5-HT7R protein in AS cells compared to controls. The chronic stimulation of the receptor with the selective agonist LP-211 failed to rescue the altered phenotype of these AS NPCs-8D.
In conclusion, these results indicate that the altered phenotype appears prematurely during neuronal differentiation in AS, together with reduced expression levels and impaired function of 5-HT7R. This observation suggests a possible involvement of 5-HT7R in the pathology, highlights the importance of performing these studies using human models of the disease.

POSTER ID: 2.16 - Developmental trajectories of neuroplasticity in mice after prenatal immune activation reveal critical windows for intervention

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Maternal health during pregnancy critically influences offspring susceptibility to neurodevelopmental disorders. The maternal immune activation (MIA) hypothesis proposes that inflammatory insults during gestation can disrupt fetal brain development, increasing the risk of neurodevelopmental disorders, including autism spectrum disorder. In this context, the present study aimed to identify potential critical developmental windows for future pharmacological interventions. MIA was induced in pregnant mouse dams by administration of the viral mimetic polyinosinic:polycytidylic acid (poly(I:C)) at gestational day 12.5, a well established model of long lasting alterations in brain function and behavior. We investigated the impact of prenatal MIA on neuroplasticity during postnatal development, focusing on adolescence and early adulthood postnatal day (PND) 40 and 60, respectively. Behavioral analyses confirmed an autism-like phenotype, with increased repetitive and compulsive-like behaviors: self-grooming was elevated at both time points, while marble burying was significantly increased at PND60. MIA induced neuroinflammatory and redox alterations, characterized by increased COX-2 expression and reduced antioxidant enzyme levels in the hippocampus, indicating a sustained imbalance between inflammatory and protective pathways. Synaptic plasticity was also affected, as shown by a dysregulation of the BDNF signaling pathway together with alterations in presynaptic and postsynaptic markers, reflecting persistent impairment of synaptic remodeling across development. In addition, ΔFosB, a marker of chronic neuronal activity and long-term transcriptional adaptation, was increased only at PND60, suggesting the emergence of late stage neuroplastic changes.
Overall, these findings demonstrate that prenatal immune activation leads to persistent, developmentally regulated alterations in synaptic plasticity, driven by the interaction between neuroinflammatory and redox mechanisms. Adolescence and early adulthood emerge as critical windows during which these alterations consolidate, highlighting a relevant period for targeted therapeutic strategies.

POSTER ID: 2.17 - Timing matters: maternal vs direct lactiplantibacillus plantarum supplementation shapes gut-brain inflammation and behavior, after early immune activation

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Exposure to immune challenges during sensitive developmental periods, such as pregnancy or early postnatal life, is a known risk factor for neurodevelopmental disorders, including autism spectrum disorder (ASD), often accompanied by gut inflammation and microbiota imbalance. In this study, we investigated whether food-derived Lactiplantibacillus plantarum (Lpb), a probiotic with anti-inflammatory properties, could counteract these alterations by influencing adult behavior, brain and gut inflammation.We used three mouse models of early-life immune activation [-prenatal (MIA), postnatal (PIA), and combined prenatal + postnatal (EIA)-] and compared two types of probiotic administration: indirect exposure through the mother during gestation and lactation, and direct post-weaning supplementation. Alongside behavioral analyses, we also examined neuroinflammatory markers, gut inflammation, and microbiota composition during adulthood.
Across both supplementation strategies, Lpb consistently increased locomotor activity and improved fear-conditioning performance. In parallel, sociability and ultrasonic vocalizations (USVs) were enhanced in Lpb treated males (irrespectively of Lpb timing), indicating a robust and consistent pro-social effect of the treatment. However, differences emerged among immune activation conditions; only EIA males showed reduced BDNF levels together with increased locomotor activity and elevated USVs, suggesting a more pronounced alteration of under this condition. These changes were restored when the probiotic was administered post weaning, not after maternal exposure. Multivariate analysis in females further highlighted that behavioral outcomes were shaped by the timing of exposure. Post-weaning supplementation was primarily associated with fear memory and anxiety-like behavior, whereas indirect maternal exposure was more closely linked to social behavior and preference for social novelty.
Overall, Lpb exerts broad beneficial effects on locomotion, emotional memory, sociability, and communicative behavior across developmental immune challenge models. However, the timing of administration could shape its impact: maternal exposure exclusively supports social behaviors, whereas post-weaning supplementation exerts wider effects modulating social- but also anxiety-related behavior, fear memory and BDNF levels.

POSTER ID: 2.18 - Study of Behavioural, Molecular, Morphological and Electrophysiological Effects of Chronic Stress in Mice

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Chronic stress profoundly affects brain function and represents a major risk factor for psychiatric and systemic disorders. Understanding its impact requires an integrated view that links behavioural, molecular, and neural mechanisms. Biological variables such as sex and circadian timing may shape stress responses, but how these factors interact with the underlying synaptic and functional changes remains unclear. This study investigates how chronic stress alters behaviour, hippocampal proteome composition, neuronal morphology, and brain activity, considering the modulatory influence of sex and circadian phase.
Male and female C57BL/6 mice were exposed to a Chronic Restraint Stress (CRS) paradigm (2 h/day for 21 days) during either the light or dark phase. Behavioural tests assessed depressive-, anxiety-, and anhedonia-like phenotypes. Based on behavioural readouts, we selected female mice for further functional, structural, and molecular studies. EEG recordings throughout the CRS protocol were used to assess stress-induced alterations in neural activity during emotional and cognitive tasks. Hippocampal dendritic architecture has being evaluated using Golgi-Cox staining, while hippocampal synaptic fractions underwent proteomic profiling to identify synaptic molecular pathways affected by stress.
We found a higher behavioural sensitivity to stress in female mice. Proteomic analyses identified stress-induced modulation of synaptic proteins, while morphological and electrophysiological investigations are expected to further clarify how chronic stress reshapes neuronal connectivity and activity dynamics.
By combining behavioural, proteomic, morphological, and functional approaches, this study provides an innovative integrated perspective on how chronic stress impacts on the brain. The results highlight the complex interplay among biological variables and molecular networks, offering new insights into mechanisms of stress in the brain.

POSTER ID: 2.19 - Neuroprotective effects of tumour necrosis factor-α-stimulated gene-6 (TSG-6) in a murine model of focal cerebral ischemia

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Tumour necrosis factor-α-stimulated gene 6 (TSG-6), a chemokine- and hyaluronate (HA)-binding protein, regulates immune and stromal cell functions in acute neurological disorders. Here, we aim to assess its modulation by cerebral ischemia and its involvement in brain pre conditioning (IPC) with the aim to understand its role in ischemic brain injury.
TSG-6 protein expression detected by western blotting was elevated (1.99-fold vs SHAM) in the ischemic cortex of adult male C57Bl6 mice subjected to 1-h MCAo followed by 24h of reperfusion, whereas pre-exposure (i.e., 72h before) to IPC (15 min MCAo) further potentiated this effect (2.25-fold vs. MCAo, $P<0.05$, ANOVA, n=6-8). Conversely, the TSG-6 plasma reduction (31.5% vs SHAM, $P<0.05$, ANOVA, n=6-8) observed at 24h of reperfusion was abolished by IPC, while IPC alone did not affect plasma levels of TSG-6. Based on their potential to regulate TSG-6, miR-23a and 23b were selected and real-time PCR analysis revealed their 1.8 and 1.7-fold elevation ($P<0.05$ vs SHAM, ANOVA, n=6-8) after MCAo in the plasma, correlating with TSG-6 reduction.
Elevation of endogenous TSG-6 levels by IPC resulted in amelioration of neurological deficits and reduction of brain infarct volume (MCAo: 92.9±3.9, IPC+MCAo: 70.6±3.8 mm3, $P<0.001$, t-test, n=12). Accordingly, intravenous administration of 30 µg of recombinant TSG-6 provided neuroprotection in ischemic mice (infarct volume in mm3: MCAo+vehicle: 102.0±6.8; MCAo+TSG-6: 72.4±10.6, $P<0.05$, t-test, n=6-7).
Thus, neuroprotection exerted by IPC is associated with the elevation of both central and peripheral TSG-6 protein levels, highlighting the relevance of this immunomodulatory protein as a promising therapeutic tool for ischemic stroke therapy.

POSTER ID: 2.20 - Activation of Stress Response Disrupts Network Activity and Synaptic Function in Shank3-Deficient Neurons

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Autism Spectrum Disorder (ASD) emerges from complex interactions between genetic predisposition and environmental influences during early development. Among high-confidence ASD genes, SHANK3 plays a central role in organizing excitatory synapses and regulating neuronal circuit assembly. Although SHANK3 haploinsufficiency alone can drive synaptic and behavioral abnormalities, environmental factors may substantially modulate neurodevelopmental trajectories, amplifying underlying genetic vulnerability. Adverse early-life experiences are increasingly associated with neurodevelopmental divergence, in part through epigenetic regulation of stress-response pathways.
To investigate the interaction between stress and Shank3 haploinsufficiency, primary neuronal cultures derived from wild-type and Shank3 mutant mice were exposed to corticosterone for 72 h to model stress responses. Corticosterone is widely used to mimic stress in vitro, as it activates glucocorticoid receptors and induces molecular and functional changes that parallel stress-related effects on neuronal development and synaptic function. Neuronal activity and synaptic functionality were assessed using a combination of multi-electrode array (MEA) recordings and whole-cell patch-clamp electrophysiology, allowing evaluation of both network-level dynamics and single-cell synaptic properties.
Corticosterone treatment induced marked functional alterations at multiple scales. MEA recordings revealed a significant reduction in global network activity, characterized by decreased firing rates, reduced burst frequency, and impaired synchronization across the neuronal network. At the single-neuron level, patch-clamp analyses showed alterations in intrinsic excitability and synaptic transmission. Together, these findings indicate a stress-induced weakening of neuronal communication and network integration, highlighting how glucocorticoid signaling can exacerbate synaptic and circuit-level vulnerabilities associated with SHANK3 deficiency during neurodevelopment.

POSTER ID: 2.21 - Targeting astrocytes with editing technologies to treat Alexander Disease

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Alexander disease (AxD) is a progressive and fatal leukodystrophy caused by gain-of-function mutations in the glial fibrillary acidic protein (GFAP), which encodes the main intermediate filament of astrocytes. Accumulation of GFAP aggregates in Rosenthal fibers leads to Central Nervous System (CNS) dysfunction with typical pathological traits, including demyelination and neuroinflammation. No cure is currently available for this fatal neurodegenerative disorder. Here, we aim at developing novel, single-dose gene editing strategies targeting Gfap as a lifetime treatment of AxD. We performed in vitro screening of single guide RNAs (gRNAs) targeting the Gfap gene and selected the best gRNA candidate inducing a robust GFAP knockdown. To optimize the in vivo brain-directed delivery of the Gfap-targeting CRISPR system, intracerebroventricular injections in mice defined the optimal AAV serotype, age of treatment, and glia-specific promoter to maximize transgene expression in astrocytes of AxD-affected brain regions. A single AAV carrying the Gfap-targeting gRNA and Staphylococcus aureus Cas9 nuclease was administered in neonatal Gfap+/R76H AxD mice, resulting in on-target editing in astrocytes (up to 30%), robust GFAP downregulation, reduced astrogliosis, and mitigated accumulation of Rosenthal fibers, a hallmark of AxD pathology, in white matter regions. To expand on the potential of gene editing as a treatment for AxD, we identified adenine base editors that correct the R76H Gfap mutation.
Overall, these data provide in vivo proof-of-concept of the efficacy of a CRISPR/Cas9 editing approach in ameliorating disease-associated phenotypes. Also, our results pave the way for the broadening of gene editing tools that apply mutation-specific treatment targeting the mutated Gfap allele in the CNS. Novel editing platforms for in vivo targeting of CNS astrocytes could benefit AxD and other neurodegenerative disorders characterized by primary astrocyte degeneration or dysfunctional/maladaptive astrogliosis.

POSTER ID: 2.22 - A High-Throughput Cell-Based BiFC Assay for Identifying Modulators of Monomeric α-Synuclein

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Alpha-synuclein (α-Syn) aggregation is a pathological hallmark of synucleinopathies, including Parkinson's disease and dementia with Lewy bodies. Due to its intrinsically disordered nature and strong propensity to misfold and aggregate, α-Syn represents a challenging therapeutic target. While most current strategies focus on aggregated species, modulation of monomeric α-Syn remains a promising yet underexplored approach to interfere with early pathogenic events. Here, we developed and validated a cell-based, high-throughput-compatible platform for the identification of small molecules that modulate monomeric α-Syn. The system is based on bimolecular fluorescence complementation (BiFC), using a split-GFP approach in which α-Syn is fused to the eleventh β-strand of GFP (GFP11) and complemented in trans by GFP1–10, allowing quantitative assessment of α-Syn levels in a cellular context.
A stable HEK293 Flp-In T-REx cell line expressing α-Syn–GFP11 under tetracycline control was generated and characterized by flow cytometry, high-content imaging, and Western blotting. The assay was implemented in a 384-well format and applied to screen ~2,200 compounds, including FDA-approved drugs and chemically diverse small molecules. Assay performance metrics ($Z' \geq 0.4$, CV < 20%) supported its robustness and suitability for high-throughput applications. The screen yielded multiple reproducible hits, including compounds previously reported to modulate α-Syn biology. Selected candidates were further validated through dose–response analyses using imaging and Western blot.
Overall, this platform provides a sensitive and scalable approach for the identification of α-Syn modulators and supports early-stage drug discovery targeting monomeric α-Syn.

POSTER ID: 2.23 - Fully human 2D models to study neurodevelopmental Disorders

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Neurodevelopmental disorders involve complex interactions between genetic, epigenetic, and environmental factors. Traditional animal models have provided invaluable insights, but often fall short in recapitulating human-specific cellular phenotypes due to species differences. Two-dimensional (2D) in vitro models, particularly those derived from human induced pluripotent stem cells (iPSCs), offer a powerful, ethically sound alternative. By differentiating iPSCs into neural progenitor cells or mature neurons, often via transcription factors such as NGN2, these monolayer cultures enable high-throughput screening of disease mechanisms, drug responses, and gene-editing outcomes. For instance, patient-derived 2D neurons reveal bridging the gap to personalized medicine while complementing emerging 3D organoid approaches. We recently optimized a generation protocol for glutamatergic neurons (iGluNeurons; Servetti et al., 2025; Servetti et al., 2026). To develop fully human 3D in vitro models, we are testing three protocols to generate human astrocytes (hAstros): (1) NGN2 induction, (2) differentiation from pluripotent stem cells, and (3) small-molecule-based methods. We evaluate hAstros via immunostaining and multi-electrode array (MEA), using commercial hAstros and rat astrocytes as controls. In parallel, we are generating GABAergic induced neurons using the ASCL1 construct and assessing outcomes via immunostaining and MEA. These efforts aim to create a fully human 3D in vitro model incorporating patient-derived components to study the mechanisms of neurodevelopmental diseases, paving the way for high-throughput disease modelling and drug screening in human-relevant systems.

POSTER ID: 2.24 - Arousal shapes history biases across timescales

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A growing body of evidence, rooted in predictive coding, suggests that perception arises from the integration of sensory input with prior expectations. As a result, perception is systematically biased by stimulus history. A key role in balancing sensory input and prior beliefs is played by the arousal system, which dynamically upweights new sensory evidence and supports belief updating. Here, we tracked pupil size, as a proxy for arousal, to test whether it predicts how stimulus history affects the perception of numerosity. We tested 49 participants, who verbally estimated the numerosity of dot arrays while their pupil size was continuously recorded. For each participant, we characterized the distribution of pre-stimulus pupil size and classified trials into high- and low-arousal states (relatively dilated and constricted pupils, respectively). Pupil size was systematically larger at the beginning of the experimental session and gradually decreased over time. By modeling and removing this global trend, we dissociated two pupillary indices: a session-level component (capturing slow pupil-size changes) and a trial-by-trial fluctuation component (capturing moment-to-moment arousal changes). The slower pupil-size changes correlated with global indices of performance, as more dilated pupils were associated with faster reaction times, reduced precision, and stronger central tendency—i.e., a greater regression of estimates toward the mean of the experienced numerosity distribution. In contrast, the trial-by-trial fluctuations specifically predicted the impact of recent stimulus history, with trials with relatively larger pupils (higher arousal) showing a stronger repulsive bias from the immediately preceding stimulus, consistent with an adaptation-like effect. Overall, we show that pupil-size changes over different timescales predict variations of perceptual performance and its dependence on stimulus history, at both a global level (precision and central tendency) as well as at a local level (serial dependency).

POSTER ID: 2.25 - Microglia-neuron crosstalk in the remodelling of peritumoral circuits in glioblastoma

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Glioblastoma (GBM) progression is sustained by bidirectional interactions between tumor cells and the tumor microenvironment (TME), where neuronal activity promotes tumor growth. Among TME components, microglia acquire tumor-supportive phenotypes, but their role in GBM-induced neuronal dysfunction and neuron–tumor communication remains unclear. GBM cells are also connected to each other and to neurons via microtubes enabling intra and intercellular communication via Ca2+-signaling. Here, we show that microglia are key drivers of peritumoral network hyperexcitability and neuron–GBM cells coupling. Patch-clamp recordings from layer 5 peritumoral neurons (PTNs) in GL261 GBM-bearing mice revealed increased excitability, characterized by depolarized resting membrane potential, reduced rheobase, and enhanced firing frequency. Pharmacological microglia depletion with PLX5622 (CSF1R inhibitor) restored neuronal excitability and reduced susceptibility to epileptiform activity. Mechanistically, glioblastoma decreased SK2 channel expression and medium afterhyperpolarization (mAHP), while microglia depletion rescued SK2 function, as confirmed by increased SK-mediated currents in PTNs, identifying SK2 channels as downstream effectors of microglia-dependent changes in neuronal excitability in GBM. Calcium imaging in GCaMP6-expressing GL261 cells revealed spontaneous Ca2+ activity partially driven by neuronal firing, as shown by tetrodotoxin sensitivity. This neuron-dependent component was abolished after microglia depletion, demonstrating that microglia are required for neuron-to-GBM cells calcium coupling. Consistently, PLX5622 treatment reduced tumor proliferation. Overall, our findings identify microglia as active regulators of neuronal excitability and neuron–GBM communication, promoting a hyperexcitable microenvironment that supports tumor progression. Targeting microglia may represent a novel strategy to restore neuronal homeostasis in glioblastoma.

POSTER ID: 2.26 - Do in vitro neurons learn? Network plasticity in 2D and 3D brain models through electrophysiological Pong training

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Background: iPSC derived-two-dimensional (2D) and three-dimensional (3D) in vitro brain models are increasingly used to recapitulate structural and functional features of the human nervous system. However, network connectivity and neuronal plasticity remain largely unexplored, despite being central to many neurological disorders.
Aim: This work aims to characterize the electrophysiological network activity of 2D and 3D neural cultures and to investigate how it reshapes in response to structured electrophysiological stimuli.
Methods: Cultures are placed on a 60-electrode MEA chip and recorded through a CL1 instrument (Cortical labs). Network plasticity is probed through repetitive sessions of a Pong-like paradigm. Prior to training, a single electrophysiological assessment is performed to identify fixed functional controlling areas — motor (paddle control), sensory (ball position encoding), and modulatory (reward/punishment stimuli). Each subsequent session consists of rest, silent, and active game phases. Training is performed daily for 4–5 consecutive days.
Results: 2D culture configurations and neural organoids derived from multiple iPSC lines have been characterized. Preliminary analyses compare signal and network features - including spike rate, burst dynamics, and inter-area connectivity - as well as Pong performance metrics such as hit/miss ratio and rally length. Results suggest differences across culture types in both baseline network properties and adaptive responses to the stimulation protocol, though further experiments are ongoing to consolidate these findings.
Future perspectives: This approach holds promise for application to disease-specific models – such as Alzheimer's disease and other complex neurological conditions - opening avenues to investigate plasticity and learning impairments, as well as the impact of pharmacological interventions on network connectivity and adaptability.

POSTER ID: 2.27 - Functional evaluation of novel Kv7 channel modulators in human iPSC-derived glutamatergic neurons using MEA

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Kv7 are voltage-gated potassium channels mainly expressed in neurons, that plays a critical role in regulating neuronal excitability. They are involved in epilepsy, depression and neurodegenerative diseases and represents an important therapeutic target. The Kv7 activator retigabine (ezogabine), was approved for epilepsy treatment, but was withdrawn due to its side effects. Recently, our research group identified two retigabine analogues: compound 60 (C60), a Kv7 activator, and compound 106 (C106), a Kv7 inhibitor. In the present study, we differentiated induced-pluripotent stem cells (iPSCs) in glutamatergic neurons, functionally characterized them using Multi-Electrode Arrays (MEA) recordings and evaluated the ability of these compounds to modulate neuronal excitability.iPSC line expressing doxycycline-inducible neurogenin 2 (NGN2) were differentiated in human excitatory neurons and co-cultured with primary mouse astrocytes. Immunofluorescence experiments were performed to assess neuronal differentiation by evaluating the expression of neuronal markers. Neuronal activity and network formation were analyzed using MEA recordings.Immunostaining at day in vitro 50 (DIV 50) confirmed the expression of neuronal markers such as Nestin and MAP2. Over time, we observed a progressive increase in spontaneous extracellular electrical activity. At DIV50, the percent of the active electrodes was 56±3%. As neuronal networks matured, they displayed synchronous bursting activity, indicative of functional synaptic connectivity. Analysis of network burst parameters showed that at DIV50 neurons exhibited synchronized bursting at a frequency of 0.21±0.05 Hz. Application of 0.1 µM of C60 reduced the percentage of active electrodes of about 84% and the frequency of synchronized network bursts of about 80%. In contrast, treatment with 0.1 µM of C106 increased both parameters. Our findings demonstrate that MEA is a powerful tool for the functional characterization of iPSC-derived glutamatergic neurons. Moreover, pharmacological modulation of Kv7 channels using activators and inhibitors significantly alters neuronal excitability and network activity, supporting their relevance as therapeutic targets in neurological disorders.

POSTER ID: 2.28 - Behavioral effects of a novel compound, prt, on pain and locomotion in a mouse model of neuropathic pain

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Background: Neuropathic pain (NP) is a complex chronic condition, which remains one of the most challenging types of pain to treat. NP significantly impairs quality of life, while current therapies often show limited efficacy or cause undesirable side effects. Therefore, there is a growing need to develop novel compounds with improved therapeutic profiles. The newly synthesised compound, PRT, exhibiting potential anti-inflammatory and analgesic properties, represents a promising candidate for further investigation in NP. The aim of the study was to evaluate the effects of repeated PRT administration on pain sensitivity and locomotor activity.
Methods: Male C57BL/6J mice underwent spared nerve injury (SNI) surgery to induce NP. Two weeks post-surgery, animals received the following treatments: PRT (30 mg/kg), ibuprofen (30 mg/kg), or vehicle, administered intraperitoneally three times daily for three consecutive days. Following the final administration, the animals underwent the von Frey test (mechanical hypersensitivity), the cold plate test (thermal hypersensitivity) and the open field test (locomotor assessment analysed with AnyMaze® tracking system).
Results: PRT slightly improved mechanical hypersensitivity at 15 and 45 minutes after the last dose, yet cold hypersensitivity was significantly reduced compared with vehicle-injected animals up to 180 minutes after the last drug administration. In the open field test, repeated administration of ibuprofen did not affect the locomotor activity; however, PRT significantly reduced total distance travelled and average speed of movement.
Conclusion: Repeated administration of PRT produced a significant reduction in thermal hypersensitivity and a modest effect on mechanical hypersensitivity in neuropathic mice. However, these analgesic-like effects were accompanied by a decrease in locomotor activity.
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POSTER ID: 2.29 - Proteomic and transcriptional profiling of iPSC-derived mesodiencephalic dopaminergic neurons reveals novel molecular targets and deregulated pathways in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder resulting from the complex interplay between genetic and environmental factors. Our group previously demonstrated a polygenic model of inheritance based on the combination of rare variants in PD-associated genes. In this study we aimed to identify deregulated molecular pathways and potential biomarkers in PD, using an integrated proteomic and transcriptional approach in human iPSC-derived dopaminergic neurons. Mesodiencephalic dopaminergic neurons (mdDA) were obtained after 45 days of in vitro differentiation of iPSCs derived from five healthy controls (CTR) and six PD patients carrying one or more PD-associated mutations. Proteomic profiling, by mass spectrometry, enabling the identification of 7844 proteins. Applying stringent thresholds based on fold change and statistical significance ($FC \leq 0.70$; $FC \geq 1.30$; $p \leq 0.01$), we detected 394 downregulated and 535 upregulated proteins. Among the most altered proteins, we observed strong downregulation of Calpastatin (CAST) and TPPP3, both involved in alpha-synuclein aggregation, and significant upregulation of LSM7, previously associated with PD, CXCR4 linked to neuroinflammation, and BCAT1 involved in branched-chain amino acid metabolism and previously reported as altered in PD plasma. Furthermore, Ingenuity Pathway Analysis (IPA) revealed significant alterations in processes mainly associated with mitochondrial function and glucose metabolism. Specifically, we observed widespread dysregulation of key enzymes involved in glycolysis (ENO2, ALDOA/C, PKM, PFKL), oxidative phosphorylation (CAT, UQCR10, COX7C), and mitochondrial dynamics (OPA1, MFN1, LONP1). These findings were also confirmed at the transcriptional level using Real-Time PCR (qPCR) and Digital PCR (ddPCR). Overall, Calpastatin, TPPP3, CXCR4 and BCAT1 emerged as potential novel biomarkers and pharmacological targets. Finally, our study expands current knowledge of PD mechanisms and highlight new molecular targets, supporting a polygenic model where rare variants contribute to disease. Ongoing experiments are assessing reduced CAST levels, calpains activity and alpha-synuclein accumulation under basal and stress conditions in PD-derived mdDA neurons.

POSTER ID: 2.30 - Identification of Master Regulators and novel genetic variants in Parkinson's disease through ARACNe3and NaRnEA Multi-omics integration

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Parkinson's disease (PD) is a complex and multifactorial neurodegenerative disorder with increasing prevalence, whose genetic basis is not yet fully understood, making it an optimal candidate for studies based on multi-omics integration. In this context, by integrating transcriptomic, proteomic and genomic data, we reconstructed the Gene Regulatory Network of the PD Substantia Nigra (ARACNe3), the brain region most affected in PD pathogenesis. Analysis of transcription factor-associated subnetworks (NaRnEA) identified a panel of key Master Regulators (MRs) driving gene expression deregulation in the Substantia Nigra. To biologically validate these bioinformatic results, we confirmed the cellular expression of the identified MRs by analysing their protein abundance through a proteomic dataset conducted on dopaminergic neurons differentiated from iPSCs of five healthy controls (CTR) and six PD patients. The results showed significant differences in the protein abundance of MRs between groups, supporting the relevance of the bioinformatic analysis. Furthermore, to evaluate whether our approach was able to detect new PD-associated genes, we selected a panel of 88 genes, from Whole Exome Sequencing (WES) data of 804 PD patients and 664 CTR samples. From this data we found and tested 1049 common variants (Minor Allele Frequency, MAF > 0.01) through an association analysis. This analysis identified 14 new genetic variants, in 10 genes, significantly associated with PD risk onset. Interestingly, some genes, including RORC, YY1, and SATB2, have been previously described in PD-related mechanisms. Notably, several associated variants show high deleteriousness scores, suggesting their potential impact on protein function. We plan to investigate the role of these genes and variants, evaluating the possible changes in their expression and alterations in the underlying molecular mechanisms and extend this analysis in our Single-cell data derived from differentiated neuron. Altogether, our multi-omics integration approach proved effective in uncovering new genetic determinants of complex diseases.

POSTER ID: 2.31 - TAAR1 Agonism in Fragile X Syndrome: Preliminary insights on its therapeutic potential

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Lack of Fragile X ribonucleoprotein 1 (FMRP) due to mutations in the X chromosome fragile X messenger ribonucleoprotein 1 gene (FMR1) causes Fragile X syndrome (FXS), which is characterized by neurodevelopmental abnormalities leading to intellectual disability and autism spectrum disorder. Currently, there is no cure for FXS. FMRP is expressed both in neurons, particularly during development, and in glial cells, with recent evidence suggesting that glial FMRP contribute to synaptic dysfunction in FXS. Trace amine associated receptor 1 (TAAR1) is a G-coupled protein receptor activated by endogenous and exogenous trace amines such as β-phenylethylamine, tyramine, 3-iodothyronamine and cyclohexylamine and possessing the ability to modulate the dopaminergic and glutamatergic system. TAAR1 is a promising target in the treatment of neurodevelopmental disorders such as schizophrenia. Based on this evidence, we have hypothesized its possible role as pharmacological target in FXS. In our experiments we used the Fmr1 knock-out (KO) mouse model of FXS, which exhibit different features of the disease including elevated susceptibility to audiogenic seizures and increased body weight. Our findings showed a reduction of TAAR1 mRNA expression in Fmr1 KO mice brain. Moreover, Fmr1 KO mice treated chronically with RO5263397, a selective agonist, showed a reduction of body weight and decreased severity of audiogenic seizures. On the other hand, in vitro assays on primary astrocytes investigating viability under inflammatory (Lipopolysaccharide) or oxidative stress (Hydrogen dioxide) challenges haven’t shown a significant modulation by the agonist in both Fmr1 KO and WT astrocytic cultures, suggesting that TAAR1 is not involved in cytoprotective pathways in this cell type. These findings provide an initial framework for proposing TAAR1 as target in FXS.
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POSTER ID: 2.32 - Age-dependent changes of metabotropic glutamate receptors expression in synapses and astrocytes in a Fragile X Syndrome mouse model

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Fragile X syndrome (FXS) represents a major genetic cause of intellectual disability and autism and arises from the loss of fragile X messenger ribonucleoprotein 1 (FMRP), which ultimately leads to altered synaptic plasticity and cellular dysfunction. Metabotropic glutamate (mGlu) receptors play a crucial role in modulating excitatory neurotransmission and have been implicated in the pathophysiology of FXS. mGlu5 receptor signalling has been extensively studied in FXS; however, the contribution of other mGlu receptor subtypes remains largely unexplored, despite their known involvement in cognitive disorders. The aim of this study was to characterize age-dependent alterations of mGlu receptor subtypes in synaptic and astrocytic compartments of the Fmr1 knockout (KO) mouse model. Expression of mGlu5, mGlu3 and mGlu7 receptors was analysed in cortical synaptosomes from young adult and aged mice and in astrocytes isolated by magnetic-activated cell sorting (MACS), while functional consequences were investigated in cultured mouse astrocytes. Our results show that mGlu5 and mGlu3 receptors display differential expression in MACS-isolated astrocytes and cortical synaptosomes from young adult and aged Fmr1 KO mice, whereas mGlu7 levels remained unchanged compared to WT controls. Activation of mGlu3 receptors influenced stress granule formation in Fmr1 KO astrocytes, consistent with its protective role in cellular stress responses. Overall, these findings reveal subtype-specific alterations of mGlu receptors in synapses and astrocytes in FXS and point to mGlu3 and mGlu5 as potential targets for modulating astrocytic dysfunction and age-related disease progression.
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POSTER ID: 2.33 - Fluorescent cortico-spinal assembloids for modeling TARDBP- associated ALS and TDP-43 dynamics

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting upper and lower motor neurons. Approximately 10% of cases are associated with pathogenic variants in TARDBP, which encodes the RNA/DNA-binding protein TDP-43. Mislocalization, aggregation, and intercellular propagation of TDP-43 are central hallmarks of the disease and contribute to neuronal dysfunction and degeneration. However, the mechanisms underlying corticospinal tract vulnerability and TDP-43 propagation remain poorly understood, partly due to the lack of human models capable of recapitulating long-range neuronal interactions. Here, we developed a human fluorescent corticospinal assembloid model to investigate TARDBP-associated ALS. Four fluorescent iPSC lines were generated: mutant and isogenic controls expressing either cytoplasmic mScarlet or GFP–TDP-43, enabling simultaneous assessment of cellular morphology and TDP-43 localization. Cortical and spinal organoid identity and maturation were validated using lineage-specific markers prior to fusion. Organoids were then combined to generate differently color-coded corticospinal assembloids, subsequently characterized using neuropathological and electrophysiological approaches. Fluorescent reporters enabled robust discrimination and tracking of mutant and isogenic control populations within multispectral assembloids, while resolving TDP-43 subcellular localization and cellular morphology. Fluorescence-activated cell sorting (FACS) identified a double-positive population across all assembloid conditions, with proportions varying according to assembloid type. Consistently, preliminary observations revealed that cells at the cortico-spinal interface in mixed mutant–control assembloids exhibited overlapping mScarlet and GFP–TDP-43 signals, suggesting intercellular redistribution of TDP-43, with potential differences across assembloid types. Human fluorescent corticospinal assembloids provide a versatile 3D platform to investigate mutation-associated cellular phenotypes and TDP-43 dynamics in ALS. This dual-reporter system represents a powerful tool for studying TDP-43 localization and intercellular interactions, offering new insights into disease mechanisms and corticospinal vulnerability.

POSTER ID: 2.34 - Studying stress in BV2 microglial cells: from model development to translational perspectives

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Chronic stress is a major risk factor for neuropsychiatric disorders. At the brain level, stressors elicit activation of the HPA axis and consequent glucocorticoid (GC) release. While acute GC signalling promotes adaptive responses through balance between mineralocorticoid (MR) and glucocorticoid (GR) receptors, chronic exposure leads to a dysregulated GR-driven state, consistently implicated in such conditions. Microglia, highly responsive to stress-related cues, act as mediators of the stress-induced neurobiological changes underlying neuropsychiatric disorders.
To model the impact of chronic exposure to stress on microglial function in vitro, the BV2 murine microglial cell line was treated for 96h with increasing concentrations of corticosterone (CORT), the principal GC in rodents, to mimic prolonged signalling. CORT treatment induced a GC profile consistent with chronic stress, as it exhibited an 'MR-GR switch' imbalance shifted towards GR. This was coupled with an impairment of its FKBP5-mediated negative feedback mechanism. In line with existing literature, these data support the model's validity and broader applicability for investigating stress-driven alterations across cellular pathways.
First, the inflammatory response was evaluated given microglia's role in neuroinflammation, key feature of neuropsychiatric conditions: CORT triggered a pro-inflammatory reaction characterised by increased interleukin-6 levels. While previous results showed a dose-dependent response across all tested concentrations, CORT induced a non-linear modulation of anti-inflammatory arginase-1, with upregulated expression at intermediate concentrations followed by a return to baseline, suggesting an adaptive response at lower stress levels, whereas the capacity to counteract the challenge might no longer be sustained beyond a certain threshold. A similar pattern was found for antioxidant catalase activity, which displayed a significant decrease only at higher concentrations.
Hence, this model may offer valuable insights into stress-related molecular changes and serve as a platform to evaluate potential therapies. Furthermore, it could complement existing in/ex vivo models to improve our understanding of such alterations in humans.

POSTER ID: 2.35 - Identification of novel variants in GRK5 Parkinson's disease gene: functional impact on protein stability, intracellular dynamics and autophagic response

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons, resulting from a complex interplay between genetic and environmental factors. In this study, we investigated the genetic role of rare and common variants in GRK5 gene in PD pathogenesis. Whole-exome sequencing (WES) data from a large cohort of PD patients and healthy controls were analyzed to identify and prioritize candidate variants based on allele frequency, predicted deleteriousness, and genetic burden. Among 44 identified common GRK5 variants (minor allele frequency, MAF > 0.01), one missense variant (p.P41L) showed significant association with PD ($p = 0.0005$; OR = 3.2) in our cohort and in an independent dataset ($p = 0.004$). Notably, eQTL analysis from the GTEx database revealed a significant correlation between p.Leu41 risk allele and increased GRK5 mRNA expression in the human brain substantia nigra. In addition, nine highly penetrant rare variants (MAF < 0.001), present only in cases, were identified within key functional domains of the protein. In silico analyses of vibrational entropy and interatomic interactions predicted that these variants exert destabilizing effects on the structure and dynamics of GRK5. To functionally characterize the GRK5 mutations, we generated GRK5-GFP plasmids carrying the identified variants by site-directed mutagenesis. Through subcellular localization analyses in SH-SY5Y and HeLa cells, under both basal and metabolic stress conditions (starvation), we observed altered intracellular distribution associated with specific variants. In GRK5-mutant cells, expression analyses revealed dysregulation of SQSTM1 and mTOR transcript levels under stress conditions, suggesting an impaired autophagic response and altered cellular homeostasis. Overall, these findings support a modulatory role of GRK5 in PD pathogenesis, linking genetic variability to altered protein stability and defective stress-response mechanisms. Future studies will validate these results in patient-derived dopaminergic neurons and investigate the interplay between GRK5 variants and BCL2 to explore the autophagy-apoptosis axis.

POSTER ID: 2.36 - Neuroinflammation-Driven Cognitive Impairment in a Parkinson's Disease Model: Synaptic Dysfunction and Therapeutic Potential of Pomalidomide

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by both motor and non-motor symptoms. Among the latter, cognitive impairment is particularly debilitating, often preceding motor deficits and significantly affecting quality of life (Backstorm, 2018; Garcia-Ptacek, 2016). Recent evidence implicates pathological α-synuclein (α-Syn) in triggering microglial reactivity and promoting neuroinflammation, which may drive synaptic dysfunction and cognitive decline (Palmas, 2022). To investigate these mechanisms and evaluate therapeutic strategies, we employed a PD rat model based on the intranigral infusion of human α-Syn oligomers (H-αSynOs). Three months post-infusion, H-αSynOs-infused rats exhibited cognitive deficits despite no overt neuronal loss in the anterior cingulate cortex (ACC). Histological analyses revealed marked neuroinflammation, including increased microglial TNF-α expression, along with mitochondrial dysfunction, evidenced by reduced neuronal COX4 levels. Immunofluorescence analyses demonstrated an increase in microglial phagocytosis of presynaptic Synapsin. This microglia-mediated engulfment correlated with impaired synaptic integrity, as indicated by decreased levels of synaptic proteins (PSD-95, Homer1, and Synapsin), reduced synapse number, decreased dendritic spine density, and a shift toward immature spine morphology. To address these alterations, animals were treated with pomalidomide (POM), an immunomodulatory imide drug known to modulate microglial states. Chronic POM administration significantly improved cognitive performance and restored key synaptic and neuronal markers. Synapsin and Homer1 expression was fully normalized, COX4 levels were partially recovered, and dendritic spine density markedly increased. Importantly, these effects were associated with reduced microglial TNF-α expression and decreased Synapsin engulfment, indicating attenuation of neuroinflammation and microglia-mediated synaptic loss. Taken together, these findings identify microglia-driven synaptic dysfunction as a key contributor to early cognitive decline in PD and support immunomodulation with POM as a promising therapeutic strategy.

POSTER ID: 2.37 - Disrupted Cystatin B-dependent mechanisms impair early human neuronal development

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To investigate the origin of neuronal hyperexcitability in EPM1, we analyzed iPSCs-derived cerebral organoids and identified a defect in ventral progenitor specification, associated with a shift toward excitatory neuronal lineages. This phenotype is associated with altered Sonic Hedgehog signaling, a key regulator of dorso-ventral patterning, which is required for proper ventral progenitor specification, suggesting that impaired morphogen availability contributes to the observed shift in neuronal identity (Forero et al., 2024).
Building on this, we investigated the molecular mechanisms underlying these alterations during human neuronal development. Interactome analysis performed on control cortical organoids revealed an interaction between CSTB and the TRiC/CCT chaperonin complex, specifically enriched in ventral lineages. We hypothesized that defects in CSTB-CCT interaction led to some of the cellular alterations in EPM1. Interestingly, disruption of this interaction at early stages of neuronal differentiation leads to defects in protein synthesis, cytoskeletal organization and vesicle trafficking, impairing extracellular vesicles (EVs) secretion. To functionally assess the role of the CCT-complex in EPM1, we performed CRISPRi-mediated silencing of the TCP1 subunit of the CCT complex in control cells, and strikingly, we detected the same EVs secretion defects characterizing EPM1 neurons. To further define the role of CSTB in this context, we performed rescue experiments in EPM1 cells. Intriguingly, restoring CSTB protein level in EPM1 cells rescues protein synthesis defects, while simultaneous silencing of one of the CCT-complex components, via CRISPR-i, abolishes this rescue. Thus, we demonstrated that CSTB functions critically depend on its interaction with the CCT-complex.
Together, these findings support a model in which CSTB-CCT cooperation regulates neuronal development by controlling intracellular protein synthesis, and potentially coupling EVs-release mechanisms with progenitor fate specification.

POSTER ID: 2.38 - Dysregulation of endosomal pathway driven by Synj1 overexpression affects neuronal homeostasis

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The endolysosomal system is a critical cellular hub. The ubiquitous inositol-phosphatase Synaptojanin1 (Synj1; locus 21q22.11), whose excessive expression was observed in the post-mortem brains of individuals with Down syndrome (DS), plays a critical role for the homeostasis and functions of early endosomes (EEs). Remarkably, Synj1-driven alterations of the structure and dynamics of EEs and impairment of recycling trafficking were found in trisomic fibroblasts, highlighting that they can contribute to the pathogenetic mechanism. However, the impact of the functional interplay between endosomal pathway and Synj1 on neuronal homeostasis is relatively unclear. By taking advantage of techniques to differentiate iPSCs into neural precursors (NPCs) and by applying deep imaging analysis combined with biochemical approach we show that the overexpression of Synj1 leads to an enlargement of EEs and perturbation of their dynamics in NPCs recapitulating the endosomal alterations observed in DS cells. Interestingly, Synj1 overexpression alters the typical morphological features of differentiated neurons. Of note, by transcriptomic analysis the expression levels of hundreds of genes, including key modulators of synaptic activity and neurogenesis, resulted deregulated upon SYNJ1 overexpression in the neuronal SH-SY5Y cells, altogether underscoring its critical role in maintaining neuronal structure and function. Consistently, the expression levels of some neural proteins (e.g., D2DR, TH) are perturbed in SH-SY5Y cells at both at steady-state and after terminal differentiation.
Overall, our data highlight a functional cross-talk among endolysosomal trafficking, Synj1 and neuronal homeostasis from one side, to the other point out a critical role of Synj1 in DS neuropathogenesis.

POSTER ID: 2.39 - Evaluating a Novel Therapeutic Agent as an Alternative to NSAIDs for Neuropathic Pain: Evidence from an SNI Mouse Model

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Background: Neuropathic pain remains a significant clinical challenge due to its complex underlying mechanisms and limited responsiveness to conventional pharmacotherapy. Commonly used analgesics such as NSAIDs are largely ineffective, and carry known risks of gastrointestinal and hepatic toxicity. This has driven the search for new, more targeted treatment strategies. PRT is a novel anti-inflammatory compound characterized by a highly favorable safety profile. Given the limitations of current NSAID therapies, PRT warrants thorough investigation as a potentially superior therapeutic alternative for neuropathic pain.
Objectives: This study compared the analgesic efficacy and systemic safety of PRT with a standard NSAID in the management of neuropathic pain.
Methods: Neuropathic pain was induced using the spared nerve injury (SNI) model of the sciatic nerve in male C57BL/6J mice. Subjects received intraperitoneal (i.p.) administration of PRT [30 mg/kg] three times per day for 3 days, while control groups were administered either a vehicle or ibuprofen [30 mg/kg]. Comparative pain outcomes regarding mechanical and thermal hypersensitivity were assessed using von Frey and cold-plate tests. Furthermore, post-mortem blood, pancreatic, and liver parameters were analyzed to compare the systemic and metabolic impacts of both compounds.
Results: PRT outperformed ibuprofen, showing robust, dose- and time-dependent reductions in mechanical and thermal hypersensitivity. While both treatments lowered AST levels, only PRT reduced LDH. Other systemic markers remained comparable, except for erythrocyte levels, which decreased solely in the ibuprofen group.
Conclusion: The present study demonstrates that PRT exerts a substantial analgesic effect on neuropathic pain, significantly outperforming ibuprofen while maintaining a comparable, yet safer, metabolic profile.
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POSTER ID: 2.40 - Endothelial Kv7.5 Channels Regulate Blood–Brain Barrier Integrity and Seizure Susceptibility

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Blood-brain barrier (BBB) disruption and consequent alterations of ion transport play a pivotal role in the onset and progression of many neurological diseases. Notably, BBB breakdown represents a risk factor for epilepsy, and seizures can damage brain endothelial cells (BECs), thus further exacerbating the disease. We recently demonstrated that KCNQ-encoded voltage-gated potassium Kv7 channels are expressed in BECs and regulate BBB permeability. Moreover, we showed that Kv7.5 was selectively reduced in brain microvessels (BMVs) from epileptic rats, and that pharmacological activation of Kv7 channels reduced BECs damage induced by kainic acid (KA). Building on these evidence, in this study we investigated if KCNQ5 deletion affected BBB function and seizure susceptibility in vivo, and endothelial permeability in vitro; moreover, we evaluated the possible modulatory effects of carnosic acid (CA), a rosemary-derived Kv7.5 opener. BMVs from kcnq5$^{-/-}$ rats displayed increased basal permeability, measured as fluorescein isothiocyanate (FITC)–dextran uptake, by about 45% compared to wt controls, accompanied by a selective reduced expression of the tight junction protein Zonula Occludens-1. When exposed to epileptogenic stimuli, kcnq5$^{-/-}$ rats required lower KA doses to reach status epilepticus and exhibited shorter seizure latency compared to wt animals. Interestingly, while KA markedly increased BBB permeability in wt rats, FITC-dextran uptake in Kv7.5-deficient animals remained unchanged, suggesting pre-existing maximal damage.
Consistently, KCNQ5$^{-/-}$ BECs derived from induced pluripotent stem cells displayed reduced trans endothelial electrical resistance values by about 40% compared to isogenic controls, suggesting impaired barrier function. Finally, CA treatment (10 mg/kg, 30 minutes prior the induction of seizures) reduced both seizure severity and BBB disruption in wt rats, whilst did not ameliorate seizure outcome in *kcnq5*$^{-/-}$ rats. Together, these findings identify Kv7.5 as a critical determinant of BBB integrity and seizure susceptibility, highlighting its potential as a therapeutic target in epilepsy and other disorders characterized by impaired BBB.

POSTER ID: 2.41 - Neuroprotective effects of Kv7 channel activators in experimental models of amyotrophic lateral sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron (MN) degeneration, muscle weakness, and respiratory failure. Multiple pathological mechanisms are involved, including excitotoxicity, oxidative stress, protein aggregation, and disruption of ionic homeostasis. In ALS, a reduction of Kv7-mediated potassium currents has been shown to contribute to MN hyperexcitability, an early hallmark of the disease. The Kv7 activator retigabine exerts neuroprotective effects in ALS-MNs, unfortunately, retigabine is no longer available for clinical use, due to toxicity unrelated to its mechanism of action. Therefore, the aim of this study was to evaluate the possible neuroprotective effects of two novel Kv7 activators identified by our research group, namely compound 60 (C60) and JNJ-37822681 (JNJ), in cellular models of ALS.
We generated stable NSC-34 motor neuron-like cell lines overexpressing either C9orf72 sequence or a mutated form of the superoxide dismutase-1 (SOD1-G93A), two gene-variants associated to ALS. Cell viability and proliferation were assessed by Water Soluble Tetrazole (WST) assays, intracellular reactive oxygen species (ROS) by fluorometric detection, and endoplasmic reticulum (ER) stress by quantifying C/EBP-homologous protein (CHOP) mRNA levels.
Overexpression of C9orf72 reduced NSC-34 viability by 40%, while SOD1-G93A clones showed a 40% decrease in proliferation. In both models, C60, JNJ, and retigabine did not rescue cell survival or proliferation. However, Kv7 activators markedly prevented the increase in intracellular ROS levels induced by acute exposure to H_2O_2 in both C9orf72 and SOD1-G93A models. Furthermore, C60, JNJ, and retigabine attenuated CHOP upregulation in NSC-34-C9orf72 cells, indicating a reduction in ER stress.
These results suggest that C60 and JNJ, by reducing ER stress and oxidative damage, may represent new potential therapeutic tools in ALS. Experiments on human iPSC-derived motor neurons carrying ALS-causing mutation and in vivo models of ALS are ongoing to further support our findings and validate their therapeutic potential.

POSTER ID: 2.42 - EXPLORING α-SYNUCLEIN/α-TUBULIN INTERPLAY IN A MOUSE MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder, primarily characterized by the loss of dopaminergic neurons in the *substantia nigra* (SN) and the formation of α-synuclein (α-Syn)-positive cytoplasmic inclusions termed Lewy bodies. Like other mostly sporadic diseases, PD etiology involves multiple factors, such as genetics, aging, immunity, and environmental exposures. These aspects could differentially impact men and women.
Since its identification as the first autosomal dominant gene for PD, α-Syn is known to play a key role in PD's pathogenesis. Several intracellular α-Syn partners, including microtubule cytoskeleton, appear to be crucial in α-Syn pathology. In human PD brains (Braak stage 6), changes in acetylated α-tubulin localization are associated with α-Syn aggregate formation, suggesting that α-tubulin acetylation could trigger α-Syn aggregation.
However, the longitudinal interplay between α-Syn and acetylated α-tubulin remains underexplored. Thus, taking advantage of a transgenic mouse model (*i.e.*, the SYN120 tg mouse), which expresses a mutant truncated and pro-aggregating form of α-Syn under the tyrosine hydroxylase promoter mimicking key features of PD pathology, this study aims to characterize this interplay in specific dopaminergic brain areas at different time points (8 and 13 months) in both sexes. Through immunofluorescence coupled with advanced confocal microscopy analysis, we describe nucleus specific, stage-dependent, and sex-related changes in the α-Syn/acetylated α-tubulin interplay within the SN and ventral tegmental area. By integrating regional and cellular analyses, we aim to identify early molecular and circuit-related signatures of PD onset and progression and their potential differences in the two sexes.
Preliminary results suggest that, in both sexes, acetylated α-tubulin rearrangement is involved not only in α-Syn aggregation pathways but also in neuronal aging itself. Further analysis will help elucidate the role of cytoskeletal components in both protein aggregation mechanisms and neuronal aging processes, as well as neurodegeneration.

POSTER ID: 2.43 - Rare variant combinations drive lipid–mitochondrial dysfunction and neuronal excitability defects in Parkinson's disease iPSC-derived neurons

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Parkinson's disease (PD) is characterized by a complex genetic architecture. Our group recently demonstrated a polygenic model of inheritance involving rare variants in novel PD-risk genes whose biological impact remains uncharacterized. To address this gap, we differentiated mesodiencephalic dopaminergic neurons derived from six iPSC lines from PD patients carrying rare highly penetrant variants and five healthy subjects, all expressing mature neuronal markers including TH, MAP2, VMAT2, and DAT.
To investigate the functional impact of these variants, we integrated electrophysiological analysis with proteomic and lipidomic profiling. Three out of six PD-derived lines, carrying mutations in *KIF21B*, *SLC6A3*/*HMOX2*, and *TMEM175/AIMP2*, showed more severe and convergent cellular dysfunction, suggesting a genotype-dependent effect in PD pathophysiology. Patch-clamp experiments revealed a ~20-fold reduction in membrane capacitance ($p<0.001$), impaired action potential firing and significant alterations in excitatory postsynaptic current amplitude and kinetics ($p<0.001$), indicating synaptic dysfunction in these three lines.
Multi-omics profiling revealed a convergent signature across the same three lines, centred on lipid mitochondrial dysfunction. Proteomic and expression analyses reflected increased levels of key enzymes including ENO1-2, PFKP, GPI, CS, and COX7C, involved in glycolysis and oxidative phosphorylation. Lipidomic analysis highlighted increased free fatty acids, acylcarnitines, and sphingolipids, with reduced gangliosides, a profile consistent with impaired mitochondrial metabolism and membrane plasticity directly explaining the observed electrophysiological dysfunction. Consistently, ATP quantification confirmed a 50% reduction at baseline, with further decreases upon stress conditions. Strikingly, altered circulating levels of Krebs cycle metabolites, malonate, pyruvate, and succinate, were confirmed in a cohort of 300 PD patients ($p<0.0001$), extending the cellular findings to a systemic metabolic signature.
Finally, whole-exome sequencing analysis identified variants in dysregulated metabolic enzymes associated with PD risk. Altogether, these results provide patient-derived neuronal signatures linking rare variant combinations to lipid–mitochondrial dysfunction and enable the discovery of candidate PD risk genes.

POSTER ID: 2.44 - Mevalonate Pathway Inhibition as a Radiosensitization Strategy in Group 3 Medulloblastoma

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Introduction: Medulloblastoma (MB) is the most frequent malignant pediatric brain tumor. Standard therapies are burdened by severe neurological sequelae, making the identification of specific molecular targets urgent. The mevalonate (MVA) pathway, crucial for protein prenylation and cell proliferation, is frequently dysregulated in oncology. This study evaluates the efficacy of pharmacological blockade of the MVA pathway as a targeted strategy for MB.
Material and method: Inhibitory efficacy was tested on three medulloblastoma cell lines (ONS76, D425, and MB002SU). Cell viability was quantified using the MTT assay following treatment with a panel of inhibitors operating at different levels of the MVA cascade: iPA (isopentenyladenosine), simvastatin, zoledronate, and naftifine. Furthermore, the expression of FDPS (Farnesyl pyrophosphate synthase), a key enzyme of the pathway, was evaluated via immunohistochemistry (IHC) to validate target presence.
Results and discussion: Viability assays revealed a highly heterogeneous efficacy among the compounds. Markedly superior results were obtained with iPA at a concentration of 10 µM and zoledronate at 30 µM, which induced a drastic and significant reduction in cell survival across all tested lines. Simvastatin and naftifine showed suboptimal or negligible effects. IHC analysis confirmed strong FDPS expression, justifying the dependence of these cells on the pathway's activity. However, although the iPA/zoledronate axis proves unequivocally cytotoxic in vitro, the translational perspective faces a severe clinical barrier: bisphosphonates like zoledronate exhibit high affinity for bone tissue and virtually zero blood-brain barrier (BBB) penetration. Achieving 30 µM concentrations in the in vivo brain tumor microenvironment is unfeasible with current formulations.
Conclusion: MVA pathway blockade via iPA and zoledronate sharply suppresses medulloblastoma viability in vitro, with a strong rationale supported by FDPS expression. Nevertheless, the actual therapeutic potential of these compounds for central nervous system tumors remains null in the absence of advanced drug delivery systems capable of breaching the BBB.

POSTER ID: 2.45 - Beyond the acute phase: long-term molecular scars and gut brain axis dysregulation following DSS colitis

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Inflammatory bowel disease (IBD) affects the central nervous system (CNS). However, the direct impact of peripheral inflammation on the CNS remains largely unknown. Emerging evidence suggests that inflammatory peripheral insults may impact on the risk for developing neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases. However, whether this gut-brain crosstalk represents a transient symptomatic sequel or a permanent shift toward neurological vulnerability remains an open question.
Using a murine model of dextran sodium sulphate (DSS)-induced colitis, we recently demonstrated that a single DSS challenge extends its impact to the CNS by disrupting the circadian clock and altering CNS fluid distribution. This cascade impairs glymphatic distribution and waste clearance, as well as the metabolic profile and, consequently, modulates neurotransmitter release. At the cellular level, peripheral inflammation disrupts neuro-astrocyte communication, driving synaptic dysfunction and ultimately impairing behavioral regulation.
Whether the CNS alterations can fully recover from peripheral inflammatory insults remains an unresolved question in the field. Within this context, we characterized the 28-day recovery period in DSS-challenged mice to differentiate temporary CNS symptoms from lasting systemic dysfunction. The brain shows a dynamic recovery where neuroinflammatory markers (NF-kB, IL-1β) and astrocytic proteins (AQP4, GFAP) normalize over time, demonstrating significant neural resilience. However, structural and metabolic scars persist, including lateral ventricle dilation and a sustained increase in complement C3, signaling chronic central immune dysregulation. Furthermore, prolonged reductions in brain taurine (Tau) and creatine (Cr+PCr) concentrations indicate a state of impaired energy homeostasis and reduced neuroprotection, which may predispose the CNS to delayed neuropsychiatric *sequelae*.
Our data define how a single intestinal insult initiates a prolonged window of neurological vulnerability. This new steady state may act as a primed substrate, altering how the organism responds to subsequent inflammatory challenges.

POSTER ID: 2.46 - Pupillometry Reveals Emotional Contagion Deficits and Arousal Regulation in CDKL5 Deficiency Disorder

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Emotional contagion (ECo) represents a fundamental component of empathy and has been extensively characterized in rodents. Socio-emotional impairments are often reported in neurodevelopmental disorders; therefore, characterizing ECo in translational mouse models is essential to bridge behavioral phenotypes with underlying neural circuitry. CDKL5 Deficiency Disorder (CDD) is a rare X-linked neurodevelopmental disorder characterized by early-onset seizures, severe developmental delay, intellectual disability, sleep disturbances, and autistic-like behaviors. CDKL5 knock-out mice, a translational model of CDD, exhibit hyperactivity and impulsivity, suggesting dysregulation of arousal systems that may impact socio-emotional processing. We recently demonstrated that pupillometry enables the quantification of ECo in mice by capturing both covert and overt changes in arousal and emotional processing. Using this approach, we investigated ECo in CDKL5 knock-out mice. We found that CDD mice display heightened responses to direct aversive stimulation but reduced sensitivity to the emotional state of a conspecific, indicating a dissociation between self-directed and socially driven emotional responses. Orexin signaling is a central regulator of the sleep-wake cycle and plays a key role in arousal and emotional modulation. We therefore investigated the effects of Suvorexant, a dual orexin receptor antagonist approved for insomnia, to explore its therapeutic potential in restoring arousal balance in CDD. Suvorexant reduced locomotor hyperactivity in CDKL5 knock-out mice without affecting wild-type littermates and significantly rescued the altered orienting response to visual stimuli, indicating improved arousal regulation. Together, these findings provide new insight into emotional and arousal dysfunctions in CDD, and provide a framework for future mechanistic and therapeutic studies.

POSTER ID: 2.47 - In vitro analysis of the anti-inflammatory properties of novel compound PRT

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Background: Glial cell activation is a central feature of neuroinflammation in central nervous system (CNS) pathologies. In response to injury, infection, or neurodegeneration, microglia and astrocytes shift toward reactive phenotypes, releasing pro-inflammatory factors that can exacerbate neuronal damage. This study analysed the influence of a new compound, PRT, on inflammatory mediators in primary microglial and astrocyte cultures to evaluate its potential to modulate central pain signalling.
Methods: In the *in vitro* experiments, primary microglia and astrocytes were treated with varying concentrations of PRT (1, 10, and 50 μM) alone or in combination with LPS (100 ng/mL) to induce an inflammatory response. Protein production of inflammatory mediators (IL-1β, IL-6, IL-10, TNFα) and the pERK1/2 pathway was subsequently analysed using ELISA and Western Blot assays. The compound was evaluated for cytotoxicity to assess its potential effects on cell viability (MTT assay).
Results: PRT exhibited a favourable safety profile, with no signs of cytotoxicity observed. Moreover, the protein induction measurements revealed a cell-specific action. While ELISA assays indicated that PRT did not significantly reduce the secretion of LPS-induced cytokines after 24 hours, Western Blot analysis showed that PRT significantly attenuated the LPS-induced increase of pERK1 and pERK2 protein levels in microglial cultures.
Summary: These findings suggest that PRT exerts targeted anti-inflammatory effects by modulating the ERK signaling pathway in microglia, establishing it as a promising therapeutic candidate for conditions driven by neuroinflammation.
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POSTER ID: 2.48 - BDNF and CNR1 as epigenetic biomarkers in bipolar disorder: insights from gene expression and DNA methylation analyses

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Bipolar disorder (BD) is a complex, multifactorial psychiatric condition whose diagnosis still relies predominantly on clinical criteria, highlighting the urgent need for reliable molecular biomarkers to enable early diagnosis and improved patient stratification. In this context, particular interest has been directed toward *CNR1*, encoding the type 1 cannabinoid receptor, and *BDNF*, a key regulator of neuroplasticity and neuronal survival. The present study investigated gene expression and epigenetic modulation of these two genes in order to elucidate the molecular mechanisms underlying BD. Analyses were performed on peripheral blood mononuclear cells (PBMCs) collected from BD patients at different clinical stages and from healthy controls. Gene expression was assessed by Real-Time qPCR, while DNA methylation was analyzed by pyrosequencing at specific CpG sites within the gene promoters of interest. Results show that both *BDNF* and *CNR1* are significantly downregulated in patients compared to controls. The downregulation of *BDNF* was confirmed after stratification by sex and clinical stage, being particularly pronounced at stage 3, whereas for *CNR1* the trend was maintained predominantly in males. At the epigenetic level, increased methylation of *BDNF* promoter I was observed, reaching significance especially in males, while *CNR1* displayed reduced methylation percentages with a similar pattern after sex-based stratification. Taken together, these findings suggest that *BDNF* and *CNR1*, along with their epigenetic profiles, may represent promising molecular biomarkers of BD, with potential implications for early diagnosis and patient stratification.

POSTER ID: 2.49 - Investigating DNER-IgG Pathogenicity in Paraneoplastic Cerebellar Ataxia via Patient-Derived Monoclonal Antibodies

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Paraneoplastic cerebellar ataxia (PCA) associated with Immunoglobulin G (IgG) targeting Delta and Notch-like epidermal growth factor-related (DNER) is a rare syndrome linked to Hodgkin lymphoma. DNER, a transmembrane protein, is highly expressed in Purkinje cells. However, the pathogenic mechanisms of DNER-IgG remain unknown.
We hypothesize that DNER-IgG induces altered membrane localization or internalization of DNER. To test this, we generated patient-derived DNER-specific monoclonal antibodies (mAbs) and are assessing their pathogenicity *in vitro* and *in vivo.* For mAb generation, we used LIBRA-seq on cerebrospinal fluid (CSF) B cells and *in vitro* differentiation of peripheral B cells followed by single-cell RNA and V(D)J sequencing. From the latter approach, V(D)J sequences from 15 *in vitro* expanded clones identified by scRNA-seq were cloned and expressed in HEK c18 cells, yielding 15 mAbs screened for DNER specificity. Additionally, LIBRA seq on 900 CSF cells identified 244 B cells; V(D)J sequences from 34 selected clones were similarly cloned, resulting in the first DNER-specific mAb (mAb301) ever produced from a LIBRA-seq positive human cell. DNER specificity of mAb301 was confirmed via live cell-based assays, immunofluorescence on mouse brain sections, and live staining on rat hippocampal neurons. Also, affinity assays on mAb301 demonstrated strong reactivity up to a 1:10,000 dilution. Ongoing internalization assays with DNER-transfected cells and pHrodo technology aim to evaluate the ability of mAb301 and patient serum to induce DNER internalization. To explore in vivo pathogenicity, mAb301 and patient IgG will be delivered via intracerebroventricular infusion using osmotic pumps in mice.
This study provides the first human recombinant DNER-specific mAb generated from a LIBRA seq–identified B cell from a patient with PCA and establishes a platform to investigate the pathogenic mechanisms of DNER-IgG. These findings may contribute to a better understanding of disease mechanisms and support the development of targeted therapeutic strategies.

POSTER ID: 2.50 - TIRESIA: Digital Twin, Immunity, And microRNAs In The Acute Phase Of Stroke Evolution

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Stroke is one of the leading causes of death and long-term disability worldwide. Despite advances in reperfusion therapies, clinical outcomes remain highly heterogeneous due to the complex cascade of events triggered after cerebral ischemia. In this context, small non-coding RNAs such as miRNAs have emerged as promising biomarkers and potential therapeutic targets. Moreover, Artificial Intelligence (AI) and Digital Twin technologies are opening new perspectives for personalized medicine and predictive modeling in neurological diseases.
The aims of this project are:
To identify and characterize circulating miRNA signatures associated with acute ischemic stroke in blood of both human patients and experimental mouse models, obtained through transient Middle Cerebral Artery Occlusion (tMCAO);
To integrate molecular data into an AI-based predictive model for the development of a patient-specific Digital Twin.
Ischemic stroke was induced in 8-week-old male C57BL/6 mice through tMCAO followed by reperfusion at different time points (1h, 3hrs, 24hrs after stroke). Blood and tissue samples were collected during the acute phase after ischemia. Selected stroke-related miRNAs involved in this pathological condition were then analyzed through Real-Time PCR. Preliminary results indicate a time-dependent regulation of specific miRNAs associated with post-ischemic neuroinflammatory responses. In particular, miR-let-7a and miR-143-3p were significantly upregulated during the early post-ischemic phase, in line with their pro-inflammatory activity and their involvement in impaired neuron–glia communication. Conversely, miR-107-3p showed an initial downregulation followed by a subsequent increase during reperfusion, suggesting its possible contribution to endogenous neuroprotection, vascular remodeling, and tissue-repair processes.
Overall, these findings suggest that the integration of miRNA-based molecular signatures with AI-driven Digital Twin technologies may represent an innovative strategy for identifying new biomarkers and pharmacological targets in ischemic stroke-related neuroinflammation.
Finally, TIRESIA project seeks to develop advanced predictive tools to support prognostic stratification and personalized therapeutic management in patients with acute ischemic stroke.