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Brayn conference

CATANIA 2026

30 SEPTEMBER • 2 OCTOBER

BOOK OF ABSTRACTS

9th BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

UNIVERSITÀ DI CATANIA - DIPARTIMENTO DI SCIENZE UMANISTICHE (DISUM)
AULA MAGNA MONASTERO DEI BENEDETTINI • PIAZZA DANTE 32, CATANIA

www.braynconference.com

Dear Young Neuroscientists,

Welcome to the **9th BraYn Conference – Brainstorming Research Assembly for Young Neuroscientists**, held this year in the vibrant city of Catania. Conceived by young researchers and built around their scientific curiosity, BraYn has grown into a **unique European meeting entirely dedicated to the next generation of neuroscientists**. Year after year, it brings together an increasingly diverse community with a shared ambition: to exchange ideas, build collaborations, and contribute to shaping the neuroscience of the future.

BraYn was founded on a simple conviction: some of the most exciting scientific advances emerge when disciplinary boundaries become permeable, different perspectives meet, and researchers at different stages of their careers engage in genuine scientific dialogue. This spirit continues to define our Conference.

The scientific program of the 9th edition reflects the extraordinary breadth of contemporary neuroscience, spanning molecular and cellular mechanisms, neural circuits and brain function, neurological disease, advanced technologies, and translational research. **Neuroinflammation**, **neurodegeneration**, **neuro-oncology**, **neurodevelopment**, **neurophysiology and neural plasticity**, **neuroimaging and clinical neurology** come together within the same scientific environment, encouraging connections that might otherwise remain unexplored.

This year, we are particularly pleased to further expand this scientific landscape with the introduction of a new session, **Cognitive Neuroscience and Neurotechnologies**, developed in collaboration with the **Italian Society of Psychophysiology and Cognitive Neurosciences (SIPF)**. The session brings cognition and emerging neurotechnologies into the interdisciplinary framework of BraYn, exploring how approaches ranging from neuroimaging and electrophysiology to non-invasive brain stimulation and multimodal methodologies can help us understand –and increasingly interrogate – the neural mechanisms underlying perception, memory, attention, decision-making, and behaviour.

Its introduction reflects the direction in which neuroscience itself is moving: toward an increasingly integrated understanding of the brain, in which molecular and cellular mechanisms, neural circuits, cognition, behaviour, technology, and disease can no longer be considered in isolation.

But BraYn is more than its scientific program.

It is a community in which young researchers are not simply an audience, but the protagonists of scientific discussion. It is a place to present new data, challenge ideas, meet future collaborators, learn from established scientists, and sometimes start projects that did not exist before entering the room. As we gather in Catania for our 9th edition, we invite you to make the most of these days: ask questions, discuss your science, cross disciplinary boundaries, meet people you do not yet know, and let your curiosity guide you.

Because the future of neuroscience will not emerge from a single discipline, laboratory, or generation. It will be built through connections, openness, creativity, and the courage to explore new ideas together. Thank you for being part of this journey and for bringing your science, enthusiasm, and curiosity to BraYn.

Welcome to BraYn Conference 2026.
Welcome to Catania.
Welcome to the next generation of neuroscience.

The BraYn Team

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CATANIA 2026 • 9th Edition

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Gaetano Gargiulo	Molecular Oncology Max-Delbrück-Center for Molecular Medicine (MDC) Berlin (Germany)
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Andrea Malaspina	UCL BRC Queen Square MND Centre, Institute of Neurology London (UK)
Emanuela Pasciuto	VIB-UAntwerp Center for Molecular Neurology Antwerp (Belgium)
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Morgane Thion	Institut Pasteur, Paris (France)
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COGNITIVE NEUROSCIENC & NEUROTECHNOLOGIES

Cognitive Neuroscience and Neurotechnologies session will explore how emerging and established neurotechnologies are transforming the investigation of human cognition. Advances in tools such as non-invasive brain stimulation, electroencephalography (EEG), neuroimaging, and multimodal approaches are enabling researchers to move beyond descriptive accounts of brain function and toward a mechanistic and causal understanding of cognitive processes. This session will focus on core domains of cognitive neuroscience, including attention, memory, executive functions, perception, and decision-making, highlighting how neurotechnological approaches can be used to probe and modulate brain activity at different spatial and temporal scales. Particular emphasis will be placed on the integration of experimental paradigms with advanced methodologies to investigate brain dynamics, network-level interactions, and mechanisms of neural plasticity. The session will also address translational and applied perspectives, discussing how neurotechnologies can contribute to the understanding and modulation of cognitive dysfunctions in neurological and psychiatric conditions. By bridging basic research, methodological innovation, and clinical applications, this session aims to provide a comprehensive overview of current advances and future directions in cognitive neuroscience. This session is co-organized and endorsed by the Italian Society of Psychophysiology and Cognitive Neurosciences – Società Italiana di Psicofisiologia e Neuroscienze Cognitive.

NEURODEGENERATION

Neurodegeneration is a scientific session focused on key aspects of a large number of diseases characterized by progressive damage of the nervous system that leads to irreversible neuronal death. The ND session will update on the more recent advances in the field. Specifically, the session will mainly focus on Parkinson's disease (PD), a slowly progressive syndrome that begins insidiously, gradually worsens in severity, and usually affects one side of the body before spreading to involve the other side, and Alzheimer's disease (AD), the most common type of dementia and an irreversible, neurodegenerative and progressive central nervous system disorder that slowly destroys memory and thinking skills, and, eventually, other mental abilities. Other neurodegenerative diseases, such as tauopathies, narcolepsy, depression and psychiatric disorders, will be also discussed in this session.

NEURODEVELOPMENT

Neurodevelopment session will cover the mechanisms and the genetics underlying human and animal brain development in health and disease. This session will focus on neurodevelopmental disorders, including autism spectrum disorder, intellectual disability, and epilepsy. The session will also discuss about the contribution of both genetic and environmental factors, as well as the role of neuronal and non-neuronal cells in the etiopathology of these disorders. This session will delve into the genetics and the molecular and cellular neurobiology underlying brain development. In this session we will also discuss on the implementation of novel therapeutic strategies to tackle neurodevelopmental disorders.

NEUROINFLAMMATION

Neuroinflammationis is a scientific session focused on exploring the mechanisms of the inflammatory response initiated in the central nervous system (CNS) by resident cells or triggered by infiltrating immune cells, which causes the neuronal dysfunctions observed in inflammatory and neurodegenerative disease of the CNS. The NI session mainly focuses on basic and clinical research in multiple sclerosis (MS), Neuromyelitis Optica Spectrum Disorder (NMOSD), Myasthenia gravis (MG) and other inflammatory diseases of the CNS that have a significant impact on the lives of young adults. Although the scientific discoveries of recent decades have improved the therapeutic approaches used for the treatment of such pathologies, many questions still remain unanswered. The NI session aims to discuss the basic pathogenic mechanisms governing CNS inflammation, the role of immune system in CNS autoimmunity, and the importance of genetic and environmental factors in the development of neuroinflammatory diseases, with a patient-centered focus.

NEUROIMAGING & CLINICAL NEUROLOGY

Neuroimaging & Clinical Neuroloy is a comprehensive scientific session exploring the intersection of advanced neuroimaging techniques and clinical neurology applications. This session delves into the utilization of various neuroimaging methodologies to probe the structure, function, and physiology of the nervous system, alongside the translational aspects of clinical neurology. The session covers two primary neuroimaging approaches: structural imaging, which aids in the diagnosis of large-scale intracranial diseases and injuries, and functional imaging, crucial for diagnosing metabolic diseases like Alzheimer's and facilitating neurological and cognitive psychology research. Techniques such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Electroencephalography (EEG), and Positron Emission Tomography (PET) will be discussed in the context of their applications alone or in combination to investigate neurological diseases. Moreover, the session emphasizes the integration of neuroscience data and basic research with clinical neurology to enhance understanding and treatment of nervous system disorders.

NEURO-ONCOLOGY

Neuro-Oncology is a scientific session focused on tumors of the central nervous system, addressing the biological mechanisms that drive their initiation, progression, and therapeutic resistance. Positioned at the interface between neurobiology and oncology, this session explores how brain tumors interact with and remodel the neural microenvironment to sustain growth, invasion, and survival. Particular attention will be given to neuron–tumor communication, circuit and synaptic alterations, and the contribution of glial and immune components within the brain tumor niche. The session will also include brain metastases and the mechanisms that regulate adaptation and growth within the central nervous system. By integrating mechanistic insights from neuroscience with translational neuro-oncology, this session aims to highlight brain-specific vulnerabilities and support the development of innovative therapeutic strategies for tumors of the nervous system.

NEUROPHYSIOLOGY & NEURAL PLASTICITY

Neurophysiology & Neural Plasticity session will cover the molecular mechanisms underlying the function of the nervous system and the capacity of the nervous system to modify itself, functionally and structurally, in response to experience and injury. All levels of function and plasticity changes will be discussed, from receptors and cellular physiology to systems and behaviour. This session will delve into approaches such as molecular and cellular neurobiology, functional neuroanatomy, neurochemistry, neuropharmacology, electrophysiology, and behavioural analysis, in both in-vivo, ex-vivo and in-vitro models in invertebrate or vertebrate species, including humans.

Day 1 • SEPTEMBER 30

09:30 Registration

10:15 Opening Ceremony (Chairpersons: G. Ferrara, G. Calabrese, N. Iraci, G. Giglia)

BRAYN STARTING GRANT SESSION

Chairpersons: V. Chiurchiù, M. Catalano, P. Infante

10:45 Starting Grant 2025 Winner | **Francesca Dragoni**
Promoter and 5'UTR Methylation profile of MAP1B and TARDBP in sporadic Amyotrophic Lateral Sclerosis: A Longitudinal Study across Bulbar and Spinal Phenotypes.

11:00 Lectio Magistralis | **Takao Hensch** (Chairwoman: E. Vannini)
Unlocking critical periods of brain development.

12:00 Lunch Break

SESSION 1 • NEURODEVELOPMENT ORAL COMMUNICATIONS

Chairpersons: E. Tagliatti, A. Borreca, E.S. Maftei

13:00 Lecture | **Morgane Thion** (Chairwoman: E. Tagliatti)
From Colonization to Vascular Guardians: Microglia in Early Brain Development.

13:30 **Veronica Krenn** • *Modelling Neuroimmune Interactions During Human Brain Development Using Brain Organoids.*

13:45 **Francesca Puleo** • *Targeted mitochondrial modulation in different cell-types rescues cognitive deficits in prenatal THC-exposed rats.*

14:00 **Chiara Calabretta** • *Spatial Molecular Profiling by MALDI-MSI to Support Histopathological Diagnosis in Drug-Resistant Epilepsy.*

14:15 SpeedTalk | **Vanessa Aragona** • *Unlocking the guardian of the brain: Choroid Plexus and neurodevelopmental insights.*

14:20 SpeedTalk | **Lorenzo Cifarelli** • *Brain-wide mapping of perineuronal nets and parvalbumin-expressing neurons across postnatal development identifies age- and sex-dependent circuit alterations in behaviorally characterized Fmr1 KO mice.*

14:25 SpeedTalk | **Aurora D'Alessio** • *Early butyrate rescues mucus barrier dysfunction in a preclinical model of autism spectrum disorder.*

14:30 SpeedTalk | **Mariagrazia Longo** • *RENKCTD11 loss drives cerebellar dysfunction and behavioral deficits in neurodevelopmental disorders.*

SESSION 2 • NEURO-ONCOLOGY
ORAL COMMUNICATIONS

Chairpersons: E. Vannini, E. Stanzani, M. Velasco, B.F. Tremonti

14:35 Lecture | **Gaetano Gargiulo** (Chairwoman: E. Vannini)
(Targeting) cell states in glioblastoma.

15:05 **Xingzi Lin** • *Rewiring anti-tumor immunity: fecal microbiota transplantation activates NK cells to control glioma in a mouse model.*

15:20 **Pablo Blanco Carlón** • *The mechanical landscape of GBM: How Piezo1 converts force into cancer.*

15:35 **Marta Scalera** • *Reinforcing peritumoral GABAergic inhibition modulates Glioblastoma progression and enhances immunotherapy efficacy.*

15:50 **Alessandra Maria Vitale** • *From Multimodal AI to Biological Twins: Integrating Deep Learning on Magnetic Resonance Imaging and Histological Data with Patient-Derived 3D Glioblastoma Models for Precision Medicine.*

16:05 **Daniele De Pasquale** • *Biomimetic Fluidic Model of the Glioblastoma-Affected Neuro-vascular Unit as an Advanced In Vitro Platform.*

16:20 SpeedTalk | **Arianna Ioni** • *Modulation of Extracellular Vesicles release targeting A2A Adenosine Receptor in Glioblastoma.*

16:25 SpeedTalk | **Elisa De Santis** • *The FLASH Effect: Assessing Survival and Functional Brain Integrity.*

16:30 SpeedTalk | **Oliviero Leonardi** • *Fluorescence-traced spatial profiling of tumor-neuron interactions in glioma-like assembloids.*

16:35 SpeedTalk | **Antonella Lettieri** • *Unraveling STAG2-Driven Neuro-Oncogenesis in Medulloblastoma for new therapeutic targeting.*

16:40 SpeedTalk | **Nicoletta Palermo** • *Targeted Temozolomide delivery by Hyaluronic Acid-gold nanoparticle–liposome enhances antitumor activity in 2D and 3D models of Glioblastoma.*

16:45 SpeedTalk | **Sebastian Andres Garcia Mena** • *Investigating non-genetic drivers of intra-clonal competition in PDGF-driven glioma using a multiomic barcoding strategy.*

16:50 Coffee Break

SESSION 3 • COGNITIVE NEUROSCIENCE AND NEUROTECHNOLOGIES ORAL COMMUNICATIONS

Chairpersons: G. Giglia, G. Gambino, V.F. Dattola

17:30 Lecture | **Vincent Van De Ven** (Chairman: G. Giglia)
A Network Perspective of Non Invasive Brain Stimulation.

18:00 **Sean Arthur Cully** • *Divergence between MP2RAGE and MPRAGE for detecting learning-induced structural brain changes.*

18:15 **Umberto Quartetti** • *a-tDCS Over Ventral Premotor Cortex Selectively Enhances Processing Of Goal-Oriented Biological Motion.*

18:30 SpeedTalk | **Aurora Meli** • *Modulation of executive functions in healthy humans through Opuntia ficus-indica fruit consumption and non-invasive brain stimulation.*

18:35 SpeedTalk | **Anthony Faiola** • *Investigating the Feasibility and Efficacy of a Virtual Reality Cognitive Training Platform for Post-Stroke Patients with Mild Cognitive Impairment.*

18:40 Poster Session 1 & Social Networking

20:40 Closing remarks

Day 2 • OCTOBER 1

SESSION 4 • NEURODEGENERATION
ORAL COMMUNICATIONS

Chairpersons: M. Medelin, E. Battocchio, G. Pollaci

09:00 Lecture | **Soyon Hong** (Chairwoman: E. Battocchio)
Neuro-glia-immune mechanisms of synapse loss.

09:30 **Ilaria Tagliabue** • *Chronic GCase inhibition induces lipid remodeling and neurodegenerative alterations in human midbrain organoids.*

09:45 **Irene Giustolisi** • *Preclinical development of an optimized bicistronic lentiviral vector enabling effective HSC Gene therapy for GM2 gangliosidoses.*

10:00 **Raluca Elena Abalai** • *Progressive Sensory Dysfunction Driven by Altered Skin-Nerve Remodeling in the Flvcr1 P221S Mouse Model.*

10:15 **Michelle Brilka** • *TWIK2 as an immunomodulatory factor in EAE.*

10:30 **Cecilia Delprete** • *Cutaneous phosphorylated alpha-synuclein topography across synucleinopathies: a distal gradient and MSA-specific compartment signature.*

10:45 **Federica Carrillo** • *Rare variant combinations drive convergent lipid-mitochondrial dysfunction in Parkinson's disease patient-derived dopaminergic neurons.*

11:00 **Martina D'Assoro** • *Targeting hyperphosphorylated Tau with intrabodies to inhibit pathological Tau aggregation.*

11:15 BraYn Educational Symposium ▶ **BD Biosciences**
Giusy Capuano • *Unveiling the Hidden Biology: a new-to the world Spectral Technology with Real-Time Imaging to reveal new dimensions in Flow Cytometry.*

11:30 SpeedTalk | **Diana Ioana Boiangiu** • *Niclosamide improves neuroprotective pathways in a C9orf72 mouse model of ALS/FTD.*

11:35 SpeedTalk | **Martina Milani** • *Early microglial dysfunction is associated with altered cerebellar synaptic development in Friedreich's Ataxia.*

11:40 SpeedTalk | **Elisabetta Pasella** • *Plasma and CSF Biomarker Profiling in Cerebral Amyloid Angiopathy: Correlations with Clinical and Neuroradiological Features.*

11:45 SpeedTalk | **Merve Begüm Bacınoğlu** • *Prion detection in the urine of individuals at genetic risk for fatal familial insomnia.*

11:50 SpeedTalk | **Marta Di Fabrizio** • *Unraveling myelin abnormalities in synucleinopathies.*

11:55 SpeedTalk | **Giuseppe Caruso** • *Development of an in vitro human brain-based 3D system to study the modulation of neuroinflammatory mechanisms and carnosine neuroprotective activity.*

12:00 Poster Session 2 & Lunch break

SESSION 5 • NEUROINFLAMMATION
ORAL COMMUNICATIONS

Chairpersons: M. Bottero, A. Baldassarro, M. Rasile, G. Carrera

14:30 Lecture | **Emanuela Pasciuto** (Chairman: V. Chiurchiù)
Harnessing the therapeutic potential of brain resident T cells to reduce neuroinflammation.

15:00 **Veronica Ceci** • *The Choroid Plexus as a hub of defective resolution mechanisms in Multiple Sclerosis.*

15:15 **Valentina Salari** • *Selective inhibition of the p2x7 receptor in microglia as a novel therapeutic strategy for epilepsy.*

15:30 BraYn Educational Symposium ▶ **Beckman Coulter**
Valerio Chiurchiù • *Decoding inflammation-resolution failure by high-dimensional flow cytometry in Parkinson's disease.*

15:45 BraYn Educational Symposium ▶ **Beckman Coulter**
Marta Pirronello • *High-parameter flow cytometry uncovers Marginal Zone–Like B cells as Cladribine's main target in Multiple Sclerosis.*

16:00 **Antonella Iaconis** • *Chronic CMIT/MIT Inhalation Disrupts the Lung–Brain Axis and Promotes α-Synuclein-Associated Dopaminergic Neurotoxicity.*

16:15 **Giulia Rossi** • *Neuroinflammatory modulation as a strategy to target neuronal pathogenic mechanisms in Huntington's Disease.*

16:30 Lecture | **Clara Grazia Chisari** (Chairmen: V. Chiurchiù, G. Ferrara)
Role of Immunomodulation in Immune-Mediated Demyelinating Disease: Current Perspectives.

16:45 BraYn Educational Symposium ▶ **Revvity**
Carmen Marangoni • *Simplifying Neurodegenerative and Neuroinflammatory Biomarker Quantification with HTRF and AlphaLISA assays.*

17:00 SpeedTalk | **Gemma Gorla** • *NGAL and VCAM-1 Are Upregulated in Cervical Arterial Dissection: A Plasma Biomarker Study.*

17:05 SpeedTalk | **Lucrezia Floro** • *Crosstalk between APOE ε4 Genotype, PPARγ, and Astrocyte Reactivity in Alzheimer's Disease Progression.*

17:10 SpeedTalk | **Su-Chun Huang** • *Longitudinal Characterization of Visual Pathway Involvement in Two Novel OSP- and MOBP-induced Experimental Autoimmune Encephalomyelitis Models.*

17:15 Closing remarks

Day 3 • OCTOBER 2

SESSION 6 • NEUROPHYSIOLOGY & NEURAL PLASTICITY
ORAL COMMUNICATIONS

Chairpersons: G. Calabrese, E. Boda, V. Castagnola

09:30 Massimo Addesse • *Dissecting Foxg1 Dependent Engram Regulation Through Genetic Manipulation and Network Profiling.*

09:45 Francesca De Chirico • *Bacteriophage M13 as a Precision Nanoplatform for Crossing the Blood–Brain Barrier.*

10:00 Ester Bruno • *Mapping histamine release sites: a 3D computational analysis of histaminergic dendritic varicosities.*

10:15 Sebastiano Alfio Torrisi • *Memantine prevents acute stress-induced memory deficits by reversing sex-dependent glutamatergic alterations in the dorsal hippocampus.*

10:30 Lecture | **Luca Peruzzotti-Jametti** (Chairman: M. Cambiaghi)
The Immunometabolic Landscape: How Microglial Metabolism Shapes CNS Inflammation.

11:00 Coffee Break

11:30 SpeedTalk | Silvia Pascarella • *Early-Life Stress Shapes Cerebellar Circuits Associated with Opioid Use Disorder Vulnerability.*

11:35 SpeedTalk | Sarah Grippi • *From Hyperexcitability to Drug Resistance: integrating behavioral and electrophysiological biomarkers in a longitudinal pilocarpine model of Temporal Lobe Epilepsy.*

11:40 SpeedTalk | Giuseppe Chiantia • *Alterations of GABAergic transmission in models of neurotoxicity and neurodevelopmental disorders: molecular, synaptic, and functional mechanisms.*

11:45 SpeedTalk | Giacomo Mazzoni • *Selective upregulation of Nav1.6 underlies PRRT2-linked cerebellar dysfunction.*

11:50 SpeedTalk | Maria Ciuro • *Smoothened modulation affects disease progression in SOD-1G93A mouse model of amyotrophic lateral sclerosis.*

SESSION 7 • NEUROIMAGING & CLINICAL NEUROLOGY ORAL COMMUNICATIONS

Chairpersons: V. Pieri, A. Bombaci, U. Manera, S. Tafaro

11:55 SpeedTalk | **Giulia Vasirani** • *Early cladribine administration prevents connectomic and molecular changes in experimental multiple sclerosis.*

12:10 SpeedTalk | **Jinlong Liu** • *Functional Network Reorganization Following VIM-MRgFUS for Parkinson's Disease.*

12:15 SpeedTalk | **Mahan Shafie** • *Network-Level Genetic Organization of Human Cortical Folding.*

12:20 Poster Session 3 & Lunch break

14:30 Lecture | **Andrea Malaspina** (Chairman: U. Manera)
New treatments and biomarkers for MND.

15:00 **Sara Cabras** • *Brain metabolic correlates of plasma biomarkers in Amyotrophic Lateral Sclerosis.*

15:15 **Giuseppe Ciullo** • *Differential recruitment of the thalamic ventral intermediate nucleus during observation and execution of goal-directed grasping actions.*

15:30 **Gemma Gorla** • *Biomarker Discovery in Sneddon Syndrome: an Exploratory Plasma Proteomic Study.*

15:45 Closing remarks & BraYn Awards
(Chairpersons: G. Ferrara, M. Romeo, G. Calabrese, N. Iraci, G. Giglia, M. Catalano)

SESSION 1 • NEURODEVELOPMENT

Veronica Krenn
Modelling Neuroimmune Interactions During Human Brain Development Using Brain Organoids

Francesca Puleo
Targeted mitochondrial modulation in different cell-types rescues cognitive deficits in prenatal THC-exposed rats

Chiara Calabretta
Spatial Molecular Profiling by MALDI-MSI to Support Histopathological Diagnosis in Drug-Resistant Epilepsy

SESSION 2 • NEURO-ONCOLOGY

Xingzi Lin
Rewiring anti-tumor immunity: fecal microbiota transplantation activates NK cells to control glioma in a mouse model

Pablo Blanco
The mechanical landscape of GBM: How Piezo1 converts force into cancer

Marta Scalera
Reinforcing peritumoral GABAergic inhibition modulates Glioblastoma progression and enhances immunotherapy efficacy

Alessandra Maria Vitale
From Multimodal AI to Biological Twins: Integrating Deep Learning on Magnetic Resonance Imaging and Histological Data with Patient-Derived 3D Glioblastoma Models for Precision Medicine.

Daniele De Pasquale
Biomimetic Fluidic Model of the Glioblastoma-Affected Neurovascular Unit as an Advanced In Vitro Platform

SESSION 3 • COGNITIVE NEUROSCIENCE & NEUROTECHNOLOGIES

Sean Arthur Cully
Divergence between MP2RAGE and MPRAGE for detecting learning-induced structural brain changes

Umberto Quartetti
a-tDCS Over Ventral Premotor Cortex Selectively Enhances Processing Of Goal-Oriented Biological Motion

SESSION 4 • NEURODEGENERATION

Ilaria Tagliabue
Chronic GCase inhibition induces lipid remodeling and neurodegenerative alterations in human midbrain organoids

Irene Giustolisi
Preclinical development of an optimized bicistronic lentiviral vector enabling effective HSC Gene therapy for GM2 gangliosidoses

Raluca Elena Abalai
Progressive Sensory Dysfunction Driven by Altered Skin-Nerve Remodeling in the Flvcr1 P221S Mouse Model

Michelle Brilka
TWIK2 as an immunomodulatory factor in EAE

Cecilia Delprete
Cutaneous phosphorylated alpha-synuclein topography across synucleinopathies: a distal gradient and MSA-specific compartment signature

Federica Carrillo
Rare variant combinations drive convergent lipid-mitochondrial dysfunction in Parkinson's disease patient-derived dopaminergic neurons

Martina D'Assoro
Targeting hyperphosphorylated Tau with intrabodies to inhibit pathological Tau aggregation

SESSION 5 • NEUROINFLAMMATION

Veronica Ceci
The Choroid Plexus as a hub of defective resolution mechanisms in Multiple Sclerosis

Valentina Salari
Selective inhibition of the p2x7 receptor in microglia as a novel therapeutic strategy for epilepsy

Antonella Iaconis
Chronic CMIT/MIT Inhalation Disrupts the Lung–Brain Axis and Promotes α-Synuclein-Associated Dopaminergic Neurotoxicity

Giulia Rossi
Neuroinflammatory modulation as a strategy to target neuronal pathogenic mechanisms in Huntington's Disease

SESSION 6 • NEUROPHYSIOLOGY & NEURAL PLASTICITY

Massimo Addesse
Dissecting Foxg1 Dependent Engram Regulation Through Genetic Manipulation and Network Profiling

Francesca De Chirico
Bacteriophage M13 as a Precision Nanoplatform for Crossing the Blood–Brain Barrier

Ester Bruno
Mapping histamine release sites: a 3D computational analysis of histaminergic dendritic varicosities

Sebastiano Alfio Torrisi
Memantine prevents acute stress-induced memory deficits by reversing sex-dependent glutamatergic alterations in the dorsal hippocampus

SESSION 7 • NEUROIMAGING & CLINICAL NEUROLOGY

Sara Cabras
Brain metabolic correlates of plasma biomarkers in Amyotrophic Lateral Sclerosis.

Giuseppe Ciullo
Differential recruitment of the thalamic ventral intermediate nucleus during observation and execution of goal-directed grasping actions

Gemma Gorla
Biomarker Discovery in Sneddon Syndrome: an Exploratory Plasma Proteomic Study

POSTER SESSION 1 • September 30, 18:40-20:40

COGNITIVE COGNITIVE NEUROSCIENCE & NEUROTECHNOLOGIES

CNN 01 | Source Dynamics During Action and Observation: The Impact of Live, Video, and Avatar Formats on Alpha and Beta ERD • **Valentina Fatima Dattola**

CNN 02 | Investigating the Feasibility and Efficacy of a Virtual Reality Cognitive Training Platform for Post-Stroke Patients with Mild Cognitive Impairment • **Anthony Faiola**

CNN 03 | Modulation of executive functions in healthy humans through Opuntia ficus-indica fruit consumption and non-invasive brain stimulation • **Aurora Meli**

NEURODEGENERATION

ND 01 | "Exogenous α-Synuclein Reshapes Presynaptic Vesicle Dynamics and Synaptic Function in Hippocampal Neurons" • **Anthony Battaglia**

ND 02 | Retinal alterations resemble brain pathology in a rat model of Parkinson's disease induced by intranigral infusion of α-synuclein oligomers • **Chiara Burgaletto**

ND 03 | Modelling fatal familial insomnia with patient-derived and chemically aged human cerebral organoids • **Eleonora Busani**

ND 04 | When Glia Turn Toxic: Progranulin Deficiency as a Driver of Neuronal Damage • **Anna Maria Carrese**

ND 05 | The CD33-ganglioside axis in late-onset Alzheimer's disease: from environmental triggers to structural variants • **Anna Maria Carrese**

ND 06 | Head-to-head comparison of Human Painless NGF with Wild-Type NGF in the 5xFAD Alzheimer's Disease Model • **Elea Coulomb**

ND 07 | Impact of hyperglycemic metabolic stress in hAPPswe neurons: transcriptomic and morphological insights • **Manuela Galeotti**

ND 08 | Proteomic Profiling of iPSC-Derived mesodiencephalic dopaminergic neurons revealed mitochondrial dysfunction, metabolic dysregulation and novel biomarkers for Parkinson's disease • **Mariarca Marciano**

ND 09 | Aβ42 Differentially Modulates Neurogenesis and Extracellular Vesicle Signaling in SVZ-Derived Neurospheres • **Francesca Massenzio**

ND 10 | Comparative study of Ella and Lumipulse immunoassay for pTau217 detection in dementia • **Evelyne Minucchi**

ND 11 | Longitudinal dynamics of serum neurofilament light chain and TARDBP expression in amyotrophic lateral sclerosis: a 12-month follow-up study • **Chiara Morandi**

ND 12 | Fluid Biomarkers in Cerebral Amyloid Angiopathy Patients: Proteomic Signatures and Biomarker Validation • **Giuliana Pollaci**

ND 13 | Atypical Glycosylation of the Prion Protein Reshapes the Molecular–Phenotypic Spectrum of Sporadic Prion Disease • **Graziella Rabbeni**

ND 14 | Whole-organoid imaging of senescence-associated neurodegeneration • **Esi Taci**

ND 15 | Defining microglial mechanisms underlying neurodegeneration in Friedreich's Ataxia through iPSC-based approaches • **Silvia Tortoriello**

NEURODEVELOPMENT

NDEV 01 | Unlocking the guardian of the brain: Choroid Plexus and neurodevelopmental insights • **Vanessa Aragona**

NDEV 02 | Brain-wide mapping of perineuronal nets and parvalbumin-expressing neurons across postnatal development identifies age- and sex-dependent circuit alterations in behaviorally characterized Fmr1 KO mice • **Lorenzo Cifarelli**

NDEV 03 | Enhanced oxidative stress-induced damage and cellular senescence are associated with FMRP deficiency in a mouse model of Fragile X Syndrome • **Lucia Di Pietro**

NDEV 04 | RENKCTD11 loss drives cerebellar dysfunction and behavioral deficits in neurodevelopmental disorders • **Mariagrazia Longo**

NDEV 05 | Post-Transcriptional Strategies to Enhance FOXG1 Expression • **Elena Sabina Maftei**

NDEV 06 | Exploring the Role of Oxidative Stress in Megalencephalic Leukoencephalopathy with Subcortical Cysts (MLC) • **Sara Sposito**

NEUROINFLAMMATION

NI 01 | A human innervated gut model for chronic visceral pain • **Sara Angrisani**

NI 02 | Immune Dysregulation and Impaired Pro-Resolving Pathways in epilepsy • **Giulia Carrera**

NI 03 | Dual sEH/LOX-5 inhibition improves mitochondrial resilience in in vitro and in vivo models of Parkinson's disease • **Marco Falcone**

NI 04 | Investigating the Role of Early-Life Inflammation in Neurodevelopmental Alterations Associated with WHIM Syndrome • **Veronica Marroccoli**

NI 05 | Characterization of the dynamics of hematopoietic niches in the skull bone marrow by multiphoton microscopy • **Giada Pessina**

NI 06 | Physical exercise as a non-pharmacological treatment in a Zebrafish model of neuroinflammatory disease • **Beatrice Pistolato**

NI 07 | Allopregnanolone potentiation of P2X4 receptor in microglia involves lysosome trafficking pathway • **Federica Torrisi**

NI 08 | PBMC secretome from Autoimmune Encephalitis patients drives neuronal hyperexcitability in neurons from mouse medial entorhinal cortex slices • **Maria Vittoria Zavaglia**

NEURO-ONCOLOGY

NO 01 | The FLASH Effect: Assessing Survival and Functional Brain Integrity • **Elisa De Santis**

NO 02 | Therapeutic efficacy of an EGFRvIII-Retargeted, IL-12-Expressing oncolytic HSV for the treatment of glioblastoma • **Antonella Di Loreto**

NO 03 | Investigating non-genetic drivers of intra-clonal competition in PDGF-driven glioma using a multiomic barcoding strategy • **Sebastian Andres Garcia Mena**

NO 04 | Modulation of Extracellular Vesicles release targeting A2A Adenosine Receptor in Glioblastoma • **Arianna Ioni**

NO 05 | Blood brain barrier in vitro model for the study of cancer cell extravasation and metastasis • **Fatima Jawad**

NO 06 | Fluorescence-traced spatial profiling of tumor-neuron interactions in glioma-like assembloids • **Oliviero Leonardi**

NO 07 | Targeted Temozolomide delivery by Hyaluronic Acid-gold nanoparticle–liposome enhances antitumor activity in 2D and 3D models of Glioblastoma • **Deborah Mannino**

NO 17 | Patient-derived 3D Glioblastoma spheroids recapitulate microanatomical complexity and enable functional investigation of tumor progression pathways • **Giuseppa D'Amico**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP 01 | Role of Nicotinic Acetylcholine Receptors Expressed by Cholinergic Neurons in the Control of Striatal Activity and Mice Behaviour • **Amanda Rosanna Alves Barboza**

NP 02 | Cerebellum-hippocampal interactions during limbic seizure-like events in the in vitro isolated guinea-pig brain • **Gaia Bruno**

NP 03 | Brainstem involvement in the development of PTSD • **Leonardo Cardone Sforzini**

NP 04 | Engineering Paracellular and Transcellular Pathways to Enable Therapeutic Delivery Across the Blood–Brain Barrier • **Valentina Castagnola**

NP 05 | Development of EIS-miR-25-Loaded Exosomes as a Potential Nanotherapeutic Strategy for Post-Stroke Neurogenesis • **Martina Cese**

NP 06 | REST-dependent tuning of the synaptic GluN2A/GluN2B balance selectively gates NMDAR-dependent LTP • **Ninfa Liccardi**

NP 07 | Neuroactive Polymer Films Modulate Neuronal Activity and Synaptic Network Plasticity • **Serena Munaò**

POSTER SESSION 2 • October 1, 12:00-14:30

COGNITIVE NEUROSCIENCE & NEUROTECHNOLOGIES

CNN 04 | Academic Stress as a Predictor of Poor Sleep Quality among International Medical Students • **Tamar Aladashvili**

CNN 05 | Visuospatial grounding of spatial semantics: evidence from transcranial direct-current stimulation modulation of the right posterior parietal cortex during a lexical decision task in virtual reality • **Fabrizio Di Giovanni**

CNN 06 | Tracing thoughts: decoding prefrontal circuit dynamics in the MAM model of schizophrenia • **Barbara Parisi**

CNN 07 | Time-resolved aperiodic dynamics during event segmentation in attention-deficit/hyperactivity disorder • **Xianzhen Zhou**

NEURODEGENERATION

ND 16 | The AQP4 A215T mutation causes a common maturation defect across AQP4 isoforms in megalencephalic leukoencephalopathy with subcortical cysts type 4 (MLC4) • **Ilaria Asci**

ND 17 | Prion detection in the urine of individuals at genetic risk for fatal familial insomnia • **Merve Begüm Bacınoğlu**

ND 18 | Development of new therapeutic approaches for targeting dysfunctional cortico-striatal circuit in HD • **Elisabetta Battocchio**

ND 19 | Niclosamide improves neuroprotective pathways in a C9orf72 mouse model of ALS/FTD • **Diana Ioana Boiangiu**

ND 20 | Development of an in vitro human brain-based 3D system to study the modulation of neuroinflammatory mechanisms and carnosine neuroprotective activity • **Giuseppe Caruso**

ND 21 | Astrocyte-derived extracellular vesicles in Parkinson's disease: multi-omics profiling of neuroprotective pathways and post-translational modifications • **Maria Cullurà**

ND 22 | Novel GRK5 variants in Parkinson's disease: identification and functional characterization of their effects on protein dynamics and stress response • **Francesca D'Amato**

ND 23 | Unraveling myelin abnormalities in synucleinopathies • **Marta Di Fabrizio**

ND 24 | Effect of discontinuous immunization with 12A12 Monoclonal Antibody on AD phenotype of aged 3xTg Mice • **Cristian Fiorucci**

ND 25 | Amyloid-Beta 1–42 Aggregation States Alter Mitochondrial Bioenergetics and Copper Homeostasis in SH-SY5Y Cells • **Alessia Floramo**

ND 26 | Prevalence of antibodies against neuronal antigens and serum biomarker profile in patients with cognitive impairment of recent onset • **Ilaria Gligora**

ND 27 | Early microglial dysfunction is associated with altered cerebellar synaptic development in Friedreich's Ataxia • **Martina Milani**

ND 28 | Optimizing neurorehabilitation in retinal degeneration: coupling subretinal P3HT nanoparticles with sensory stimulation • **Edoardo Porzano**

ND 29 | Ryanodine receptor-mediated calcium release regulates presynaptic function and is altered in Alzheimer's Disease models • **Michela Redana**

ND 41 | Precision Prevention of Neurodegenerative Diseases: Lifestyle, Natural Bioactives and Artificial Intelligence Across the Life Course • **Erika Smeriglio**

NEURODEVELOPMENT

NDEV 07 | Early butyrate rescues mucus barrier dysfunction in a preclinical model of autism spectrum disorder • **Aurora D'Alessio**

NDEV 08 | Tracking therapeutic efficacy in Angelman syndrome: a novel neuroimaging biomarker • **Lorenzo Dadà**

NDEV 09 | Molecular and functional characterization of adult microglia in NRG1 TM HET mice, a mouse model of neurodevelopmental disorder • **Maria Ilaria Marino**

NDEV 10 | Integrating micro-Raman Spectroscopy and Atomic Force Microscopy for biological longitudinal characterization of human Cortical Brain Organoids. • **Chiara Piferi**

NDEV 11 | Modulation of Nicotinic Receptors in Inhibitory Prefontal Interneurons and Their Effect on Behavior in ASD Mouse Models • **Ana Paula Rodrigues de Paulo**

NDEV 12 | Neuronal activity control by products of Foxg1-responsive, Tdark Rnf genes • **Claudia Sgherza**

NEUROINFLAMMATION

NI 09 | EBV infection drives neuroinflammation in MS brain through mechanisms of viral immune evasion • **Lucia Benincasa**

NI 10 | Hematopoietic and innate immune cells as novel targets of AgRP neuropeptide signaling • **Marta Bottero**

NI 11 | Microglia-to-astrocyte connexin 43 transfer potentially contributes to the chronification of pain hypersensitivity after astroglial connexin 43 deletion • **Simona Denaro**

NI 12 | Inhibition of NLRP3 through MCC950 reduces neuroinflammation and increases BDNF signaling in a mouse model of Huntington's Disease • **Flavia Dioguardi**

NI 13 | EBI2-Mediated Signaling in Glia and Its Implications for Remyelination in Organotypic Cerebellar Cultures • **Fatimah Fatimah**

NI 14 | Immunomodulatory and Remyelinating Properties of the Oxysterol Analogue CF3-7α,25OHC in Mouse Models of Multiple Sclerosis • **Klaudia Konieczna-Wolska**

NI 15 | SKYBONE: Viral lineage tracing of skull bone marrow-derived immune cells in a murine model of multiple sclerosis • **Stella Maglio**

NI 16 | Aspects of neuroinflammation in treatment-resistant schizophrenia • **Vladimir Mokhov**

NEUROIMAGING & CLINICAL NEUROLOGY

NIM 01 | Investigating blood-brain barrier interactions of Gadolinium-based contrast agents using an in vitro human model • **Alberto Cornet-Gomez**

NIM 02 | The role of cognitive reserve in Amyotrophic Lateral Sclerosis with C9ORF72 expansion: a 2-[18F]FDG-PET study • **Enrico Matteoni**

NIM 03 | Monitoring disease activity through GFAP and Neurofilament Light Chain longitudinal changes in SOD1-related ALS patients receiving antisense oligonucleotide therapy • **Salvatore Tafaro**

NEURO-ONCOLOGY

NO 08 | Dual-Targeted Peptide Nanofibers for Selective Co-Delivery of Temozolomide and 3-Bromopyruvate in Glioblastoma • **Teresa Barra**

NO 09 | Photoglioblaster: Dual-Triggered Phage Therapy for Precision Targeting of Glioblastoma • **Elena Causa**

NO 10 | Radiation therapy triggers a reshaping of transcriptional landscape of healthy microglia cell line analyzed through long reads RNA sequencing • **Giuseppe Nicolò Conti**

NO 11 | Unraveling STAG2-Driven Neuro-Oncogenesis in Medulloblastoma for new therapeutic targeting • **Antonella Lettieri**

NO 12 | Gut-brain axis in brain tumor development: exploring the impact of bile acids on glioma • **Martina Moroni**

NO 13 | Assessing the in vitro and in vivo effects of monoclonal antibodies against Glioblastoma • **Francesca Michela Narcisi**

NO 14 | "In vitro and in vivo models to assess the impact of ionizing radiation on calcium dynamics in Glioblastoma" • **Filippo Rossi**

NO 15 | Cancer-neuronal crosstalk in glioblastoma: neuronal-dependent mechanisms in tumor progression • **Chiara Saulle**

NO 20 | P2X7R Blockade Restrains Glioblastoma Development and Regulates Tumor–Microglia Interactions • **Noemi Marino**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP 08 | PEDOT:PSS Functionalized with Halloysite Nanotubes Promotes Neuronal Maturation and Early Network-Oriented Organization • **Alessandra Armeli**

NP 09 | Adult hippocampal neurogenesis shapes ideomotor action control in mice • **Warsha Barde**

NP 10 | Molecular biomarkers of unmyelinated peripheral nerve regeneration: a systematic immunofluorescence screening in human skin biopsies • **Sara Ben Abid**

NP 11 | Gene supplementation therapy restores motor deficits in Gnao1[C215Y] mice and proves broad efficacy in C. elegans model • **Ludovica Maria Busdraghi**

NP 12 | Cognition at the core of metabolic syndrome: linking neurometabolic load to cognitive and affective vulnerability in high-fat diet rat model. • **Marina Correnti**

NP 13 | REST supports GABAergic control of gamma-like network activity • **Raffaella Grimaldi**

NP 14 | Lysolecithin injection in the hippocampus of guinea-pigs: focal demyelination induced in vivo • **Cindy Paletta**

POSTER SESSION 3 • October 2, 12:20-14:30

COGNITIVE NEUROSCIENCE & NEUROTECHNOLOGIES

CNN 08 | BBB-on-a-Flow: Fabrication of Blood-Brain Barrier Vessel-Like Structures within a Millifluidic Bioreactor • **Caterina Allodola**

CNN 09 | Extra Hand Finger Augmentation in Congenital Oligodactyly: Design Rationale and Transcranial Magnetic Stimulation Based Neuroplasticity Assessment • **Antonio Cangelosi**

CNN 10 | Social Reactivity and Socio-Sensory Functioning in Autism: A Multimodal Study Integrating Eye-Tracking, Autonomic Measures and EEG • **Chiara Marraffa**

CNN 11 | Stress, Sleep Quality, and Educational Migration Context among International Medical Students • **Ekaterine Mikautadze**

NEURODEGENERATION

ND 30 | Epigenetic regulation in Krabbe disease: microRNAs as putative biomarkers and therapeutic targets • **Fabio Allia**

ND 31 | Targeting VDAC1 to Prevent Amyloid-β-Mediated Mitochondrial Dysfunction in neurodegenerative disorders • **Fabrizio Cavallaro**

ND 32 | Exploring the Vault Complex in Alzheimer's Disease: First Characterization of MVP, VPARP, TEP1 and vtRNA Expression in Aβ1-42-Treated Differentiated SH-SY5Y Cells • **Giovanni Luca Cipriano**

ND 33 | How could the sensory dysfunction be restored in the 3xTg-AD mouse model of Alzheimer's Disease? • **Simona Francia**

ND 34 | Transcriptional Master Regulators and novel genetic determinants of Parkinson's disease identified by multi-omics network analysis • **Giuseppe Giurin**

ND 35 | Nrf2/NOX2 Pathway Dysregulation and Oxidative Stress Biomarkers in Gaucher Disease–Associated Parkinsonism: Insights Into a Potential Therapeutic Target • **Davide Labellarte**

ND 36 | Effects of ATM alteration on microglial responses in Ataxia Telangectasia • **Cristiana Lucia Rita Lipari**

ND 37 | Modeling riboflavin transporter deficiency using patient-derived iPSC motor neurons reveals mitophagy defects • **Chiara Marioli**

ND 38 | Dysregulation of Pro-Regenerative Program Emerges as a Candidate Mechanism of Small Fiber Neuropathy • **Sara Palermo**

ND 39 | Plasma and CSF Biomarker Profiling in Cerebral Amyloid Angiopathy: Correlations with Clinical and Neuroradiological Features • **Elisabetta Pasella**

ND 40 | Biochemical and Functional Characterization of a β4GalNT1 Knock-Out Murine Neuroblastoma Cell Model • **Carola Pedroli**

ND 42 | Interleukin-9 induces calretinin expression in spinal neurons and may restore its downregulation in amyotrophic lateral sclerosis • **Costanza Stacchiotti**

ND 43 | Membrane and mitochondrial defects drive sensory neuron dysfunction in FLVCR1 related neuropathies • **Diletta Isabella Zanin Venturini**

NEURODEVELOPMENT

NDEV 13 | CYFIP1 Haploinsufficiency Drives Altered Contactomics, Reactive Phenotype and Metabolic Reprogramming of Microglia • **Domenico Corasiniti**

NDEV 14 | Caenorhabditis elegans as an integrated in vivo platform for dissecting neuronal function and dysfunction and enabling therapeutic discovery in neuroscience • **Martina Di Rocco**

NDEV 15 | Ion channel repertoire shapes early spontaneous activity and subtype identity in developing cortical neurons • **Sara Mancinelli**

NDEV 16 | Influence of microenvironmental factors on hippocampal neural stem cell fate in vitro • **Gemma Palazzolo**

NDEV 17 | Listening to Neurons: TREM2-Dependent Microglial Support of Neuronal Fitness • **Paola Poliseno**

NEUROINFLAMMATION

NI 17 | A tri-polymer nanoplastic mixture triggers gut-brain axis dysfunction: focus on inflammation and synaptic plasticity • **Federico Ciceri**

NI 18 | Crosstalk between APOE ε4 Genotype, PPARγ, and Astrocyte Reactivity in Alzheimer's Disease Progression. • **Lucrezia Floro**

NI 19 | SPMs System Imbalance contributes to Neuroinflammation in Alzheimer's Disease • **Giulia Forte**

NI 20 | NGAL and VCAM-1 Are Upregulated in Cervical Arterial Dissection: A Plasma Biomarker Study • **Gemma Gorla**

NI 21 | Longitudinal Characterization of Visual Pathway Involvement in Two Novel OSP- and MOBP-induced Experimental Autoimmune Encephalomyelitis Models • **Su-Chun Huang**

NI 22 | Exploring the Role of ATM Signalling in HSV-1-Infected Monocytes and Its Potential Contribution to Neuroinflammation • **Federica Mastrolembo Barnà**

NI 23 | An innervated and Immunocompetent Human Skin Equivalent of Psoriasis • **Michela Oliviero**

NI 24 | Targeting GLT-1 and PPAR-α: Dual Therapy with Ceftriaxone and PEA Alleviates Neuropathic Pain and Neuroinflammation in SNI Mice • **Milena Melake Teweldemedhin**

NI 25 | Neuroinflammatory Remodeling and Inflammasome Activation in the Superior Colliculus of an MPTP Induced Parkinsonian Rat Model • **Giovanna Vermiglio**

NEUROIMAGING & CLINICAL NEUROLOGY

NIM 04 | Functional Network Reorganization Following VIM-MRgFUS for Parkinson's Disease • **Jinlong Liu**

NIM 05 | Network-Level Genetic Organization of Human Cortical Folding • **Mahan Shafie**

NIM 06 | Early cladribine administration prevents connectomic and molecular changes in experimental multiple sclerosis • **Giulia Vasirani**

NEURO-ONCOLOGY

NO 16 | MEOX2 as a Putative Driver of Therapy Resistance in Glioblastoma Stem Cells • **Vincenza Aliperti**

NO 18 | Combining TMS and Action Observation to probe the fronto-parietal network integrity in glioma patients: a proof-of-concept study • **Laura Ferrari**

NO 19 | Functional Targeting of MEX3A Through RNA-Based Therapeutic Strategies in Glioblastoma • **Giulia Ferri**

NO 21 | Mechanistic insights into Nucleotide Excision Repair in Glioma Stem Cells • **Nathan Christian Molinari**

NO 22 | Targeting glioblastoma stem cells with biomimetic vesicles for boron neutron capture therapy • **Beatrice Federica Tremonti**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP 15 | Alterations of GABAergic transmission in models of neurotoxicity and neurodevelopmental disorders: molecular, synaptic, and functional mechanisms • **Giuseppe Chiantia**

NP 16 | Smoothened modulation affects disease progression in SOD1G93A mouse model of amyotrophic lateral sclerosis • **Maria Ciuro**

NP 17 | Cortisol-Driven Dysregulation in Human Hippocampal Progenitor Cells: Insights into the Protective Role of Exercise-Derived Factors • **Patrizia Genini**

NP 18 | From Hyperexcitability to Drug Resistance: integrating behavioral and electrophysiological biomarkers in a longitudinal pilocarpine model of Temporal Lobe Epilepsy • **Sarah Grippi**

NP 19 | Selective upregulation of Nav1.6 underlies PRRT2-linked cerebellar dysfunction • **Giacomo Mazzoni**

NP 20 | Early-Life Stress Shapes Cerebellar Circuits Associated with Opioid Use Disorder Vulnerability • **Pellegrino Lippiello**

NP 21 | Cortico-striatal circuit mechanisms mediate D1 dopamine agonist effects on memory capacity through cAMP/PKA signalling • Diletta Cavezza

COMMUNI ORAL CATIONS

BraIn
CATANIA 2026 • 9th Edition

Modelling neuroimmune interactions during human brain development using brain organoids

Veronica Krenn [1]

[1] *University of Milan-Bicocca, Dip. Biotecnologie e Bioscienze, Milan, Italy*

Neuroimmune interactions are increasingly recognized as key regulators of brain development and contributors to neurodevelopmental disorders (NDDs), a heterogeneous group of conditions representing a growing health challenge. Among these interactions, microglia and type I interferon (IFN) signalling are important players whose dysregulation has been implicated in diverse neurodevelopmental conditions, including those associated with prenatal stress, intrauterine infections and Down syndrome. Understanding where and in which cell populations neuroimmune signalling operates during human brain development is therefore a central question. Much of our current understanding derives from animal models, yet species-specific differences in microglial biology, IFN signalling, and brain development limit translation to humans. To address this gap, we use human brain organoids as a tractable platform for studying neuroimmune interactions during early neurodevelopment. Building on previous work, we established a fluorescent reporter system for quantitative analysis of cytokine responsiveness in brain organoids. Combining this reporter with quantitative imaging and spatial analyses to map IFN responses across cell populations and spatial domains, we uncovered widespread IFN responsiveness across major neuronal populations together with striking spatial heterogeneity in signalling activity. Using dose-titration and reporter analyses we identified both cell-intrinsic programmes and cytokine concentration as key determinants of this heterogeneity, supporting the existence of spatially defined neuroimmune niches during human brain development. In parallel, we describe our efforts towards the development of microglia-containing brain organoids as human models of neuroimmune interactions. Together, these studies establish brain organoids as versatile platforms for human neuroimmunology, accelerating disease modelling and therapeutic discovery in NDDs.

Targeted mitochondrial modulation in different cell-types rescues cognitive deficits in prenatal THC-exposed rats

Francesca Puleo [1] - Gianluca Lavanco [2] - Valentina Castelli [3] - Anna Brancato [2] - Martina Cicero [3] - Astrid Cannich [4] - Giovanni Marsicano [4] - Luigi Bellocchio [4] - Carla Cannizzaro [5]

[1] University of Palermo, Dept. of Biomedicine, Neuroscience and Advanced Diagnostics, PALERMO, Italy - [2] University of Palermo, Dept. of Sciences for Health Promotion and Mother and Child Care "G. D'Alessandro", Palermo, Italy - [3] University of Palermo, Dept. of Biomedicine, Neuroscience and Advanced Diognostics, Palermo, Italy - [4] INSERM U1215, NeuroCentre Magendie, Endocannabinoids and Neuroadaptation, Bordeaux, France - [5] University of Palermo, Dept. of Biomedicine, Neuroscience and Advanced Diagnostics, Palermo, Italy

Prenatal exposure to Δ9-tetrahydrocannabinol (pTHC), a partial agonist at endocannabinoid receptors, alters brain development, impacting neural function and behavior later in life. The endocannabinoid system regulates cell differentiation during gestation, supporting neuron-glial interactions, synaptic plasticity, and metabolic homeostasis. While pTHC behavioral consequences are well-documented, its cellular mechanisms remain unclear. Mitochondrial dysfunction in neuronal and glial cells is a critical nexus in neurodevelopmental disorders, regulating proliferation, differentiation, and energy metabolism essential for brain maturation. This study investigated pTHC-induced adverse brain development by examining disrupted neuron-glia differentiation and hippocampus-dependent memory. We hypothesized that pTHC alters cell populations via mitochondrial mechanisms and that targeted modulation could restore cognition. Memory was assessed via the Novel Object Recognition Test (NORT). Immunofluorescence quantified neuronal (NeuN+) and glial (GFAP+) densities in the hippocampus of pTHC-exposed and control adolescent rats. To rescue cognitive deficits, we utilized a chemogenetic approach (mitochondria-targeted-DREADD-Gs) to selectively modulate signaling in hippocampal neuronal and astroglial populations. Results confirmed memory deficits (impaired NORT) in pTHC-exposed animals. Immunofluorescence revealed pTHC significantly increased GFAP+ astrocyte density in the CA1 region, driving an altered glial-neuronal ratio. Crucially, chemogenetic Gs-activation yielded cell- and compartment-specific cognitive rescue: memory was restored by stimulating Gs signaling at either the neuronal plasma membrane or astroglial mitochondria. Our data indicate pTHC-induced memory disruption is linked to altered neuron-glia differentiation. Memory deficits can be functionally rescued by boosting astroglial mitochondrial and neuronal plasma membrane signaling. Targeted cell-specific metabolic modulation may represent a promising intervention to remediate adverse pTHC behavioral outcomes.

Spatial molecular profiling by MALDI-MSI to support histopathological diagnosis in drug-resistant epilepsy

Chiara Calabretta [1] - Dalia De Santis [1] - Niccolò D'Agaro [2] - Giulia Capitoli [2] - Marco de Curtis [1] - Rita Garbelli [1] - Cinzia Cagnoli [1]

[1] Fondazione IRCCS Istituto Neurologico Carlo Besta, Clinical and Experimental Epileptology Unit, Milano, Italy - [2] School of Medicine and Surgery, University of Milano-Bicocca, Bicocca Bioinformatics Biostatistics and Bioimaging B4 Center, Monza, Italy

Epilepsy is a neurological disorder affecting over 70 million people worldwide. Epilepsy surgery is an effective treatment for patients with focal drug-resistant epilepsy (DRE), accounting for approximately 30% of cases unresponsive to pharmacological therapy. Histopathological diagnosis of resected brain tissue relies on microscopic evaluation, including specific staining and immunohistochemistry, remains the gold standard for diagnosis. However, neuropathological classification is often challenging, and several inter-observer studies have highlighted the presence of uncertain or discordant diagnoses, particularly for specific pathologies. These discrepancies may directly impact prognosis and influence clinical decision-making during patient follow-up. In this study, we applied MALDI mass spectrometry imaging (MALDI-MSI) to formalin-fixed paraffin-embedded (FFPE) brain tissue samples from patients with focal DRE, including three distinct histopathological groups: focal cortical dysplasia (FCD), temporal lobe epilepsy, and cryptogenic epilepsy, to assess concordance with histopathology and explore its potential to capture tissue heterogeneity through spatial molecular profiling. Unsupervised analyses, including spatial segmentation and principal component analysis, demonstrated a good correspondence between molecular patterns and histological features, and enabled the separation of sample subgroups according to diagnosis. Furthermore, MALDI-MSI allowed the visualization of spatial distributions of known diagnostic markers through multiplex analysis within a single tissue section. Building on these findings, ongoing work focuses on the identification of novel molecular biomarkers using high-dimensional statistical and multivariate approaches. These data-driven strategies aim to define molecular signatures associated with specific histopathological classes with the goal of improving diagnostic accuracy and supporting prognostic stratification in epilepsy surgery.

Rewiring anti-tumor immunity: fecal microbiota transplantation activates NK cells tocontrol glioma in a mouse model

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Glioblastoma (GB) is the most lethal primary brain tumor, with a 5-year survival rate of approximately 5%. Growing evidence indicates that patients with GB exhibit an altered gut microbiota (GM), highlighting the role of the gut-brain axis in tumor progression. Fecal microbiota transplantation (FMT), the transfer of healthy fecal material to a diseased recipient, represents a direct strategy to modulate GM composition. This study investigated the impact of healthy-donor FMT on GB progression and the underlying immune mechanisms. Using a syngeneic GL261 glioma model, mice received FMT or vehicle, with or without antibiotic (ABX) pre-treatment. Microbiota composition, survival, and immune responses were evaluated. We previously demonstrated that FMT significantly reduced tumor volume compared with control-treated animals. Moreover, FMT prolonged survival, with the strongest benefit observed in ABX-FMT mice. Microbiota profiling revealed significant shifts in bacterial community composition following FMT, identifying distinct microbial taxa associated with treatment response. Flow cytometry analyses demonstrated an increase in the cytotoxic activity of both natural killer (NK) and $CD8^+$ T cells within the brains of ABX-FMT mice. Consistently, NK cells isolated from these animals displayed enhanced killing activity against GL261 cells in vitro. Furthermore, ABX-FMT mice exhibited increased frequencies of circulating IL-15Rα^+ dendritic cells together with enhanced IL-15 internalization by NK cells, suggesting a potential mechanism underlying NK-cell activation in vivo. Mechanistic in vitro study further suggested that bacterial extracellular vesicles (BEVs) present exclusively in the serum of ABX-FMT mice enhance IL-15Rα trans-presentation by DCs, thereby promoting NK-cell licensing and activation. Collectively, these findings identify a microbiota-BEV-dendritic cell-NK cell axis that contributes to glioblastoma control and may represent a novel therapeutic target for GB.

The mechanical landscape of GBM: How Piezo1 converts force into cancer

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Glioblastoma multiforme (GBM) is defined as IDH1-WT grade IV astrocytic tumor and is one of the most aggressive, untreatable and less understood types of cancers, so deeper understanding of its biology is an unmet clinical need. In this context, we focus on mechanosensation, the ability of cells of sensing mechanical and physical forces. The tumor mechanoenvironment is highly altered compared to healthy tissues, which is why mechanosensation has begun to be extensively studied in cancer. Specifically, we are studying Piezo1, a mechanosensitive non-selective calcium channel located in cell membranes. We developed a transgenic mouse model of Piezo1 overexpression specifically in astrocytes (Piezo1Tg/Gfap-Cre) that develop gliomas with 45% of incidence, postulating Piezo1 as a new oncogene in glioma. To explore the molecular mechanisms driving the oncogenic role of Piezo1, we performed a single-cell RNA-Seq of transgenic mice brains in pre-tumoral stage, observing an inflammaging phenotype. The inflammaging-associated changes (SASP release, increased H2AX foci, β-galactosidase expression) favor the progressive accumulation of molecular disruptions such as the loss of tumor suppressors (p21, p16, p53) and the overactivation of STAT3 pathway, which are the ultimate responsible for glioma onset. We corroborated that Piezo1 drives GBM malignancy via STAT3 in a cohort of the Hospital 12 de Octubre (Madrid, Spain), worsening prognosis in male patients. Moreover, U251 human GBM cell line showed increased aggressiveness upon Piezo1 overexpression (higher clonogenicity, higher migration and IL6 & CSF1 release), that was reverted after treatment with the JAK/STAT inhibitor tofacitinib. Together, our results show a novel molecular mechanism by which Piezo1 can drive the onset and progression of GBM through JAK/STAT, opening new horizons in GBM research, helping to better understand this complex disease and offering new potential therapeutic strategies for GBM.

Reinforcing peritumoral GABAergic inhibition modulates Glioblastoma progression and enhances immunotherapy efficacy

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Gliomas are highly aggressive brain tumors characterized by profound alterations in neuronal network activity within the peritumoral microenvironment. Parvalbumin (PV)-expressing GABAergic interneurons are essential for maintaining cortical excitatory–inhibitory balance, and their dysfunction has been implicated in glioma progression and neurological symptoms. This study investigated how chemogenetic modulation of peritumoral PV-interneurons and pharmacological enhancement of GABAergic signaling affect tumor growth, cortical function, and survival in murine glioma models. Selective inactivation of PV-interneurons via hM4D(Gi)-DREADDs worsened glioma-associated symptoms, accelerating weight loss, motor impairment, and epileptiform activity without affecting tumor volume or survival. Conversely, chronic activation using hM3D(Gq)-DREADDs significantly reduced tumor proliferation and mass, preserved motor performance, stabilized cortical activity, and partially maintained visual processing. To explore translational applications, the GABA-B receptor agonist Baclofen was tested in GL261 and CT-2A glioma models. Molecular and in vitro analyses indicated that Baclofen does not directly inhibit glioma proliferation, supporting an indirect mechanism mediated by the tumor microenvironment. However, Baclofen moderately reduced tumor volume in the GL261 model but not in the more aggressive CT-2A model, suggesting a contex-dependent microenvironment-mediated mechanism. Importantly, combining Baclofen with anti–PD-L1 immunotherapy produced a strong synergistic effect in vivo, markedly reducing tumor burden, inducing complete tumor eradication in 66% of treated mice, significantly extending survival, and generating durable antitumor immune memory. Together, these findings identify reinforcement of peritumoral GABAergic inhibition as a promising adjuvant strategy to stabilize cortical circuits and enhance immunotherapeutic efficacy in glioma.

From multimodal AI to biological twins: integrating deep learning on magnetic resonance imaging and histological data with patient-derived 3D glioblastoma models for precision medicine

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Glioblastoma (GBM) is one of the most aggressive primary brain tumors, characterized by poor prognosis and limited therapeutic options. A major limitation is the lack of an experimental model able to reproduce tumor complexity while interpreting large-scale patient-derived data.
In this context, the concept of "biological twin" is emerging as a powerful strategy to digitally and experimentally mirror disease and investigate therapeutic approaches and responses.
In our work, we implemented a deep learning (DL)-based framework to analyze multiple GBM patient-derived datasets, including magnetic resonance imaging and hematoxylin and eosin-stained histological sections from tumor biopsies. Advanced convolutional neural networks were employed to extract spatial features and identify latent patterns across radiological and histopathological domains, thereby enabling multimodal integration of macroscale tumor architecture and microscale cellular organization.
In parallel, we generated GBM patient-derived 3D spheroids, providing a biologically relevant and highly representative in vitro counterpart that was subjected to an extensive morpho-phenotypic characterization. The obtained results showed that this 3D model preserved key features of the native tumor, including spatial organization and cellular heterogeneity, as evidenced by the non-uniform distribution of specific molecular markers and the generation of functional cellular niches. This model will be further integrated into our DL-based framework to enhance its robustness and biological relevance.
Overall, by combining AI-driven image analysis with patient-derived 3D tumor models, this study proposes a translational framework for generating a GBM biological twin. This approach will allow the integration between computational and experimental oncology, with the potential to improve disease modeling, enable more accurate prediction of therapeutic responses, and advance the implementation of precision medicine strategies.

Biomimetic fluidic model of the glioblastoma-affected neurovascular unit as an advanced in vitro platform

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Glioblastoma (GBM) remains a poorly understood and highly aggressive brain tumor, characterized by frequent recurrence and limited therapeutic options. A major challenge in GBM treatment is the inability of many potentially effective drugs to cross the blood–brain barrier (BBB), the selective interface that protects the central nervous system.
Current in vitro BBB models are commonly based on static Transwell systems, which fail to reproduce the physiological shear stress generated by blood flow. As a result, drug transport and uptake dynamics often differ from those observed in vivo.
Here, we present a biomimetic in vitro platform designed to model a GBM-affected neurovascular unit (NVU) using exclusively primary human cells. Primary cells were isolated from GBM surgical specimens, phenotypically characterized, and separated into distinct cellular populations. In parallel, patient-derived GBM tumoroids were generated.
The resulting cell populations were seeded into a custom-designed perfused bioreactor according to the histological organization of the human NVU. The device consists of a three-dimensional dual-compartment structure. The upper channel reproduces the BBB and contains endothelial cells, astrocytes, and pericytes. The lower compartment, directly connected to the upper channel, contains GBM tumoroids together with healthy microglia, astrocytes, and neurons, mimicking the brain parenchymal environment. The bioreactor was engineered through fluid-dynamic modeling to generate a physiological wall shear stress of approximately 15 dyn/cm^2, comparable to that of human cerebral capillaries.
This platform provides a physiologically relevant tool for investigating BBB permeability, assessing the distribution of therapeutic agents within brain tissue, and evaluating treatment responses in GBM and surrounding neural cells. The model may support the development and preclinical testing of novel therapies for glioblastoma.

Divergence between MP2RAGE and MPRAGE for detecting learning-induced structural brain changes

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Motor skill training can produce neuroplastic changes observable in vivo by magnetic resonance imaging (MRI). However, the choice of acquisition sequence may affect inferences about the nature of these changes. Here, we use a longitudinal fine-motor skill learning data set (n=63) with up to 7 weekly-collected brain scans at 7 Tesla with the magnetisation-prepared 2 rapid acquisition gradient echoes (MP2RAGE) sequence to investigate whether any changes in grey matter volume and cortical thickness of the pre- and postcentral gyri can be observed over the study period. Additionally, we recovered magnetisation-prepared rapid gradient echo (MPRAGE) images from the MP2RAGE images, enabling comparisons of estimates derived from these widely used acquisition sequences. Participants learned to perform a configural sequence task using their nondominant left hand and did so in conjunction with the weekly MRI sessions. We extracted grey matter volume and cortical thickness from the brain images and fit multivariate Bayesian multilevel regression models to assess morphological changes in the right hemisphere, where learning-related changes are expected to happen to a greater extent, and the left hemisphere, where they are not. We find substantially greater changes in grey matter volume and cortical thickness in the right, compared to the left, hemisphere precentral and postcentral gyri, consistent with learning-induced brain plasticity. However, these effects were not invariant to the choice between MP2RAGE and MPRAGE: the direction of effects was opposite in the right hemisphere depending on the sequence. These results highlight that even relatively short-term motor learning may induce observable neuroplasticity effects. However, the observed divergence between acquisition sequences suggests that we may be led to different, even opposite, conclusions regarding potential underlying neuroplasticity mechanisms at play.

a-tDCS over ventral premotor cortex selectively enhances processing of goal-oriented biological motion

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The perceptual encoding of movements performed by living beings is of fundamental importance in the performance of numerous biologically relevant behaviors. However, to date, unresolved questions remain about the complex network supporting whole-body biological motion (BM) processing. This study aims to explore the extent to which the mirror neuron system, focusing on the ventral premotor cortex (vPMC), is involved in this cognitive function, hypothesizing a prevalent involvement in the processing of goal-oriented actions. Eleven healthy participants underwent a transcranial direct current stimulation (tDCS) protocol targeting the left vPMC across four sessions: no stimulation, sham stimulation, anodal tDCS, cathodal tDCS. During each session, participants performed a BM discrimination task using point-light displays depicting biological and non-biological movements, which were either goal-oriented or non-goal-oriented. Goal-oriented actions were defined as movements explicitly directed toward an external object or outcome. All stimulation sessions were randomized across participants, and the order of stimulus presentation was randomized within and across experimental conditions. Task performance was assessed in terms of accuracy and reaction times. Anodal tDCS selectively reduced reaction times during the recognition of goal-oriented BM actions, compared with the “no stimulation” condition ($p=0.001$). No significant effects were observed on accuracy across conditions, nor did cathodal tDCS or sham stimulation produce significant changes in reaction times. These preliminary results show a specific involvement of the vPMC in the perceptual encoding of goal-oriented BM, supporting a task-dependent contribution of mirror neuron system nodes within the broader biological motion network.

Chronic GCase inhibition induces lipid remodeling and neurodegenerative alterations in human midbrain organoids

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Deficiency of β-glucocerebrosidase (GCase), a lysosomal hydrolase involved in glucosylceramide (GlcCer) catabolism, is associated with neurodegenerative disorders such as Gaucher disease and GBA-associated Parkinson's disease. Reduced GCase activity leads to GlcCer accumulation within lysosomes, although the mechanisms linking lipid dysregulation to neuronal dysfunction remain poorly understood. To investigate these processes, I developed a human in vitro model based on induced pluripotent stem cell-derived midbrain organoids, which recapitulate key structural and cellular features of the human midbrain, including dopaminergic neuronal populations. Chronic GCase deficiency was induced by treating organoids for 30 days with conduritol B epoxide (CBE), a selective GCase inhibitor. Biochemical analyses confirmed a suppression of GCase activity, accompanied by massive GlcCer accumulation. In addition, preliminary lipidomic analyses by MALDI imaging mass spectrometry revealed widespread alterations in lipid composition and distribution in CBE-treated organoids. These changes are likely linked to lysosomal impairment due to GlcCer accumulation, causing defective sphingolipid degradation and leading to altered membrane turnover. Since membrane lipids regulate neuronal signaling, membrane organization and vesicle trafficking, these alterations may contribute to neuronal vulnerability. Consistently, chronic GCase inhibition induced neurodegenerative features, including reduced expression of neuronal and dopaminergic markers and altered neuronal architecture. Overall, these findings demonstrate that GCase deficiency promotes both lipid dysregulation and neurodegenerative alterations in human midbrain organoids, supporting the relevance of this system as a translational human model to investigate the interplay between lysosomal dysfunction and neurodegeneration, providing a physiologically relevant platform to model early pathogenic events in GCase-related neurodegeneration.

Preclinical development of an optimized bicistronic lentiviral vector enabling effective HSC Gene therapy for GM2 gangliosidoses

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GM2 gangliosidoses (Tay-Sachs and Sandhoff disease; TSD, SD) are rare lysosomal storage disorders (LSDs) caused by HEXA and HEXB mutations that lead to β-hexosaminidase A (β-Hex) deficiency and progressive GM2 ganglioside accumulation in the central nervous system. Infantile forms are rapidly neurodegenerative and currently lack effective treatments, largely because therapeutic enzymes cannot cross the blood-brain barrier. Hematopoietic stem cell gene therapy (HSC GT) with lentiviral vectors (LVs) delivering supraphysiological levels of therapeutic transgenes has shown benefit in other neurodegenerative LSDs, but its application to GM2 gangliosidoses has been limited by insufficient β-Hex production from HSC-derived myeloid progeny. To overcome this limitation, we developed a novel bicistronic LV that coordinately overexpresses the α- and β-subunits to reconstitute functional β-Hex. Molecular and functional analyses demonstrated superior performance of the new LV compared with previous-generation constructs in patient-derived fibroblasts, in hematopoietic stem/progenitor cells isolated from mouse models of SD and TSD, and in hematopoietic stem cells from healthy donors. Importantly, LV-transduced patient-derived (SD and TSD) macrophages showed increased β-Hex activity associated with reduced GM2 accumulation. In parallel, a myeloid-to-neural cross-correction platform using macrophage-conditioned media supported efficient enzyme transfer, uptake, and functional restoration in TSD human induced pluripotent stem cell (hiPSC)-derived neuronal/glial cells, the disease-relevant targets. Collectively, these results establish a relevant human in vitro platform for assessing the therapeutic potential of HSC-GT for GM2 gangliosidoses.

Progressive sensory dysfunction driven by altered skin-nerve remodeling in the Flvcr1 P221S mouse model

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Mutations in FLVCR1 (Feline Leukemia Virus Subgroup C Cellular Receptor 1), a choline/ethanolamine importer involved in membrane lipid synthesis and heme homeostasis, is associated with rare sensory and autonomic neuropathies, though underlying mechanisms remain poorly defined. This study investigates 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 and elucidate its pathogenesis, a homozygous CRISPR-Cas9 KI mouse model carrying the FLVCR1 P221S mutation was generated. Murine embryonic fibroblasts were analyzed to assess early cellular consequences. These cells exhibited reduced ATP levels, decreased ALAS1 activity, increased TBARS, and altered membrane fluidity, consistent with impaired heme metabolism, oxidative stress, and compromised membrane integrity. To define neuron-specific vulnerability, in vitro cultures of primary sensory neurons were performed, revealing a significant reduction in both soma size and axonal outgrowth. This showed an intrinsic neuronal growth defect occurring independently of the microenvironment. To determine whether these alterations lead to functional deficits, behavioral and histological analyses were performed in vivo. From 7 months of age, mutant mice showed increased response latency to noxious heat and cold stimuli, signaling impaired thermal sensitivity and peripheral sensory pathway dysfunction. Furthermore, 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. This suggests 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 neuropathy.

TWIK2 as an immunomodulatory factor in EAE

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Demyelination, neuroinflammation and axonal damage critically shape the pathogenesis of Multiple sclerosis, yet the molecular regulators coordinating immune cell functions within the central nervous system remain unclear. TWIK2 is receiving increasing attention in the pathophysiology of MS where it is thought to play an immunomodulatory role in the inflammatory response and in the interaction between immune cells. Here, we investigated the immunomodulatory role of TWIK2 in neuroinflammation using in-vitro approaches and the murine model of Experimental Autoimmune Encephalomyelitis in TWIK2-deficient mice.
TWIK2 knockout mice exhibit an altered disease course compared to wild-type controls, characterized by attenuated clinical severity and delayed disease progression. Mechanistically, loss of TWIK2 resulted in impaired phagocytotic capacity of antigen-presenting cells (APC). In addition, TWIK2 deficiency led to altered APC functionality, shown by decreased expression of co-stimulatory molecules and reduced efficiency in priming autoreactive T cells. Our data identifies TWIK2 as regulator of immune cell function, linking phagocytosis and APC-T cell interaction to disease progression in neuroinflammation. These findings highlight TWIK2 as a potential therapeutic target for modulating neuroimmune responses in Multiple sclerosis.

Cutaneous phosphorylated alpha-synuclein topography across synucleinopathies: a distal gradient and MSA-specific compartment signature

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Synucleinopathies, including Parkinson's disease (PD), multiple system atrophy (MSA), and dementia with Lewy bodies (DLB), are neurodegenerative disorders defined by the prion-like propagation of misfolded phosphorylated alpha-synuclein (pSyn) throughout the nervous system. This pathological process extends to the peripheral autonomic nervous system, including the richly innervated skin. Skin punch biopsy with indirect immunofluorescence (iIF) targeting pSyn at serine 129 thus provides minimally invasive, in vivo access to peripheral synuclein pathology across distinct neural annexes (perivascular fibers, subepidermal plexus, sweat glands, arrector pili muscle) whose innervation patterns may reflect disease-specific differences in strain biology and autonomic circuit vulnerability. We investigated whether topographic and compartmental pSyn distribution across paired biopsy sites encode diagnosis-specific signatures. Paired cervical (proximal) and leg (distal) biopsies from 215 pSyn-confirmed patients, MSA (n=32), DLB (n=43), parkinsonism (n=38), PD (n=88), other (n=14), were processed by iIF using PGP9.5 (pan-neuronal marker) and anti- Ser129pSyn. Chi-square, paired McNemar and odds ratio analyses were applied with Benjamini-Hochberg false discovery rate (FDR) correction. Distal leg positivity differed significantly across diagnoses (χ^2=13.94, p=0.007); cervical positivity did not. MSA showed widespread distal involvement (96.9%) with an exclusive leg-dominant intra-individual asymmetry (McNemar p=0.021). Compartment profiling revealed a dual MSA-specific dissociation: marked reduction of cervical perivascular pSyn (21.7% vs. 83–92%; OR=0.047, FDR $p<10^{-7}$) and subepidermal plexus enrichment at both sites (OR=18.9 and 9.9; FDR $p<10^{-5}$). This perivascular-to-subepidermal inversion may reflect MSA-specific differences in autonomic innervation and synuclein strain tropism, opening new avenues for in vivo investigation of propagation mechanisms across synucleinopathies.

Rare variant combinations drive convergent lipid-mitochondrial dysfunction in Parkinson's disease patient-derived dopaminergic neurons

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Parkinson's disease (PD) exhibits a complex polygenic architecture involving rare variants in novel risk genes, whose functional impact remains unclear. Here, we differentiated mesodiencephalic dopaminergic neurons from six PD patient-derived iPSC lines carrying distinct rare variant combinations and five healthy controls. Three lines, carrying variants in KIF21B, SLC6A3/HMOX2, and TMEM175/AIMP2, showed a severe and convergent phenotype, indicating a genotype-dependent effect. Patch-clamp experiments revealed a 20-fold reduction in membrane capacitance ($p<0.001$), impaired action potential firing, and altered excitatory postsynaptic current amplitude and kinetics ($p<0.001$), indicating profound synaptic dysfunction. Multi-omics profiling identified a convergent lipid-mitochondrial dysfunction across the same three lines. Upregulation of key glycolytic and oxidative phosphorylation enzymes was accompanied by a 50% reduction in baseline ATP, further worsened under stress, reflecting a compensatory but ineffective bioenergetic response. Accordingly, lipidomic analysis revealed accumulation of free fatty acids, acylcarnitines, and sphingolipids, with reduced GM3, consistent with impaired mitochondrial fatty acid oxidation and membrane remodeling, directly explaining the electrophysiological dysfunction. Notably, these findings extended to circulating levels, revealing altered concentration of Krebs cycle metabolites (malonate, pyruvate, succinate) in a cohort of 300 PD patients ($p<0.0001$), extending cellular findings to a systemic metabolic signature. Finally, through Whole-exome sequencing we identified genetic variants in dysregulated metabolic enzymes associated with PD risk, supporting the validity of our approach. Together, these findings identify a functionally distinct neuronal subtype in PD defined by rare variant combinations and implicate lipid-mitochondrial dysfunction as a convergent mechanism underlying synaptic vulnerability.

Targeting hyperphosphorylated Tau with intrabodies to inhibit pathological Tau aggregation

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The aberrant hyperphosphorylation of Tau promotes its detachment from microtubules and drives its aggregation into pathological species, which represent key hallmarks of tauopathies and neurodegeneration, including Alzheimer's disease. Several therapeutic strategies are currently being explored to target pathological Tau and yet face major limitations, including limited intracellular accessibility, insufficient specificity, and potential interference with the protein's physiological functions. In this context, this project aims to selectively target the intracellular pool of hyperphosphorylated Tau while sparing physiological Tau. To achieve this, we developed intracellular antibodies (intrabodies) specifically targeting disease-relevant phosphorylated Tau epitopes to interfere with pathological Tau hyperphosphorylation and aggregation. Intrabody selection was performed using the PISA (Post-translational Intracellular Silencing Antibody) platform, a screening system integrating yeast two-hybrid technology with tethered catalysis to identify binders specific for post-translationally modified (PTM) proteins, which allows the generation of the target PTM-protein and the selective identification of PTM-dependent binders directly inside cells. Here, we used a designed Tau Proline Rich Domain as bait and screened it against a human scFv library from convalescent COVID-19 patients, based on evidence linking SARS-CoV-2 infection to increased Tau phosphorylation and neurodegenerative signatures. The screening yielded three scFvs selectively binding the phosphorylated Tau (pTau), and epitope mapping for one candidate. The candidates demonstrated good expression levels and co-immunoprecipitated with pTau in mammalian cells, validating their selectivity to pTau at certain sites. Ongoing studies aim to evaluate their functional activity in cellular models, assessing their ability to bind and selectively degrade hyperphosphorylated Tau, to inhibit its pathological aggregation.

The choroid plexus as a hub of defective resolution mechanisms in multiple sclerosis

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The choroid plexus (ChP) is not only responsible for CSF production, but also acts as a key neuroimmune barrier by controlling immune cell trafficking within the CNS. Chronic or dysregulated ChP inflammation has been associated with several neurological disorders, including multiple sclerosis (MS). The key players in resolving inflammation are termed Specialized pro-resolving lipid mediators (SPMs), and include lipoxins, resolvins, protectins, and maresins, who altogether actively regulate resolution of inflammation and tissue repair. However, their role in controlling ChP inflammation and MS remains poorly understood and not investigated. Here, by flow cytometry we investigated how inflammatory activation affects SPM biosynthetic pathways and receptor expression in human ChP epithelial cells. Inflamed ChP strongly upregulated key SPM-biosynthesizing enzymes, (i.e. COX-2, 12/15-LOX), and receptors (i.e. ALX/FPR2, GPR32). Consistently, lipidomic analysis of CSF-like supernatants showed increased levels of SPMs, particularly lipoxins (LXA4/B4), resolvins (RvE1, RvD1–3), maresins (MaR1) and protectins (PD1), compared with non-inflamed controls, suggesting that ChP is a neuroimmune barrier actively involved in inflammation and resolution. These findings were confirmed by lipidomic analysis of CSF from MS patients, revealing a profound impairment of SPM profiles that were associated with disease stage, and by immunohistochemistry of post-mortem ChP tissue of MS patients, which showed significantly reduced levels of key SPM enzymes (i.e. 12/15-LOX). Finally, the therapeutic potential of SPMs was assessed in the EAE mouse model of MS. Treatment with a combination of RvD3 and LXB4 significantly ameliorated disease severity, preventing demyelination and oligodendrocyte loss. Overall, these results identify the ChP as an important regulator of inflammatory resolution in the CNS, which is altered in MS, and support SPM-based therapies as promising strategies for MS treatment.

Selective inhibition of the p2x7 receptor in microglia as a novel therapeutic strategy for epilepsy

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Epilepsy is one of the most common chronic neurological diseases. Over 30% of patients are, however, unresponsive to current anti-seizure medications (ASMs), with ASMs not significantly impacting on disease progression and causing adverse side effects. Consequently, there is a pressing need to identify new, safer, and disease-modifying treatment strategies effective in pharmacoresistant epilepsy. Several studies have provided compelling evidence for a role of the ATP-gated P2X7 receptor (P2X7R) in seizure generation and the development of epilepsy. However, its cell-specific contribution to epileptogenesis remains poorly understood. Using cell-type specific knock-out mice, we demonstrated that these effects are highly dependent on the targeted cell type, with a specific knock-out of P2X7R in microglia reducing seizures and neuroinflammatory markers and amiliorating epileptogenesis. These findings demonstrate a cell type-specific, proconvulsive role of P2X7R in microglia and identify microglial P2X7R signaling as a key driver of seizure-associated neuroinflammation. Based on these results, we are developing a targeted therapeutic strategy involving antisense oligonucleotides (ASOs) encapsulated in lipid nanoparticles (LNPs) engineered to selectively target microglia. This approach aims to selectively silence P2X7R within the seizure focus while minimizing off-target effects. Ongoing studies are evaluating the efficiency, specificity, and therapeutic potential of this strategy in vitro and in preclinical models of epilepsy. Together, our findings highlight microglial P2X7R as a critical mediator of seizure pathology and support its selective targeting as a promising disease-modifying strategy for epilepsy.

Chronic CMIT/MIT inhalation disrupts the lung–brain axis and promotes α-synuclein-associated dopaminergic neurotoxicity

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Chronic exposure to airborne toxicants is increasingly recognized as a driver of systemic neuroinflammation through dysregulation of the lung–brain axis. In this context, we investigated whether prolonged inhalation of chloromethylisothiazolinone/methylisothiazolinone (CMIT/MIT), a biocidal mixture associated with humidifier disinfectant–related lung injury, promotes neurodegenerative-like alterations through pulmonary-derived inflammatory signaling. Adult CD1 mice were exposed to aerosolized CMIT/MIT for 28 days. Pulmonary remodeling, systemic oxidative stress, blood–brain barrier (BBB) integrity, neuroinflammation, dopaminergic neurotoxicity, and behavioral alterations were evaluated through histological, biochemical, and behavioral analyses. CMIT/MIT exposure induced severe pulmonary fibrosis, alveolar-capillary barrier disruption, and marked inflammatory cell infiltration in bronchoalveolar lavage fluid, accompanied by elevated IL-1β, TNF-α, and IL-6 levels. These pulmonary alterations were associated with systemic oxidative stress characterized by increased ROS/RNS production, lipid peroxidation, NLRP3 inflammasome activation, and depletion of antioxidant defenses. CMIT/MIT caused significant downregulation of the tight junction proteins Occludin and ZO-1, indicating BBB impairment. Brain tissue displayed extensive reactive gliosis, oxidative damage, and neuroinflammatory cytokine release. Importantly, chronic exposure promoted α-synuclein accumulation, DAT downregulation, and loss of TH-positive dopaminergic neurons, together with severe motor, cognitive, and anxiety-like behavioral deficits. Our findings identify chronic CMIT/MIT inhalation as a potent inducer of lung-driven neurotoxicity and demonstrate that pulmonary oxidative-inflammatory dysfunction can propagate systemically to compromise cerebral homeostasis. These results highlight the lung–brain axis as a critical mechanistic pathway linking environmental exposure to neurodegenerative vulnerability.

Neuroinflammatory modulation as a strategy to target neuronal pathogenic mechanisms in Huntington's disease

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Huntington's disease (HD) is an inherited neurodegenerative disorder caused by mutant huntingtin (mHTT), leading to progressive motor, cognitive, and psychiatric impairment. In addition to classical neurodegenerative mechanisms, chronic inflammation plays a key role in disease progression. The NLRP3 inflammasome has emerged as a potential therapeutic target, although its function in neuronal cells remains incompletely understood. Here, we investigated the effects of pharmacological NLRP3 inhibition using MCC950 in mouse striatal progenitor cells (STHdh) expressing either wild-type or mutant huntingtin. Pro-inflammatory stimulation activated the NLRP3 pathway, resulting in increased caspase-1 and gasdermin D expression, particularly in mHTT cells, correlated to an enhanced susceptibility to inflammatory cell death, like as pyroptosis, compared to wild-type cells. MCC950 treatment significantly reduced markers of NLRP3-dependent inflammatory responses. MCC950 also partially improved mitochondrial metabolism under inflammatory conditions selectively in mutant cells, suggesting a protective effect on bioenergetic homeostasis in pathological conditions. However, cell viability was not restored, indicating the contribution of additional cell death pathways under neuronal inflammatory conditions. The increase of caspase-3/7 activity in mutant cells suggests concurrent activation of apoptosis alongside pyroptosis, supporting a potential role for PANoptosis under inflammatory stress. In differentiated neuronal models, MCC950 partially rescued both caspase-1 activation and cell viability. Ongoing studies using iPSC-derived human neurons aim to validate these findings in disease-relevant systems. Overall, our data identify MCC950 as a modulator of inflammasome activation and mitochondrial dysfunction in HD cell models, supporting combinatorial therapeutic strategies targeting both inflammatory and neurodegenerative mechanisms.

Dissecting Foxg1-dependent engram regulation through genetic manipulation and network profiling

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The allocation of neurons to a memory engram is shaped by local inhibitory circuits: increased inhibition from surrounding interneurons reduces the number of principal neurons recruited into the engram and limits their long-term reactivation, constraining engram size and stability. Since engram neurons display elevated excitability regulated by transcriptional programs, we investigated the role of Foxg1, a master regulator of telencephalic development and postnatal neuronal function that controls progenitor proliferation, interneuron and pyramidal differentiation, dendritic growth, synaptic plasticity, and excitability.
Using AAV-mediated Foxg1 gain- and loss-of-function, TRAP2 activity-dependent tagging, contextual fear conditioning, and IEG profiling, we found that Foxg1 modulates engram dynamics across hippocampal, amygdala, cortical, and hypothalamic regions. Foxg1 upregulation enhances neuronal recruitment into training and retrieval engrams, increases encoding-recall overlap, and induces engram shrinkage, suggesting more selective allocation. Instead, Foxg1 downregulation reduces recruitment and stability, revealing a bidirectional and region-specific control partly mediated by Foxg1-dependent modulation of excitability.
On these grounds, we aim to dissect how Foxg1 shapes inhibitory circuit function and engram allocation at cellular and network levels. To do so, we are developing lentiviral vectors to selectively label and manipulate interneuron populations and will combine Foxg1 overexpression or deletion with calcium imaging on primary mouse neurons. This strategy will provide initial functional evidence on how Foxg1-depedent transcriptional programs influence interneuron excitability, inhibitory tone, and pyramidal neuron recruitment. The project will ultimately integrate genetic manipulation, circuit-level analyses, and in vivo approaches to clarify how Foxg1 orchestrates molecular and inhibitory mechanisms that shape memory allocation and persistence.

Bacteriophage M13 as a precision nanoplatform for crossing the blood–brain barrier

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The blood-brain barrier (BBB) is a highly selective and dynamic interface that protects the central nervous system (CNS) and maintains brain homeostasis. However, this barrier also severely limits the delivery of therapeutics to the brain, causing many promising drugs to fail to reach effective concentrations in the CNS and restricting treatment options for neurodegenerative disorders. Although synthetic nanomaterials have been explored to enhance transcellular transport across brain endothelial cells, their performance in complex biological systems is often limited by protein corona formation and reduced targeting specificity. To address these challenges, we investigated M13 bacteriophages as versatile, biocompatible, and biomimetic nanovectors for BBB crossing and neuronal targeting. M13 phages possess several advantageous properties: a high loading capacity linked to their capsid proteins, enabling efficient fluorescent labeling and tracking; a genome suitable for extensive genetic modification; and a filamentous structure that supports targeted interactions with cellular pathways. Here, we demonstrate that M13 phages can actively traverse endothelial barriers through specific intracellular trafficking mechanisms, a process further enhanced by targeting peptides. Throughout transport, the phages preserve both structural integrity and biological functionality. We also show that engineered M13 phages efficiently target infected neuronal cells and promote successful BBB translocation. This homing capability remains stable even in complex biological environments characterized by high protein content and cellular density, conditions that typically impair conventional nanoparticles. Overall, our findings provide mechanistic insight into the interaction between engineered bacteriophages and the BBB and highlight the potential of M13 phages as adaptable nanoplatforms for targeted therapeutic and diagnostic applications in CNS disorders.

Mapping histamine release sites: a 3D computational analysis of histaminergic dendritic varicosities

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Histaminergic (HAergic) neurons of the tuberomammillary nucleus (TMN) are the brain's exclusive source of neuronal histamine, projecting to nearly every major brain region. Unlike classical neurotransmitters, HA is primarily released through varicosities distributed along neuronal fibers, whose synthesis and release are regulated by H3 autoreceptors. Stress-induced functional heterogeneity within the HA neuronal population suggests that distinct subpopulations may engage different brain circuits according to behavioural demands. Here, we present a computational framework for the 3D morphometric characterization of dendritic varicosities in HAergic neurons, aimed to investigate stress-induced structural plasticity. Adult histidine decarboxylase (Hdc)-Cre mice received bilateral injections of pAAV-Syn-ChR2(H134R)-GFP into the TMN to label HAergic neurons. Viral vector expression in TMN HAergic neurons was verified by immunohistochemistry on 20 µm coronal brain sections, using anti-HDC and anti-mCherry primary antibodies. Confocal image stacks were acquired (Nikon A1, 40x objective). Neuronal structures were reconstructed using SENPAI, a segmentation framework which leverages image intensity and Hessian-based second-order derivatives to highlight tubular and protruding neuronal structures. The dendritic skeleton was extracted from the segmented volume and the primary dendritic path was identified. Path irregularities due to image noise or dendritic tortuosity were removed with the Douglas-Peucker algorithm. Varicosities were detected on the 3D mesh of the segmentation using the Shape Diameter Function (SDF), which estimates local structural thickness to identify cross-sectional enlargements along the dendrite. For each varicosity, morphometric parameters including, count, size and position along the dendritic arbor, were estimated. This framework provides, to our knowledge, the first morphometric reference values for dendritic varicosities in HAergic neurons.

Memantine prevents acute stress-induced memory deficits by reversing sex-dependent glutamatergic alterations in the dorsal hippocampus

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Acute stress impairs hippocampal-dependent memory, but the underlying glutamatergic mechanisms and their potential sex specificity remain unclear. We previously showed that 2 h of restraint stress after training in the novel object location (NOL) task produce comparable long-term spatial memory deficits in male and female C57BL/6J mice through distinct molecular adaptations in the dorsal hippocampus (dHPC). Here, we combined UHPLC-based glutamate–glutamine measurements, whole-cell patch-clamp recordings and behavioural testing to determine whether non-competitive NMDA receptor antagonists prevent acute stress-induced cognitive dysfunction in a sex-dependent manner. Acute restraint stress produced marked, time-dependent and sex-specific changes in dHPC glutamatergic homeostasis: stressed females showed an immediate increase in glutamate, glutamine and total glutamate–glutamine pool associated with reduced miniature EPSC amplitude and decreased AMPA/NMDA ratio at CA3–CA1 synapses, whereas stressed males exhibited a delayed hypoglutamatergic profile 24 h post-stress without detectable synaptic transmission deficits. Pre-stress administration of MK-801 (0.1 mg/kg, i.p.) selectively rescued NOL performance in stressed male mice, while memantine (5 mg/kg, i.p.) selectively normalised spatial memory in stressed female mice without affecting locomotion or exploration. In females, memantine also restored the AMPA/NMDA ratio at CA3–CA1 synapses to control levels, indicating a rescue of stress-induced weakening of excitatory synaptic strength. These findings reveal divergent, sex-specific glutamatergic pathophysiology underlying acute stress-induced memory impairment and support the development of sex-tailored NMDA receptor–based interventions for stress-related cognitive dysfunction.

Brain metabolic correlates of plasma biomarkers in amyotrophic lateral sclerosis.

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Objective. Amyotrophic lateral sclerosis (ALS) is characterised by loss of upper (UMN) and lower (LMN) motor neurons. Neurofilament light chain (NfL) has been proposed as a proxy of central involvement. We explored brain metabolic correlates of NfL and a panel of various plasma biomarkers. Methods. We recruited ALS patients from Turin ALS Centre, who underwent brain 2-[18F]FDG-PET. NfL, GFAP, UCHL1 and total tau (t-tau) were analysed with Simoa®, MMP-9 and MCP-1 with AlphaLISA, NfL, phosphorilated tau in position 181 (p181tau) and 217 (p217tau) with Lumipulse®. For each biomarker we performed a multiple regression analysis, adjusting for age, sex, site of onset, progression rate and cognitive status. Results. On 65 ALS patients, Simoa® and Lumipulse® NfL measurements showed a good correlation and agreement, with comparable ability to capture disease progression. UMN scores displayed a positive and negative correlation trend with NfL and p181tau, respectively. p181tau correlated inversely with t-tau and positively with p217tau, creatinkinase and atrophy. NfL showed an inverse correlation with frontal metabolism (medial, superior and precentral frontal gyri, paracentral lobule and cingulate gyrus). A trend was found between cerebellum and both t-tau (positive) and p181tau (negative). We observed a positive correlation between p181tau and anterior cingulate cortex (ACC) metabolism. Adjustment for cognitive status reduced association of limbic areas and motor cortex with NfL and of cerebellum with p181tau. Other biomarkers showed no correlation with brain metabolism. Conclusion. The negative correlation between frontal metabolism and NfL supports the role of NfL as a marker of UMN damage. The clinical and metabolic correlates of NfL, t-tau and p181tau could suggest the ACC involvement and the cerebellar compensatory role in cognitive impairment, probably affecting less explored functions, like social cognition and memory, in patients with a wider central involvement.

Differential recruitment of the thalamic ventral intermediate nucleus during observation and execution of goal-directed grasping actions

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The thalamic ventral intermediate nucleus (Vim) integrates motor information previously processed in the cortico-basal ganglia-cerebellar circuits to relay these signals to motor cortical areas. Vim represents the primary target for MR-guided Focused Ultrasound (MRgFUS) to alleviate upper-limb Essential Tremor (ET). In this fMRI study we aimed at mapping, using a high spatial resolution (voxel size=1.75 mm3), the functional activation of the Vim during observation, imagery, and execution of actions. Twenty-five healthy participants underwent six conditions: passive observation of goal-directed reaching-grasping actions (OBS ACTION); passive observation of static hand-object images; motor imagery of a reaching-grasping action on the objects; passive objects observation; execution of reaching-grasping of a sphere (EXE GRASP); execution of hand opening-closing movement. Small volume correction (SVC) was performed within the Vim ($p<0.05$ FWE). Additionally, spatial overlap was computed between Vim activation clusters and two MNI literature-based MRgFUS sweetspots for ET, as well as the lesion overlap map derived from our cohort of 70 ET patients treated with MRgFUS. SVC analyses revealed that OBS ACTION activated the Vim medial dorsal portion, whereas EXE GRASP recruited both its medial dorsal and ventral sectors. No Vim activations were reported in the remaining conditions. Notably, the MRgFUS sweetspots for ET reported in the literature, as well as our own lesion overlap hotspot, were included within the most ventral sectors of the Vim activation cluster during EXE GRASP. Overall, this study provides the first evidence that Vim is differentially recruited during action observation and action execution, thus supporting the hypothesis of its involvement in visual information processing and motor resonance mechanisms. Importantly, these results further confirm the fundamental role of this nucleus in the upper-limb motor control in both healthy subjects and ET patients.

Biomarker discovery in Sneddon syndrome: an exploratory plasma proteomic study

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Sneddon Syndrome (SS) is a rare, progressive, non-inflammatory vasculopathy affecting small and medium arteries, characterized by livedo reticularis and recurrent stroke. With an incidence of 4 cases per 1,000,000 individuals, SS predominantly affects young to middle-aged women. No diagnostic gold standard currently exists, the clinical diagnosis being based on livedo reticularis and neurological manifestations. Confirmation is achieved through brain MRI, skin biopsy, antiphospholipid antibodies, and, in selected cases, testing for ADA2 mutations. An exploratory proteomic analysis was conducted on plasma samples from SS patients by Olink Proximity Extension Assay platform, using “Neurology” and “Neuro Exploratory” panels including 184 proteins. Relative protein expression levels were compared with healthy donors and correlated with clinical variables to identify potential disease-associated biomarkers.
Correlation analysis revealed positive and negative associations between circulating proteins and clinical features (e.g. ischemic event severity). Among these, circulating Heme oxygenase 2 (HO-2) showed a strong positive correlation with skin biopsy test positivity ($r \approx 0.96$, $p = 0.022$). HO-2 is a constitutive heme-degrading enzyme that generally reduces oxidative damage and ensures cerebral blood flow, and is highly expressed in cerebral vessels, although pre-clinical models of intracerebral hemorrhage suggested also neurotoxic effects. Increased plasma HO-2 levels were specifically associated with characteristic cutaneous vascular alterations, suggesting a potential role as a diagnostic biomarker in SN. Moreover, skin biopsy positivity displayed weak or absent correlations with other biomarkers, supporting the specificity of the HO-2 association.
These preliminary findings identify HO-2 as a promising candidate biomarker with potential diagnostic and pathophysiological relevance in SS, warranting further validation in larger patient cohorts.

POSTER

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SESSION

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Source dynamics during action and observation: the impact of live, video, and avatar formats on alpha and beta ERD

CNN 01

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Perceiving others' actions is fundamental for human social interaction and relies on the Action Observation Network (AON). In modern digital environments, however, real interpersonal interactions are increasingly replaced by videos and virtual avatars, potentially altering sensorimotor processing. This study investigated how biological and artificial kinematics modulate cortical source-level Event-Related Desynchronization (ERD) during action execution and observation. High-density electroencephalography (EEG) was recorded during four conditions: motor Execution, Live Observation, Human Video Observation, and Avatar Observation. Each included two paradigms: a manipulative action (reaching-and-grasping an object) and a non-manipulative action (non-functional touch). Source-level ERD was computed using log-ratio contrasts (Manipulative−Non-manipulative) in the Alpha (8–13 Hz) and Beta (14–25 Hz) bands. Alpha modulation showed broad distributed patterns across frontal, temporal, and occipital regions, suggesting a general attentional and visuomotor involvement. Conversely, Beta modulation revealed selective condition-dependent dynamics. During Execution, manipulative trials induced stronger fronto-central modulation, while Non-manipulative actions engaged posterior regions. During Live Observation, central sensorimotor sources showed stronger modulation for Non-manipulative trials. Human Video Observation showed weaker and spatially distributed effects involving temporal and sensorimotor regions, while Avatar Observation exhibited a distributed visual-parietal engagement during Non-manipulative trials alongside a selective recruitment of the medially located Supplementary Motor Area (SMA) restricted to the virtual Manipulative condition. Overall, these findings indicate that cortical processing of observed actions is strongly shaped by both stimulus format and object-related context, with the clearest sensorimotor differentiation emerging during real action execution.

Investigating the feasibility and efficacy of a virtual reality cognitive training platform for post-stroke patients with mild cognitive impairment

CNN 02

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Globally, there are approximately 93.8M people living with the aftereffects of a stroke. Post-stroke mild cognitive impairment (PMCI) is defined as the occurrence of cognitive deterioration after stroke, ranging from minor impairment to dementia. Research has demonstrated that the prevalence of PMCI in the first year after stroke is 40%, with follow-up studies showing PMCI at 61% among 10-year survivors. This underscores the urgent need for scalable non-pharmacological interventions that leverage innovative technologies such as virtual reality (VR) to engage impaired brain circuity and promote attention/arousal in the middle cerebral artery territory—which supplies the cerebral cortex (specifically the frontal, parietal, and temporal lobes), i.e., those brain regions most frequently and severely affected by stroke. By leveraging VR's potential for producing immersive 3D visual stimuli, we can effectively engage the frontoparietal network, involved in visuospatial memory and the salience network necessary for attention. Our forthcoming study includes our VR cognitive rehabilitation training (VR-CRT) intervention, which provides the synergistic effects of selection attention training with immersive VR navigation, offering a unique mechanism of neurotransmitter stimulation. VR-CRT has advantages over traditional cognitive training programs due to its immersive 3D VR environment with 81 embedded attention exercises. In our prior study using VR-CRT we observed modest to above average improvement with neuro-oncology patients with mild cognitive impairment. The aim of our forthcoming study is to measure the feasibility and efficacy of VR-CRT on PMCI patients, observing for memory, attention, processing speed, and executive function. The central hypothesis is, PMCI patients randomized to the experimental arm (VR-CRT) will show trends toward higher total index cognitive scores from baseline, compared to the control arm using traditional cognitive therapies.

Modulation of executive functions in healthy humans through Opuntia ficus-indica fruit consumption and non-invasive brain stimulation

CNN 03

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Non-invasive brain stimulation is an established tool for modulating executive functions. Recent evidence suggests these functions may also be influenced by functional foods containing blood–brain barrier–permeable molecules. We examined whether consuming yellow–orange Opuntia ficus-indica fruits (OFIF), rich in the pigment Indicaxanthin (IX), affects visuospatial working memory (WM) and interacts with offline anodal transcranial direct current stimulation (a-tDCS) over the right dorsolateral prefrontal cortex. Eighteen healthy adults completed a single-blind, placebo-controlled study: a treatment group (n=12) ate 400g yellow–orange IX-rich OFIF, a placebo group (n=6) ate 400g white IX-devoid OFIF. Each completed two sessions (with/without fruit), performing a visuospatial N-back task (1-back, 2-back) before (baseline) and after a-tDCS. a-tDCS selectively improved the 2-back (p=.04), not the 1-back, whereas OFIF improved the 1-back (p=.01), not the 2-back. By cultivar: on the 1-back, yellow–orange OFIF increased accuracy vs no-fruit both at baseline (p<.001) and after a-tDCS (p=.001); white OFIF improved accuracy only after stimulation (p<.001), not at baseline (p=1.000). On the 2-back, yellow–orange OFIF improved accuracy at baseline (p<.001) but reduced it after a-tDCS (p=.005), while white OFIF improved it both before and after (both p<.001). At baseline both cultivars improved the 2-back, more so for yellow–orange OFIF (p=.02). a-tDCS and IX-rich OFIF appear to support WM through partly dissociable mechanisms. The lack of additive gains for combined yellow–orange OFIF and a-tDCS suggests homeostatic plasticity preventing excessive cortical excitability. The greater baseline gain for yellow–orange over white fruit indicates a specific IX effect beyond expectancy. IX-rich OFIF may thus act as a neuromodulator of prefrontal WM and interact with a-tDCS-induced plasticity, offering a new perspective on how functional foods may support cognitive performance.

Exogenous α-synuclein reshapes presynaptic vesicle dynamics and synaptic function in hippocampal neurons

ND 01

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Parkinson's disease (PD) is the second most common neurodegenerative disorder and its pathophysiology extends far beyond the classical motor symptoms associated with the disease. α-Synuclein (α-syn) oligomers are considered key mediators of PD pathology, inducing a wide range of cellular and synaptic alterations. Here, we investigated the effects of exogenous α-syn oligomers on cultured excitatory hippocampal neurons (HNs), both in network and autaptic configurations, using conventional electrophysiological approaches.

Our results show that exogenous α-syn is able to alter single action potentials (AP) parameters, more specifically by increasing the upstroke speed (US) and the after-hyperpolarization peak (AHP). Moreover, we show that exogenous α-syn significantly shifts the distribution of inter-event intervals (IEIs) toward shorter values, without affecting the amplitude of miniature excitatory postsynaptic currents (mEPSCs) in hippocampal neuronal networks. This effect suggests an enhancement of presynaptic spontaneous neurotransmitter release. However, noise analysis carried on the same recordings showed also a possible post synaptic alteration at AMPA receptors, characterized by a reduction in single-channel conductance accompanied by an increase in receptor number. Finally, analysis of synaptic responses during 20 Hz stimulation trains in HNs autaptic neurons revealed a significant increase in the size of the readily releasable pool (RRP), accompanied by a reduction in release probability, highlighting the fact that spontaneous versus evoked synaptic release is differently affected.

Overall, these findings identify α-syn oligomers as potent modulators of hippocampal synaptic function and suggest that early synaptic alterations may represent a mechanistic link between α-syn pathology and cognitive impairment in Parkinson's disease.

Retinal alterations resemble brain pathology in a rat model of Parkinson's disease induced by intranigral infusion of α-synuclein oligomers

ND 02

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Parkinson's disease (PD) is a neurodegenerative synucleinopathy, characterized by dopaminergic degeneration, pathological alpha-synuclein (α-Syn) deposition, and neuroinflammation in both motor and non-motor brain regions. Despite its motor-centric characterization, visual disturbances such as hallucinations, diplopia, altered contrast sensitivity and retinal abnormalities are well-documented non-motor changes of PD. However, the neuropathological basis of retinal dysfunction and the role of α-Syn remain poorly investigated. Given the central neuropathological role of α-Syn in PD brain, we assessed whether the retina is affected in a translational rat model of PD based on the intranigral infusion of toxic oligomers of human α-Synuclein (H-α-SynOs). Rats received stereotaxic injections of H-α-SynOs or PBS (Vehicle) into the substantia nigra pars compacta (SNpc) and were sacrificed three months post-infusion.
Retinal tissue pathological parameters, the expression patterns of selected miRNAs and inflammatory markers were assessed. The retina of rats infused with H-α-SynOs exhibited high levels of phosphorylated-α-Syn (p-α-Syn), along with a significant decrease of tyrosine hydroxylase (TH)-positive, reflecting dopaminergic neuron disfunction. Analysis of PD-associated miRNAs in the retina also revealed heightened levels of miR-384-5p, which inversely correlated with the expression of its predicted molecular target, SIRT1, in rats infused with H-α-SynOs. Consistently, H-α-SynOs infusion induced a widespread activation of retinal astrocytes and microglia, accompanied by heightened proinflammatory cytokine signaling downstream of TLR4/NFkB.
Collectively, our data reveal that H-α-SynOs extend its neuropathological effects to retinal damage, reinforcing the robustness of our preclinical model in replicating PD pathology and its translational value as a platform for testing proactive interventions targeting PD-related neurodegeneration.

Modelling fatal familial insomnia with patient-derived and chemically aged human cerebral organoids

ND 03

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Fatal familial insomnia (FFI) is a genetic prion disease caused by the D178N-129M mutation in the PRNP gene. Disease onset typically occurs around 50 years of age and is rapidly progressive, with more than half of patients dying within one year. Despite extensive research, the mechanisms underlying FFI remain poorly understood. Human cerebral organoids (hCOs) derived from induced pluripotent stem cells (iPSCs) provide a promising platform to investigate disease processes in a human-relevant context. However, existing FFI hCO models, generated by introducing the D178N mutation into commercial iPSC lines, reproduce only limited disease-associated alterations, including altered neurotransmitter levels and astrogliosis, lacking key pathological features. To address these limitations, we developed a patient-derived hCO model of FFI that preserves the genetic background of affected individuals. Particularly, peripheral blood mononuclear cells (PBMCs) from FFI mutation carriers and non-carrier relatives were reprogrammed to generate four FFI iPSC lines, three non-carrier control lines, and two CRISPR-Cas9-corrected isogenic control lines. iPSCs were differentiated into neural progenitor cells and subsequently into hCOs. Additionally, to better model the age-related neurodegenerative phenotype of FFI, hCOs were treated with the neddylation inhibitor Pevonedistat, previously shown to induce neuronal aging features. Preliminary analyses showed increased expression of the astrocytic markers S100B and GFAP and reduced expression of the neuronal markers PSD95 and β3-tubulin in FFI hCOs compared with controls. Pevonedistat treatment increased cellular senescence markers, confirming induction of neuronal aging, and reduced PrP levels. Ongoing immunofluorescence studies are further evaluating astrocytosis and neuronal loss. Overall, this patient-derived, aged hCO model represents a valuable platform for investigating FFI pathogenesis.

When glia turn toxic: progranulin deficiency as a driver of neuronal damage

ND 04

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Glial cells are central regulators of brain homeostasis, and their dysfunction is increasingly implicated in neurodegenerative diseases. Loss-of-function mutations in granulin (GRN), leading to progranulin (PGRN) deficiency, disrupt immune regulation, cellular clearance, and neuronal integrity. Reduced PGRN levels are implicated in a spectrum of neurodegenerative disorders and are also observed during aging. To dissect PGRN-dependent mechanisms, we established a disease-relevant cellular model by generating GRN-KO glial cells and assessed non-cell-autonomous effects on wild-type mature SH-SY5Y neurons exposed to PGRN-deficient glial conditioned medium. These progranulin-deficient glial cells exhibited a disease-like phenotype characterized by impaired phagocytic capacity, an altered inflammatory profile, and a senescence-like state associated with reduced motility, highlighting a critical role for PGRN in glial homeostasis. Wild-type neurons treated with glia-derived conditioned medium displayed structural and functional deficits, including synaptic instability and reduced dendritic complexity, as revealed by optical and confocal microscopy, indicating that PGRN-deficient glia release factors detrimental to neuronal integrity. Exposure to environmental and inflammatory stressors further exacerbated glial dysfunction, consistently amplifying GRN-KO-associated phenotypes. Taken together, these findings establish glial PGRN deficiency as a primary driver of non-cell-autonomous neuronal vulnerability and validate this glial model as a robust platform for mechanistic investigation. Finally, we highlight rescue strategies targeting PGRN-related pathways, including granulin-derived peptides and natural bioactive compounds, as promising approaches to restore cellular homeostasis and mitigate disease-associated phenotypes.

The CD33-ganglioside axis in late-onset Alzheimer's disease: from environmental triggers to structural variants

ND 05

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The myeloid-restricted receptor CD33 (Siglec-3) is a sialic acid-binding inhibitory receptor that dampens microglial phagocytosis of amyloid-β through the ITIM-mediated recruitment of SHP-1/SHP-2. Although CD33 is a known genetic risk factor for late-onset Alzheimer's disease (LOAD), how environmental neuroinflammatory triggers, neuronal sialoglycan availability, and variation within the CD33 V-set Ig domain jointly modulate this axis remains poorly defined. In human HMC3 microglial cells, the endocrine disruptor bisphenol-A and bacterial LPS significantly up-regulated CD33 and IL-1β, while TREM2 expression remains unaffected, identifying inflammatory states associated with increased CD33 expression. In differentiating SH-SY5Y neurons, the sialyltransferases ST3GAL5 and ST3GAL2, responsible for GM3/GD3 ganglioside synthesis, were strongly induced upon retinoic acid and BDNF treatment, supporting neuronal availability of CD33 ligands. In PMA-differentiated THP-1 macrophages, exposure to the disialoganglioside GT1b modulated SYK phosphorylation, consistent with the ability of macrophage-like cells to translate ganglioside engagement into intracellular signaling. Parallel computational modeling of four V-set Ig domain missense variants (the LOAD-enriched rs2455069 p.R69G, together with F118L, R119W, and Y127H selected from ClinVar/gnomAD) were explored in silico (structural prediction and docking). The analysis identified R91 as a major alternative binding site to the canonical R119 across all variants and revealed variant-specific effects on binding-pocket conformation, while confocal immunofluorescence on transiently transfected HEK293 cells showed that R69G variant does not impair CD33 surface expression. Together, these data delineate a multi-scale framework in which inflammatory stimuli, neuronal sialoglycan availability, and CD33 V-set variation converge on the CD33 axis relevant to LOAD pathogenesis.

Head-to-head comparison of human painless NGF with wild-type NGF in the 5xFAD Alzheimer's disease model

ND 06

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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). 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 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 β-secretases. Furthermore, only hNGFp rescued 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 had distinct effects on cytokines. 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.

Impact of hyperglycemic metabolic stress in hAPPswe neurons: transcriptomic and morphological insights

ND 07

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The coexistence of Type 2 Diabetes (T2D) and Alzheimer's Disease (AD) exacerbates shared mechanisms including brain insulin resistance, chronic inflammation and metabolic dysfunctions, accelerating cognitive decline and increasing the risk of progression from mild cognitive impairment to AD. This study investigated the effects of hyperglycemic condition on primary cortical cultures derived from a familiar AD model (Tg2576) expressing the human APP gene carrying the Swedish mutation (hAPPswe). The mixed neurons/astrocytes cultures were isolated from E13.5 fetuses and, after 7 days in vitro, were exposed to control glucose levels (25 mM; normal glucose, NG) and high-glucose medium (35, 50, 75, 125 mM; HG) for 24 hours. Transcriptomic analysis (qPCR array) was performed on WT-NG, WT-HG, hAPPswe-NG and hAPPswe-HG groups, focusing on insulin and glucose metabolism pathways. Then, cultures were exposed to a glucose concentration gradient to evaluate nuclear morphology, mitochondrial potential (MitoTracker), and intracellular hAPP/hAb (6E10 antibody). Morphological readouts were analyzed by confocal microscopy and cell-based High Content Screening. In basal conditions, hAPPswe cultures show a gene expression profile related to insulin resistance condition. HG exposure resulted in a significant hAPPswe-specific dysregulation of several metabolic pathways. Moreover, HG induced an accumulation of hAPPswe/hAb both in neurons and astrocytes. Mitochondrial potential increased in WT and hAPPswe cells proportionally to glucose concentration, while showing a strong decrease at the higher level (125 mM). The two higher glucose concentrations (75, 125 mM) induced cell death in both genotypes, with a higher impact on hAPPswe neurons. Overall, these findings indicate that hyperglycemia triggers a selective vulnerability under AD background, supporting the hypothesis that metabolic stress could worsen pathologic processes and disease progression.

Proteomic profiling of iPSC-derived mesodiencephalic dopaminergic neurons revealed mitochondrial dysfunction, metabolic dysregulation and novel biomarkers for Parkinson's disease

ND 08

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Parkinson's disease (PD) is a neurodegenerative disorder resulting from the complex interplay between genetic and environmental factors. Earlier studies demonstrated a polygenic inheritance model based on rare variants in PD-associated genes. We aimed to identify deregulated molecular pathways and potential biomarkers in PD, using an integrated proteomic and transcriptional approach in human iPSC-derived mesodiencephalic dopaminergic neurons (mdDA), obtained after 45 days of in vitro differentiation 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 (FC ≤ 0.70; FC ≥ 1.30; p ≤ 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. Furthermore, Ingenuity Pathway Analysis (IPA) revealed significant alterations in mitochondrial function and glucose metabolism, with 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 confirmed at the transcriptional level by Real-Time PCR (qPCR) and Digital PCR (ddPCR). Overall, CAST, 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.

Aβ42 differentially modulates neurogenesis and extracellular vesicle signaling in SVZ-derived neurospheres

ND 09

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Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, characterized by progressive and irreversible cognitive decline, accompanied by neuroinflammation. A major pathological hallmark of AD is the generation of amyloid-beta (Aβ) peptides, particularly Aβ42, within amyloid plaques. However, the effects of Aβ42 on adult neurogenesis and extracellular vesicle (EV)–mediated inflammatory signaling remain poorly understood.
Subventricular zone (SVZ)-derived neurospheres represent a well-established in vitro model to study neurogenesis and neural stem cell (NSC) progeny. In parallel, NSC-derived EVs are emerging as key modulators of the neural microenvironment, reflecting the molecular state of their cells of origin and influencing neighboring or distant cells through bioactive cargo, including miRNAs.
Using live-cell imaging, we found that Aβ42 initially enhanced neurosphere proliferation, followed by an earlier onset of neuronal differentiation. This accelerated differentiation was associated with changes in EV size distribution and alterations in EV-associated miRNA profiles. Specifically, miRNAs involved in regulating NSC proliferation, differentiation, and pro-inflammatory signaling were significantly dysregulated following Aβ42 exposure, supporting their involvement in SVZ neurosphere behaviour.
Overall, our findings demonstrate that Aβ42 differentially modulates neurogenesis by transiently promoting proliferation while accelerating neuronal differentiation and independently reshapes EV signaling in NSC-derived neurospheres. The dysregulation of inflammation-related miRNAs suggests a mechanistic link between Aβ42 exposure, impaired neurogenesis, and neuroinflammatory signaling in AD. These findings provide new insights into disease progression and may help identify novel therapeutic targets to counteract neurodegeneration.

Comparative study of Ella and Lumipulse immunoassay for pTau217 detection in dementia

ND 10

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Dementia diagnosis still remains challenging due to the absence of robust biomarkers accessible with minimally invasive techniques. Immunoassays represent an important tool to quantify dementia-related biomarkers concentration in plasma with great sensitivity, marking a turning point for the accessibility of fluid biomarkers. PTau217 is a a well-established plasma marker in dementia, thereby facilitating the diagnosis process. The aim of this work was to assess and compare two widely used platforms, Lumipulse and Ella, based on their pTau217 detection sensitivity in plasma. Plasma samples were collected from 5 healthy controls (HC), 32 subjective cognitive decline (SCD), 24 mild cognitive impairment (MCI) and 11 Alzheimer's disease (AD) patients. PTau217 levels were measured using Lumipulse G600II automated system and for Ella immunoassay SR-X. Plasma pTau-217 concentration measured with both Lumipulse and Ella increased coherently with dementia continuum, pTau217 levels reflected also MMSE scores. Plasma pTau217 levels were then correlated with classic CSF biomarkers, such as pTau181, Aβ40 and Aβ42, showing consistency with the diagnosis. The analyses confirmed overall agreement between the two assays; although Lumipulse appeared to offer greater sensitivity. Both platforms provided concordant results, demonstrating that both represent reliable methods to assess plasma pTau217 levels in AD-dementia like patients for tracking the progression of the pathology.

Longitudinal dynamics of serum neurofilament light chain and TARDBP expression in amyotrophic lateral sclerosis: a 12-month follow-up study

ND 11

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Background: Neurofilament light chain (NfL) is a well-established biomarker of neuroaxonal damage in amyotrophic lateral sclerosis (ALS). TARDBP (TDP-43), a key RNA-binding protein involved in ALS pathogenesis, represents a central molecular hallmark of the disease. The relationship between neuroaxonal injury and TARDBP dysregulation across disease stages remains unclear. This study aimed to validate serum NfL levels and to investigate their longitudinal association with TARDBP expression.
Methods: Serum NfL was measured using the ELLA platform in 57 ALS patients and 28 healthy controls (HC). TARDBP gene expression was quantified by qPCR. Samples were collected at disease onset/baseline (T0) and after 12 months (T12). Clinical disability was assessed using the ALS Functional Rating Scale-Revised (ALSFRS-R). Cross-sectional and longitudinal analyses were performed.
Results: At T0, NfL levels were significantly higher in ALS patients compared with HC, confirming neuroaxonal damage. TARDBP expression was significantly downregulated at T0 versus controls. At T12, NfL levels significantly decreased but remained above HC. Conversely, TARDBP expression significantly increased at T12, showing a pathological upregulation pattern. NfL levels at T12 were significantly negatively correlated with ALSFRS-R scores, indicating that higher neuroaxonal damage was associated with greater functional impairment.
Conclusions: NfL is significantly elevated at disease onset in ALS and decreases over 12 months while remaining above control levels. TARDBP shows an opposite temporal pattern, with early downregulation followed by upregulation at 12 months. These findings suggest a dynamic relationship between neuroaxonal injury and TARDBP dysregulation, supporting their potential role as complementary biomarkers of disease progression.

Fluid biomarkers in cerebral amyloid angiopathy patients: proteomic signatures and biomarker validation

ND 12

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Cerebral amyloid angiopathy (CAA) is a common cerebral small vessel disease associated with spontaneous intracerebral haemorrhages, characterized by the progressive deposition of β-amyloid (Aβ) protein within the walls of cortical and leptomeningeal small arterioles. The current CAA diagnosis relies on radiological detection of late stage disease hallmarks, thus hindering an early clinical finding and potential interventions in CAA progression. There is an urgent need to clarify pathways underlying CAA for early diagnosis, patient stratification and development of effective treatments. This study was aimed to identify and validate novel biomarkers for CAA in both plasma and cerebrospinal fluid (CSF) through a proteomic approach. We analysed n=30 CAA patients versus appropriate controls, (CTRL) for both CSF (n=25) and plasma (n=30). We performed a proximity extension assay (PEA) using Olink Neurology and Neuroexploratory. Then we validate the top plasma candidate biomarkers in a patient subset, by targeted immunoassays (ELISA, SiMoA). As expected, CSF showed a low protein content with respect to plasma, with a modest alteration of some proteins. Instead, in plasma 10/184 proteins resulted significantly altered between CAA and CTRL. Among these, Brevican (p value=0.0027), a brain-specific extracellular matrix (ECM) proteoglycan, emerged as the most highly significantly downregulated in CAA patients; neurofilament light and ectodysplasin A2 receptor, the latter associated with inflammation and tissue damage, resulted as increased (p value=0.018 and 0.02 respectively). We then validated BCAN and NFL in a different patient's cohort, confirming its altered expression in plasma of CAA patients. Overall, our findings demonstrate how exploratory proteomic platforms such as Olink enable the simultaneous analysis of a large number of proteins using minimal sample volumes, facilitating the identification of disease-related molecular signatures and providing new insights into CAA pathophysiology.

Atypical glycosylation of the prion protein reshapes the molecular–phenotypic spectrum of sporadic prion disease

ND 13

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In prion diseases, the cellular prion protein, PrPC, undergoes physiological N-glycosylation at asparagine residues 181 and 197, generating di-, mono-, and unglycosylated glycoforms. Following conversion into the pathogenic isoform, PrPSc, these glycoforms give rise to the typical three-band profile detected by Western blot after protease digestion. The relative intensity of these bands represents an important molecular signature used for diagnosis and prion strain classification. In addition, the electrophoretic mobility of the unglycosylated PrPSc fragment allows the distinction between the two major molecular types, type 1 and type 2.
We investigated 16 sporadic prion disease cases showing an unusual PrPSc profile, characterized by complete proteolytic loss of the diglycosylated isoform and predominance of mono- and unglycosylated fragments. All cases were identified through the National Prion Disease Pathology Surveillance Center in Cleveland, USA, and PRNP sequencing excluded pathogenic mutations. The series showed a predominance of valine (V) homozygosity at codon 129, with 129VV accounting for 56% of cases, while methionine (M) homozygosity, 129MM, and 129MV represented 25% and 19%, respectively. Disease onset occurred as early as 22 years of age in 129MV cases, and younger onset was associated with more severe neuropathological changes. The 129MM cases were exclusively associated with PrPSc type 2-like molecular profile and lacked coarse PrP deposits, whereas 129MV and 129VV cases displayed a PrPSc type 1-like profile. Notably, 129VV cases showed the shortest disease duration and the mildest neuropathological changes.
Overall, these findings define an atypically glycosylated PrPSc phenotype across the full spectrum of PRNP codon 129 genotypes and suggest that altered PrP glycosylation can substantially reshape the expected relationship between codon 129 genotype, PrPSc molecular type, and disease phenotype.

Whole-organoid imaging of senescence-associated neurodegeneration

ND 14

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Neurodegenerative disorders (NDDs) are chronic and progressive pathological conditions characterized by incremental neuronal and synaptic loss, abnormal protein aggregation, and localized neuroinflammation. Aging is the major risk factor for NDDs, highlighting the central role of cellular senescence, a state of cell cycle arrest that contributes to aging and age-related diseases through the accumulation of dysfunctional cells.
In the nervous system, senescent cells promote neurodegeneration via the senescence-associated secretory phenotype (SASP), a pro-inflammatory program that disrupts tissue homeostasis. Persistent DNA damage triggers cell senescence; thus, the accumulation of senescent cells may worsen the neuroinflammation state, leading in NDDs by accelerating disease progression and damaging regenerative capacity.
Emerging studies identify p300 as a key epigenetic driver of cellular senescence and the SASP through de novo super-enhancers formation and epigenomic remodeling. In our recent work, we showed that p300 inhibition reduces senescence markers and delays stress-induced senescence in several human cellular models. Building on these findings, we are now investigating the link between these processes in 2D and 3D chemically-induced models of neurodegeneration. We aim to determine whether senescence contributes to neurodegenerative features and if p300 inhibition can alleviate the phenotype. To this end, we performed molecular and cellular analyses in both 2D neuronal cultures and 3D cortical organoids, including gene and protein expression. In addition, advanced 3D tissue clearing and volumetric imaging were conducted on whole-organoids to explore the spatial distribution of cellular populations, protein aggregates, and neuroinflammatory features inside the tissue architecture.

Defining microglial mechanisms underlying neurodegeneration in Friedreich's Ataxia through iPSC-based approaches

ND 15

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Friedreich's ataxia (FRDA) is an inherited neurodegenerative disease marked by progressive sensory and spinocerebellar dysfunction. While neuronal degeneration is central to the disease, increasing evidence suggests that non-neuronal cell populations may also contribute to its progression. In this study, we developed a robust protocol for generating human induced pluripotent stem cell (iPSC)-derived microglia (iMG) to characterize the functional and molecular phenotype of FRDA-patient-derived cells compared to healthy controls and investigate the potential contribution of microglial dysfunction to FRDA pathology. We confirmed iMG identity via immunofluorescence and expression analysis of microglial markers (Iba1, P2Y12, CD68, TREM2), while functional activity was assessed through migration, phagocytosis, and lipopolysaccharide (LPS)-induced activation assays. Our results demonstrated successful differentiation with no significant differences in gene or protein expression between control and FRDA microglia in basal conditions, although morphology evaluation suggested a differential phenotype. Functional assays revealed a distinct pathological profile: FRDA microglia exhibited significantly higher migration rates alongside a marked reduction in phagocytic activity and a blunted response to LPS stimulation, indicating reduced susceptibility to activation. Potential neurotoxic effects of FRDA microglia were investigated by treating control neurons with microglia-conditioned medium (MCM). Crucially, FRDA-MCM decreased mature neuronal markers, indicating a non-cell-autonomous toxic effect mediated by dysfunctional microglia. Our findings suggest that FRDA microglia possess an aberrant functional phenotype, which fails to provide neuroprotection, possibly contributing to the disease mechanisms, highlighting microglial dysfunction as a potential target for future therapeutic interventions in FRDA.

Unlocking the guardian of the brain: Choroid Plexus and neurodevelopmental insights

NDEV 01

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Cerebrospinal fluid (CSF), produced by the choroid plexus (ChP), constitutes the earliest extracellular environment of the developing brain. Through a specialized epithelial-vascular-immune interface, the ChP acts as a central homeostatic hub that governs CSF molecular composition, influencing brain maturation and circuit development. Growing evidence implicates ChP dysfunction and immune dysregulation in neurodevelopmental and neuropsychiatric conditions, including autism and schizophrenia, highlighting the ChP as a key amplifier of pathological processes. Yet, modeling this integrated structure in humans remains challenging: animal models incompletely capture human physiology, while existing in vitro systems lack key components to replicate ChP complexity beyond its epithelial compartment. To address this, we developed Vascularized and Immune-competent ChP Organoids (VIChOs) from human pluripotent stem cells, enabling the co-differentiation of ectodermal and mesodermal lineages within a single 3D system. VIChOs faithfully recapitulate native human ChP cellular heterogeneity and ultrastructural features while preserving coordinated functional competence. Using single-cell RNA sequencing, we longitudinally characterized VIChO cell-type composition, revealing a dynamic cellular landscape regulated by subtype-specific signaling pathways. By leveraging this inherent heterogeneity, we conducted hypothesis-driven perturbations that unveiled context-dependent shifts in cellular organization and secretome composition, including cytokine-responsive endo-epithelial barrier remodeling, activation and expansion of endogenous border-associated macrophages, and serotonin-evoked apocrine secretion. Functionally, VIChO-derived CSF-like fluid possesses a selective metabolic profile capable of modulating neuronal activity and synchrony in human cortical networks. Collectively, our work establishes VIChOs as a novel, scalable human ChP model in which integrated cellular complexity determines CSF metabolic composition to instruct cortical circuit development and synaptic maturation, providing a physiologically relevant platform for exploring ChP biology in health, disease, and therapeutic discovery.

Brain-wide mapping of perineuronal nets and parvalbumin-expressing neurons across postnatal development identifies age- and sex-dependent circuit alterations in behaviorally characterized Fmr1 KO mice

NDEV 02

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Fragile X syndrome (FXS), caused by loss of FMR1 function, is the most common monogenic cause of intellectual disability and autism spectrum disorder. Altered maturation of inhibitory circuits and perineuronal nets (PNNs) – extracellular matrix (ECM) structures that preferentially enwrap parvalbumin-positive interneurons (PVIs) – may contribute to FXS pathophysiology by affecting critical period closure and circuit stability. However, their brain-wide organization across development and sex remains poorly characterized. We developed a serial-section-based pipeline integrating atlas registration and AI-trained image analysis to map PNNs and PVIs in adult P60 male Fmr1 knockout and wild-type mice. We then extended the analysis to both sexes at P20, P40 and P60, combining molecular and behavioral assessments. In P60 males, Fmr1 deletion reduced PNNs and PVIs across cortex, amygdala, hippocampus and striatum. PNN loss was marked in the isocortex, while PVI density was highly reduced in the amygdala. Similar alterations in P40 males suggest that PNN/PVI abnormalities emerge postnatally and persist into adulthood. Conversely, females at P20 and P40 showed no significant PNN/PVI alterations, supporting sex-dependent vulnerability of neuroplasticity. Preliminary data suggest that behavioral abnormalities attenuate with age while PNN/PVI alterations become more pronounced, indicating that a milder behavioral phenotype does not necessarily reflect molecular normalization. This divergence may arise from compensatory mechanisms that mitigate overt deficits without restoring the molecular phenotype, while highlighting PNNs and PVIs as sensitive readouts of persistent circuit alterations in FXS. By integrating brain-wide PNN/PVI mapping with behavioral analyses, this study identifies age-, sex- and region-specific alterations in ECM organization and inhibitory maturation, helping to define vulnerable circuits and developmental windows relevant for therapeutic intervention in FXS.

Enhanced oxidative stress-induced damage and cellular senescence are associated with FMRP deficiency in a mouse model of Fragile X Syndrome

NDEV 03

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Mutations in the fragile X mental retardation 1 (FMR1) gene leads to absence of fragile X ribonucleoprotein 1 (FMRP), therefore causing Fragile X syndrome (FXS), the most common form of inherited intellectual disability and a leading monogenic cause of autism. FMRP is an RNA-binding protein, which regulates mRNA transport and translation, whose absence in the brain alters synaptic protein expression and impairs neuronal function. It also participates in the DNA damage response and is a component of stress granules (SGs). Moreover, the Fmr1 knockout (KO) mouse brain is characterized by an excess of oxidizing agents, which not only enhance susceptibility to cell injury, but can also trigger cellular senescence. Understanding how neural cells respond to the oxidative environment in the absence of FMRP is the main goal of our work.
We examined SGs formation and cell survival after exposure to oxidative stress in cultured neurons and astrocytes of wild-type (WT) and Fmr1 KO mice by immunocytochemistry and MTT assay. We also used senescence-associated-b-galactosidase (SA-b-gal) assay and Western blotting to study markers of senescence both in vitro and ex vivo. Fmr1 KO astrocytes exposed to oxidative stress exhibit a lower number of SGs and a lower viability, the latter also detectable in neurons. Fmr1 KO brains exhibited increased SA-b-gal staining and an altered expression pattern of senescent markers.
These results suggest that lack of FMRP sensitizes cells to oxidative stress-induced damage and accelerates senescence processes, possibly contributing to brain dysfunction in FXS.

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RENKCTD11 loss drives cerebellar dysfunction and behavioral deficits in neurodevelopmental disorders

NDEV 04

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Neurodevelopmental disorders (NDDs) are often associated with brain abnormalities leading to behavioral and motor deficits. While cortical regions were long considered main contributors, the cerebellum also plays a central role in complex behaviors. However, how molecular changes governing cerebellar development contribute to NDDs remains unclear. Our group identified a novel gene, RENKCTD11 (REN), as a key regulator of cerebellar development, involved in negative control of Hedgehog (Hh) signaling. REN belongs to the KCTD gene family, whose members are related to neurodevelopmental and neuropsychiatric disorders. Here, we generated and characterized a REN knock-out (REN-KO) mouse model. Early developmental analysis revealed altered cerebellar architecture in REN-KO mice compared to wild-type controls (REN-WT), associated with increased Ki-67 expression in REN-KO mice, further supporting the negative role of REN in Hh-dependent proliferation during cerebellar development. RNA-seq profiling followed by qRT-PCR validation on cerebella from 10-day-old REN-KO mice highlighted extensive transcriptional rewiring affecting pathways involved in synaptic organization, neuronal plasticity, and cerebellar circuit maturation. In parallel, we observed significant dysregulation of Hh target genes together with multiple genes previously associated with neurodevelopmental disorders, supporting the idea that REN loss disrupts molecular programs critical for proper cerebellar development and neuronal function. At the functional level, REN-KO mice at adult stage, exhibit marked behavioral abnormalities, including impaired social interaction and motor coordination. These phenotypes are accompanied with cerebellar morphological alterations, showing increased cerebellar size, a feature reported in preclinical models of several NDDs. Overall, our findings illuminate how loss of REN leads to disruption of cerebellar developmental program and contribute to NDDs and neurobehavior deficits.

Post-transcriptional strategies to enhance FOXG1 expression

NDEV 05

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FOXG1 is a master regulatory gene involved in the early stages of cortical development and, like many brain-related genes, is highly dosage-sensitive. Consequently, de novo mutations in this gene cause a spectrum of neurodevelopmental disorders collectively known as FOXG1 syndrome, characterized by brain malformations, epilepsy, and autism spectrum disorder-like features. A subset of these pathologies arises from FOXG1 haploinsufficiency, therefore they could in principle be ameliorated by increasing FOXG1 protein expression. In this project, we aim to investigate multiple post-transcriptional strategies to achieve this goal.
First, we examined three negative regulatory elements within the untranslated regions (UTRs) of FOXG1 mRNA: (1) an upstream open reading frame that negatively affects FOXG1 translation, presumably by recruiting a fraction of scanning ribosomes; (2) a set of phylogenetically conserved miRNA target sites located in the 3′UTR, associated with miRNAs that are highly expressed in the neocortex and have been shown to reduce FOXG1 expression in vitro and in vivo; and (3) naturally preferred, distal polyadenylation sites that promote the production of longer, possibly less stable mRNA isoforms.
We targeted these regions using specific antisense oligonucleotides designed to selectively interfere with their regulatory activity.
In parallel, we explored the possibility of enhancing protein production from endogenous messenger RNAs using two types of translational activators: a programmable RNA-based platform inspired by natural internal ribosome entry sites (IRESs), and a CRISPR/Cas-inspired RNA-targeting system. Both approaches promote translation by recruiting components of the translational machinery. We designed them to target structurally accessible sequences within the FOXG1 3′UTR.
If successful, these methods could complement other tools already available in our laboratory, enhancing FOXG1 expression at both translational and transcriptional levels.

Exploring the role of oxidative stress in megalencephalic leukoencephalopathy with subcortical cysts (MLC)

NDEV 06

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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.

A human innervated gut model for chronic visceral pain

NI 01

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Chronic visceral intestinal pain is a widespread and debilitating condition, affecting over 20% of adults, often associated with high rates of gut disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). This condition is driven by dysregulated communication between the intestinal epithelium, stromal microenvironment, and sensory neurons, which makes diagnosis and therapeutic planning intrinsically difficult.[1]
In this context, several in vitro platforms have been developed to investigate the cellular mechanisms and signalling pathways underlying gut pathophysiology [2]. However, current in vitro models fail to reproduce the structural complexity, extracellular matrix organization, and functional innervation of human intestinal tissue, thereby preventing the study of epithelial-neuronal crosstalk and peripheral sensitization.
To overcome these limitations, we developed a functional human endogenous connective tissue model of the intestine, comprising a bioengineered human full-thickness three-dimensional (3D) stromal equivalent that supports a polarized intestinal epithelium, following a two-step bottom-up approach.[3] This consists of two main steps: first, stromal microtissue precursors (µTPs) were obtained by seeding human intestinal myofibroblasts onto porous microbeads, allowing endogenous extracellular matrix production. Then, µTPs were assembled within a maturation chamber to form a macroscopic human endogenous connective tissue that faithfully recapitulates the morphological organization of the native intestinal stroma. The resulting 3D stromal equivalent promotes epithelial polarization and differentiation when co-cultured with intestinal epithelial cells (Caco-2).
Furthermore, to accurately model the peripheral somatosensory system, the platform was integrated with human-iPSCs derived sensory neurons, resembling the dorsal ganglion root neurons' innervation. [4] Then, chemogenic stimuli, such as capsaicin [4], were selectively applied to the luminal compartment to investigate the functional crosstalk between the intestinal and neuronal compartments. Specifically, the stimulus was expected to trigger a significant neuronal hyperactivation, effectively recapitulating the peripheral sensitization mechanisms associated with visceral pain[CA1.1]. The model was subsequently characterized by assessing barrier integrity via transepithelial electrical resistance (TEER) measurements, and by evaluating neuroinflammatory signalling and neuronal hyperactivation through electrophysiological recordings using high-density microelectrode arrays (HDMEA) devices. [5] Overall, this platform provides a novel and physiologically relevant in vitro tool to model chronic visceral pain, with potential applications in drug screening, mechanistic studies, and the development of targeted therapies.

Immune dysregulation and impaired pro-resolving pathways in epilepsy

NI 02

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Epilepsy is a neurological disorder characterized by repeated and unpredictable seizures caused by abnormal brain activity. Among its different forms, late-onset epilepsy of unknown origin (LOUE) occurs in absence of identifiable causes and is clinically associated with focal seizures. LOUE is considered as an early manifestation of age-related brain pathology driven by mechanisms enhancing cortical excitability, including cerebrovascular alterations, amyloidosis, and neuroinflammation. Recent studies suggest that neuroinflammation may result from defective resolution pathways normally mediated by omega-3-derived specialized pro-resolving lipid mediators (SPMs). Thus, we performed an immunophenotypic analysis of peripheral blood mononuclear immune cells in LOUE patients. Compared to healthy controls, we observed significant alterations in innate and adaptive immunity, including increased frequencies of non-classical monocytes, dendritic cells, NK and NKT cells, as well as CD4+ and CD8+ T lymphocytes. Functional characterization of T-cell subsets revealed enhanced production of pro-inflammatory cytokines, particularly TNFα and IFNγ, in both CD4+ and CD8+ T cells. These alterations were associated with dysregulation of pro-resolving pathways, involving SPM enzymes (i.e. COX2 and 5/12/15-LOXs) and receptors (i.e. FPR2, ChemR23, GPR18/32/37 and LgR6). Despite, several of these were upregulated in specific immune subsets, unchanged or reduced expression of either enzymes or receptors were identified among several cell subsets such as non-classical and intermediate monocytes, myeloid and plasmacytoid dendritic cells. Importantly, treatment with key SPMs like MaR1 and RvD3 reduced TNFα production by CD4+ T cells and increased the frequency of regulatory T cells. Overall, our findings identify systemic immune dysregulation and impaired resolution pathways as key features of epilepsy, providing new insights into disease mechanisms and potential therapeutic targets.

Dual sEH/LOX-5 inhibition improves mitochondrial resilience in in vitro and in vivo models of Parkinson's disease

NI 03

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Parkinson's disease (PD) is a multifactorial neurodegenerative disorder with heavy medical and societal implications worldwide. Among PD hallmarks, neuroinflammation and mitochondrial dysfunction drive the degeneration of dopaminergic neurons (DAergic) in the substantia nigra pars compacta. Despite progress in understanding inflammatory pathways, current therapies are based on single-targets, such as cyclooxygenase-2 (COX-2) and leukotriene (LT)-modulating agents. Evidence suggests a role of lipid mediators - epoxy fatty acids (EpFAs) – which regulate initiation, amplification, and resolution of inflammation, and whose dysregulation drives chronic inflammation. Soluble epoxide hydrolase (sEH) converts EpFAs to their corresponding diols, lacking anti-inflammatory properties and fueling inflammation. We investigated the potential neuroprotective effects of inhibiting sEH enzyme, singularly and in combination with lipooxigenase-5 (LOX-5), with novel compounds LO44 and LO35 respectively, to achieve a comprehensive control of inflammatory responses. In in vitro models of PD, viability experiments revealed LO35 as the most effective in conferring DAergic cells resilience to MPP+-mitochondrial dysfunction. Untargeted metabolomic analysis identified specific metabolites changes upon LO35 treatment, related to cell bioenergetics and antioxidant activity. Finally, we exposed zebrafish larvae to neurotoxin MPP+ to validate LO35 pre-treatment effect in an in vivo PD model. Behavioral assays showed increased locomotor activity and response to touch stimuli in LO35 pre-treated larvae. Overall, these findings point at regulating lipid mediators balance as a novel strategy to enhance DAergic neuroprotection and to dampen neuroinflammation. The dual targeting of this pathway may overcome current limitations and will be a further step towards the development of future effective treatments that modify the course of PD and possibly improving patient quality of life.

Investigating the role of early-life inflammation in neurodevelopmental alterations associated with WHIM syndrome

NI 04

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WHIM syndrome is a rare primary immunodeficiency caused by gain-of-function mutations in the chemokine receptor C-X-C motif chemokine receptor 4 (CXCR4), leading to leukocyte retention in the bone marrow, neutropenia, recurrent infections, and immune dysfunction. Beyond hematological manifestations, CXCR4 signaling plays a crucial role during central nervous system development. Recent evidence demonstrated that CXCR4 dysfunction is associated with cerebellar structural abnormalities and persistent psychomotor impairments, suggesting a neurodevelopmental component of the disease. The present study investigates whether recurrent inflammatory insults during early postnatal life may exacerbate the neurological phenotype associated with CXCR4 dysfunction. To model the inflammatory challenges commonly experienced by WHIM patients, mutant mice are exposed to Complete Freund's Adjuvant (CFA)-induced peripheral inflammation at postnatal day 6 (P6) and postnatal day 14 (P14), two critical windows for cerebellar maturation and circuit refinement. The inflammatory response following CFA exposure is characterized through enzyme-linked immunosorbent assay (ELISA), flow cytometry (FACS), and immunohistochemistry to assess systemic and central immune activation. Cerebellar involvement and neurodevelopmental outcomes are evaluated through a battery of developmental and psychomotor milestones previously established in CXCR4 mutant mice. Behavioral analyses are further refined using DeepLabCut, a markerless pose estimation software that enables high-resolution quantification of subtle motor abnormalities not detectable with conventional observational scoring. This approach aims to determine whether early-life inflammation synergize with CXCR4-mediated neurodevelopmental defects to worsen cerebellar dysfunction and behavioral outcomes in WHIM syndrome.

Characterization of the dynamics of hematopoietic niches in the skull bone marrow by multiphoton microscopy

NI 05

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Recent evidence has challenged the traditional concept of the central nervous system (CNS) as an immune-privileged organ, highlighting the importance of skull bone marrow (sBM) hematopoietic niches in neuroimmune surveillance and response. Moreover, the sBM is directly connected to the meninges through specialized vascular channels, enabling the rapid trafficking of immune cells to the CNS. However, the organization and functional dynamics of these niches remain poorly understood. Here, we exploited in vivo two-photon microscopy to investigate the anatomy and cellular behavior of hematopoietic niches within the sBM. We developed a minimally invasive microsurgical method for direct injection into the sBM of live mice, enabling selective labeling of precursor cell populations and more mature immune cell subsets using fluorescent antibodies. Time-lapse two-photon imaging revealed dynamic cellular responses in physiological conditions and in response to stimuli. Tracking analyses revealed the differential behavior of different cell types in terms of cell mobilization and interaction. Overall, this work establishes a novel platform for the in vivo study of sBM hematopoietic niches and their dynamics under physiological and pathological conditions. This approach provides a foundation for future investigations into the contribution of sBM-derived immune cells to CNS homeostasis and pathology, supporting the development of therapeutic strategies targeting neuroimmune interactions. Furthermore, the sBM-meningeal interface may represent a preferential route for brain access that could be exploited for targeted drug delivery while bypassing systemic administration.

Physical exercise as a non-pharmacological treatment in a Zebrafish model of neuroinflammatory disease

NI 06

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Physical exercise has emerged as a promising non-pharmacological strategy to reduce disease burden in neuroinflammatory and neurodegenerative disorders, although the mechanisms through which it preserves motor function remain poorly understood. Since inflammatory processes contribute to several forms of neuromuscular dysfunction leading to progressive motor impairment, in this study we investigated the effects of structured exercise on disease onset, severity, and motor decline in a zebrafish model of neuroinflammation, with particular attention to motor fatigue and swimming performance. Adult zebrafish (Tubingen, 5–6 months old) were subcutaneously immunized with 1 mg/mL MOG in Complete Freund's Adjuvant (CFA), while control animals received CFA alone. Clinical symptoms, swimming abilities, and mortality were monitored daily for 7–15 days following immunization. In parallel, two additional groups underwent a 15-day resistance training (RT) protocol prior to injection, and Sprint Tests (ST) were performed before and after training to assess exercise-induced functional adaptations. MOG-immunized animals displayed an early onset of symptoms characterized by progressive immobility, reduced swimming activity, and abnormal body posture, whereas CFA-injected controls showed no evident clinical alterations. Trained animals were further evaluated for resistance to motor fatigue and swimming kinematics following EAE induction. At the end of the experiments, body musculature was collected for biomechanical characterization, and spinal cord samples were processed for histological analyses. Additionally, we will inject lipopolysaccharide to induce neuroinflammatory responses in adult zebrafish, providing further validation of exercise-based interventions. Overall, our findings support the use of zebrafish as a valuable model to investigate the mechanisms through which physical exercise may mitigate neuroinflammation-associated motor dysfunction and fatigue.

Allopregnanolone potentiation of P2X4 receptor in microglia involves lysosome trafficking pathway

NI 07

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Among cholesterol metabolites, allopregnanolone (alloP) is a neurosteroid known to modulate current elicited by several ionotropic receptors, such as GABAA, NMDA and, recently, purinergic P2X4. In microglia cells, stimulation of P2X4 receptor is known to increase the release of brain-derived neurotrophic factor (BDNF), leading to a potential neuroprotective effect in a neuroinflammatory milieu. P2X4 receptors are selectively activated by a wide range of adenosine triphosphate concentrations (ATP, 3-100 µM). In our study, performing electrophysiology patch-clamp experiments in BV2 microglia cells, we found that alloP potentiates P2X4 current in a concentration-dependent manner. Current potentiation by alloP occurred in the nanomolar range (1-100 nM) when receptors were activated by ATP 5 µM, whereas a smaller but significant current potentiation was observed in the presence of ATP 50 µM and alloP 10 µM. We then investigated the trafficking pathway linked to P2X4 receptor. After activation on the plasma membrane, P2X4 receptor is internalized in lysosomes to be eventually restored into the plasma membrane. In live cell imaging experiments carried out in BV2 cells using a construct of P2X4 receptor tagged with the yellow fluorescent protein (P2X4-YFP), we observed its colocalization with the lysosomal marker lysotracker red DND-99 (1 nM).
Cells expressing P2X4-YFP were exposed to ATP 5 µM and alloP 100 nM (to mimic the higher purinergic current potentiation) and, compared to control cells, they showed an increase in fluorescence. This result suggests that P2X4 receptors are reinserted in the plasma membrane following lysosomes trafficking, and these newly inserted receptors are contributing to the increased purinergic current. Therefore, alloP might promote P2X4 activity and associated effects, showing for the first time the influence of neurosteroids in microglia cells.

PBMC secretome from Autoimmune Encephalitis patients drives neuronal hyperexcitability in neurons from mouse medial entorhinal cortex slices

NI 08

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Autoimmune Encephalitis (AEs) are rare, debilitating, yet potentially treatable conditions, often characterized by specific antibodies against intracellular or neuronal surface proteins (NIAbs and NSAbs, respectively; Dalmau and Graus, 2018). Although pathogenic mechanisms exerted by some NSAbs- associated AE have been recently clarified, it is not clear why seizures occur in some patients and why they may evolve into epilepsy. A recent study led by our lab demonstrated that arterial perfusion of the isolated guinea pig brain with the PBMCs' secretome from AE patients—but not from healthy subjects—evokes seizure-like events (SLEs). This ictogenicity is linked to a distinct cytokine profile between patients and healthy subjects (Prevosti et al., 2025). To further investigate the impact of AE patient-derived cytokines on neuronal firing, synaptic transmission and plasticity, we perfused mouse brain slices with AE patient and healthy subject-derived PBMC secretome. The intrinsic membrane properties of pyramidal neurons from medial entorhinal cortex (mEC) layers V VI were investigated under current-clamp conditions following the perfusion of AE patient-and healthy subject PBMCs secretome. Spontaneous EPSCs/IPSCs were also recorded to evaluate the capability of AE patient-derived PBMC secretome to induce SLEs. AE patient-derived PBMC secretome increased frequency of firing and evoked spontaneous SLEs in neurons from mEC, confirming the capability of AE-patient derived soluble inflammatory mediators to contribute to seizure onset. This in vitro approach could help to dissect the functional contribution of patient derived inflammatory mediators on network hyper excitability, potentially allowing future finding of new therapeutic targets.

The FLASH effect: assessing survival and functional brain integrity

NO 01

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In brain cancer therapy, balancing tumor control with the preservation of neural function remains a central challenge, emphasizing the need to maximize anti-tumor efficacy while minimizing long-term damage to the healthy brain.
FLASH radiotherapy (≥40 Gy/s), which delivers radiation at ultra-high dose rates, has emerged as an innovative modality capable of widening this therapeutic window. Preclinical studies describe the "FLASH effect" as an effect in which the normal brain tissue is selectively spared without compromising tumor control compared to conventional (CONV) irradiation.
During my PhD research, I investigated the biological and functional consequences of FLASH versus CONV radiotherapy across distinct stages of brain maturation using complementary murine models. The experimental approach incorporated cell injections, motor and behavioral tests (including Grip Strength, Gridwalk, and the Object Recognition Test), magnetic resonance imaging (MRI), immunohistochemical analyses, and electrophysiological recordings.
In adult naïve and glioma-bearing mice, both irradiation modalities successfully delayed tumor progression and improved survival at 15 and 20 Gy, with the 20 Gy FLASH cohort exhibiting the most significant survival advantage.
Furthermore, to address the unique vulnerabilities of the developing brain, where neural circuits undergo critical periods of experience-dependent plasticity, we employed the monocular deprivation (MD) paradigm, the gold standard for quantifying visual cortex plasticity, to evaluate how radiation reshapes cortical adaptability. Preliminary findings reveal dose-dependent and modality-specific differences in plasticity responses between FLASH- and CONV-treated juvenile animals. Ongoing transcriptomic profiling (RNA-seq) aims to identify the specific molecular pathways underlying these divergent functional outcomes.

Therapeutic efficacy of an EGFRvIII-Retargeted, IL-12-Expressing oncolytic HSV for the treatment of glioblastoma

NO 02

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Glioblastoma (GBM) remains one of the most lethal primary brain tumors, largely due to its invasive growth and resistance to conventional therapies. Retargeted oncolytic herpes simplex viruses (oHSVs) provide a strategy to couple tumor-restricted infection with local immune stimulation. Building on prior evidence that cytokine-armed oHSVs can induce potent antitumor immunity, we explored EGFRvIII - a tumor-restricted mutant receptor recurrently expressed in GBM - as a selective entry receptor to enhance targeting precision and safety. We generated a fully retargeted third-generation oHSV, (R-429), engineered to recognize EGFRvIII and express murine IL-12. Viral infectivity, replication kinetics, and spreading were examined in EGFRvIII-positive and -negative glioblastoma and healthy cells. IL-12 production was quantified in infected cultures and antitumor activity was subsequently assessed in vivo in mice bearing EGFRvIII-expressing glioblastoma. R-429 displayed strict EGFRvIII-dependent tropism, efficiently infecting and eliminating receptor-positive GBM cells while sparing EGFRvIII-negative cells and normal fibroblasts, even at elevated viral doses. The virus maintained robust replicative capacity and efficient spreading across tumor cells. Infected cells secreted IL-12, confirming preservation of the immunostimulatory payload. Therapeutic administration of R-429 in tumor-bearing mice resulted in a marked extension of survival compared to controls. In conclusion, the EGFRvIII-targeted, IL-12–armed fully retargeted oHSV R-429 demonstrates high specificity, preserved oncolytic activity, and immunostimulatory potential, supporting its development as a promising therapeutic strategy for glioblastoma.

Investigating non-genetic drivers of intra-clonal competition in PDGF-driven glioma using a multiomic barcoding strategy

NO 03

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We have recently shown that during gliomagenesis clonal populations diverge into subgroups with varying fitness, driving a process of selection that leads to the predominance of specific clones and the extinction of others. Crucially, this selection persists even among clonally related cells in later tumor stages, indicating that clonal divergence is a continuous process. To elucidate the mechanisms underlying this phenomenon, we focused on thne hypothesis that heritable, clone-specific gene expression patterns -potentially driven by stable epigenetic profiles- provide the transcriptional heterogeneity necessary to fuel these competitive dynamics. To investigate this, we developed an optimized lentiviral lineage tracing library compatible with single-cell RNA sequencing (scRNA-seq). Our vector encodes a high diversity genetic barcode embedded within a transcript sca old, allowing for the simultaneous recovery of clonal identity and the global transcriptome using standard 3' polyA-capture protocols. We applied this tool to a PDGF-driven murine glioma model, initiating cultures with approximately 350 distinct clones. We performed baseline scRNA seq (t0) and established three independent biological replicates, which were cultured in vitro for 2.5 months with serial collection to track longitudinal clonal dynamics via NGS and assess di erential fitness. Our results indicate that at baseline (t0), linked transcriptional and clonal identity was successfully recovered for ~85% of sequenced cells. The population displayed an immediate clonal frequency imbalance, with two dominant clones accounting for approximately 50% of the total population. By isolating the top 30 most represented clones and applying ANOVA-based F-statistics and permutation testing, we identified ~5,000 "clonal genes" characterized by significantly higher inter-clonal variance compared to intra-clonal variance. This finding provides strong evidence for a widespread, non-genetic form of clonal transcriptional memory. Furthermore, regression analysis linking baseline clone sizes to transcriptional profiles revealed functional correlations, enabling the identification of specific gene signatures associated with clonal fitness. In conclusion, our study introduces a high-resolution single-cell barcoding platform that bridges lineage tracing with transcriptomics. Applied to monoclonal glioma cultures, this approach revealed that non-genetic, clonally heritable transcriptomic states spontaneously emerge, significantly reshaping the clonal architecture of the tumor. By exploiting this methodology, we aim to identify key gene targets potentially driving clonal competition mechanisms in glioblastoma.

Modulation of extracellular vesicles release targeting A2A adenosine receptor in glioblastoma

NO 04

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Glioblastoma (GB) is the most aggressive primary brain tumor in adults characterized by high intra- and inter-tumoral heterogeneity and drug resistance, limiting the efficacy of currently approved treatments. In the complexity of tumor microenvironment (TME), the release of extracellular vesicles (EVs) is one of the emerging mechanisms mediating the crosstalk between healthy and tumor cells, enabling the transfer of bioactive molecules that promote tumor growth, invasion, and immune evasion. GB-derived EVs contribute to the functional reprogramming of microglia, driving their transition from a neuroprotective to a tumor-supportive phenotype. In this context, differential modulation of EV release by healthy and tumor cells could reshape TME interactions. To this aim, we targeted the adenosinergic signaling downstream of the A_2A receptor (A_2AR), which is a direct regulator of EV release, by using an adenosine-dependent A_2AR partial agonist. This compound could exert a dual effect: acting as an agonist in low-adenosine environments, such as healthy microglia, thereby promoting EV release, while behaving as a functional antagonist in the high-adenosine tumor microenvironment, reducing vesicle release from tumor cells. We used 2D and 3D in vitro models of glioma-microglia interaction and a pre-clinical murine glioma model to evaluate the effects of this context-dependent agent on EV secretion by tumor cells, tumor-associated microglia and homeostatic microglia. Pharmacological modulation of A_2AR signalling with partial agonist normalized EV trafficking in a contextdependent manner, reducing EV release from glioma and tumorassociated microglia while promoting EV secretion from healthy microglia. This shift in EV dynamics was associated with attenuation of glioma growth in vitro and in vivo. Taken together this support regulation of EV release as a promising therapeutic approach to reprogram tumor–microglia interactions and counteract glioma progression.

Blood brain barrier in vitro model for the study of cancer cell extravasation and metastasis

NO 05

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The blood–brain barrier (BBB) is a specialized and dynamic interface that tightly regulates cellular and molecular transport between the central nervous system (CNS) and the systemic circulation. Primarily composed of brain microvascular endothelial cells closely associated with pericytes, the BBB maintains CNS homeostasis through restrictive tight junctions and selective permeability. While essential for protecting the brain, the BBB also represents a major obstacle to therapeutic delivery into the CNS, particularly in the context of brain malignancies and metastasis. This challenge is especially relevant in triple-negative breast cancer (TNBC), an aggressive breast cancer subtype characterized by a high incidence of brain metastasis and limited therapeutic options. In this study, a physiologically relevant in vitro BBB model was established and optimized using primary human brain microvascular endothelial cells (HBMECs) and human brain vascular pericytes (HBVPs) cultured on Trans-well inserts. While astrocytes had been incorporated in previous models without pericytes, their unavailability during the present study led to the incorporation of brain-specific pericytes as the supporting neurovascular cell type. To better recapitulate the native BBB microenvironment, cells were cultured on a composite of Matrigel and porcine brain-derived extracellular matrix (ECM). Barrier integrity and functionality were evaluated using trans-endothelial electrical resistance (TEER), Evans Blue and sodium fluorescein permeability assays, immunofluorescence, quantitative real-time PCR, and Western blot analysis of BBB-associated proteins, including Claudin-5 and ZO-1. Optimization of the endothelial–pericyte co-culture system resulted in enhanced barrier properties, demonstrated by increased TEER, reduced paracellular permeability, and improved junctional protein expression and localization. The optimized BBB model was subsequently used to investigate interactions between TNBC cells and the BBB using MDA-MB-231-shCx43 cells. Live-cell confocal imaging enabled visualization of tumor cell transmigration across the endothelial barrier, demonstrating the effectiveness of the model for capturing early steps of metastasis.

The model was further employed to evaluate the cytotoxic effects of the imipridone-based compounds ONC206 and ONC212 on TNBC cells within the BBB system. Live/Dead assays demonstrated reduced tumor cell viability following drug treatment, accompanied by morphological features consistent with cellular stress and death, including cell shrinkage, rounding, membrane blebbing, and detachment. These findings highlight the utility of the established BBB model as a platform for assessing the biological activity of candidate therapeutic agents under physiologically relevant conditions.

This work establishes a reproducible and physiologically relevant in vitro BBB model integrating primary human brain endothelial cells, pericytes, and brain-derived ECM. This platform provides a valuable tool for investigating the mechanisms underlying TNBC brain metastasis and for evaluating potential therapeutic agents targeting metastatic progression.

Keywords: Blood–Brain Barrier, Triple-Negative Breast Cancer, Brain Metastasis, MDA-MB-231, ONC206, ONC212, In Vitro BBB Model

Fluorescence-traced spatial profiling of tumor-neuron interactions in glioma-like assembloids

NO 06

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Neuronal activity is increasingly recognized as a driver of glioma growth, acting both through direct synaptic contacts and indirect signaling. However, current human model systems only partially capture the spatially organized interface at which glioma cells engage neuronal tissue. To address this, we are establishing a fluorescence-traced human assembloid platform to map glioma-neurons interactions with molecular resolution. The system is based on the fusion of control and NF1/TP53 double-knockout cortical organoids, in which combined loss of these tumor-suppressors promotes a malignant, glioma-like state. In individual organoids, we have already generated preliminary bulk and single-cell RNA sequencing, electrophysiological recordings, lentiviral fluorescent tracing and histological characterization, demonstrating the feasibility of integrating molecular, functional and imaging-based readouts in this system. To interrogate glioma-neuron interactions directly, control and glioma-like organoids will be transduced with lentiviral reporters carrying distinct fluorophores and transcript-associated barcodes. After 60 days of differentiation, organoids will be fused and maintained for approximately one month to allow cellular integration and synapse formation. Fluorescence-guided spatial transcriptomics will then be used to profile different compartments of the assembloid, including neighboring neural populations and regions of physical interaction. In parallel, multielectrode array recordings will assess whether tumor integration alters neuronal network activity. By combining genetic tumor modeling, organoid fusion, spatial transcriptomics and electrophysiology, this platform will enable direct investigation of how glioma-like cells engage human neural tissue. This will provide a spatially and functionally resolved framework to identify molecular programs mediating tumor–neuron interactions and uncover mechanisms by which neural activity may support glioma progression.

Targeted temozolomide delivery by hyaluronic acid-gold nanoparticle–liposome enhances antitumor activity in 2D and 3D models of glioblastoma

NO 07

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Glioblastoma (GB) is the most common and aggressive cancer of the central nervous system, characterized by high proliferation and invasiveness. In clinical treatment, Temozolomide (TMZ) has increased patient survival, but its efficacy is limited by the development of resistance in tumor cells and its short plasma half-life. The formulation of therapeutic agents in nanoparticles is one of the most promising approaches for the treatment of GB due to the increased availability at the target site, which increases efficacy and reduces toxic effects. In the oncology field, liposomes represent an innovative drug delivery strategy, enabling the active and specific targeting of organs and cells. Meanwhile, gold nanoparticles (AuNPs) are attracting growing interest for their unique physicochemical properties and biomedical applications, including drug delivery and targeting. It was demonstrated that their integration with liposomes could improve drug loading, paving the way for new drug delivery systems. Based on this evidence, this study aims to develop liposome-decorated AuNPs conjugated with hyaluronic acid (HA) and subsequently test them in in vitro 2D and 3D models of GB to generate innovative drug delivery formulations. HA could act as a targeting molecule due to its high affinity for the transmembrane receptor CD44+, which is overexpressed in GB cells. In vitro studies using human U87 GB cell lines showed that treatment with TMZ-loaded Au-NPs/HA exerted a significant cytotoxic effect compared to free TMZ. Intracellular studies conducted by flow cytometry revealed that liposomes are efficiently internalized by the U87 cells; however, when conjugated with Au NPs/HA, a significant enhancement in cellular uptake was observed. Moreover, the nanoparticles showed a stronger capacity to trigger the apoptotic pathway compared to free TMZ. In conclusion, these results support the potential of TMZ-loaded Au NPs/HA-liposomes as targeted drug delivery systems for GB therapy.

Patient-derived 3D glioblastoma spheroids recapitulate microanatomical complexity and enable functional investigation of tumor progression pathways

NO 17

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Glioblastoma (GBM) remains the most aggressive primary brain tumor, with poor prognosis driven by extensive intratumoral heterogeneity, therapeutic resistance, and recurrence. Patient-derived three-dimensional (3D) models offer the opportunity to recapitulate key structural and functional features of GBM while providing a clinically relevant platform for investigating disease-driving pathways. We established patient-derived 3D GBM spheroids and characterized their growth dynamics, morphology, cellular organization, and ultrastructural features using scanning and transmission electron microscopy (SEM and TEM). Furthermore, morpho-phenotipical analyses of our model revealed the establishment of spatially organized functional niches, including a hypoxic core and a proliferative peripheral zone, closely recapitulating the in vivo structural organization of GBM. To explore the utility of this model for pathway-oriented studies, we investigated SCF/c-KIT signaling, a receptor tyrosine kinase pathway involved in cell growth and survival. c-KIT is a proto-oncogene encoding the stem cell factor receptor and is frequently dysregulated in several malignancies, including gastrointestinal stromal tumors and melanoma. The aim of this study was to investigate the role of c-KIT signaling in GBM, with a specific focus on its interaction with the molecular chaperone HSP90. As HSP90 is known to stabilize multiple oncogenic client proteins, we hypothesized a functional association between c-KIT and HSP90 in GBM. To address this, we evaluated their interaction and co-localization using immunoprecipitation and immunofluorescence-based analyses. In conclusion, patient-derived 3D GBM spheroids recapitulate the microanatomical complexity and heterogeneity of GBM and provide a robust platform for functional investigation of tumor progression pathways. This model supports the exploration of the c-KIT-HSP90 axis as a potential therapeutic vulnerability for targeted strategies in GBM.

Role of Nicotinic Acetylcholine Receptors Expressed by Cholinergic Neurons in the Control of Striatal Activity and Mice Behaviour

NP 01

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The cholinergic signalling is critical for regulating behaviour and degeneration of cholinergic neurons (CNs) is linked to neurological disorders as well as to unbalanced sociability and reward circuitry. The striatum is a key cholinergic hub, containing cholinergic interneurons (CINs) that express beta2 nicotinic acetylcholine receptors (beta2*nAChRs), the main striatal source of acetylcholine. Our group previously showed that deleting beta2*nAChRs in striatal CINs alters mouse behaviour and striatal Fos expression. Our hypothesis is that beta2*nAChRs deletion may reduce striatal CINs' activity, leading to increased activity of medium spiny neurons (MSNs), the main striatal neuronal population. Thus, to investigate the effects of beta2*nAChRs deletion in CNs on CINs' activity, local striatal circuits and mice behaviour, we generated a conditional knockout (ChAT(IRES)-Cre-beta2-flox/flox) mouse, expressing Cre recombinase selectively in CNs. Then, we determined the electrophysiological properties of striatal CINs and MSNs in the control and mutant mice by performing ex vivo patch clamp recordings as well as conducted behavioural tasks, including social behaviour and sensitivity to stimulants. Our results showed that the deletion of beta2*nAChRs does not affect most of the electrophysiological properties of striatal CINs and MSNs. Still, in CINs, we observe a slight decrease in the sag depth induced by hyperpolarizing currents, suggesting altered HCN channel and CINs' excitability. In terms of behaviour, our tests demonstrated that beta2*nAChRs deletion in CNs leads to a higher sociability and a lower amphetamine-induced hyperlocomotion in female mice, suggesting altered dopamine modulation by the cholinergic system in different brain regions, such as the striatum. Our goal is to better understand how to modulate CNs' activity, as this can help identify therapies for disorders involving cholinergic dysfunction, including Parkinson's and Huntington's diseases.

Cerebellum-hippocampal interactions during limbic seizure-like events in the in vitro isolated guinea-pig brain

NP 02

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Cerebellum (CRB) involvement and alterations in patients with temporal lobe epilepsy (TLE) have been described for decades. Deep cerebellar nuclei are the main output of CRB projecting to numerous brain regions. In particular, the fastigial nucleus (FN) is multisynaptically connected with the limbic system, but its role and its interaction with the hippocampus in the control of seizures is still poorly understood. Here we explore the involvement of FN during limbic seizure-like events (SLEs) and the capability of CRB to generate epileptiform discharges. Simultaneous field potential recordings were performed in the dorsal hippocampi and in the FN of the isolated whole guinea-pig brain (IWB) maintained in vitro during SLEs induced by the arterial perfusion of either the GABAergic receptor antagonist bicuculline methiodide (BMI; 50µM; 4min) or the K+-channel blocker 4-aminopyridine (4AP; 50µM; 5min). We also evaluated hippocampal and FN activation: i) during BMI-induced SLEs before and after CRB disconnection from the telencephalon, ii) after local injections of BMI (1mM) in FN and iii) after arterial perfusion of BMI (5mM; 1min) in the CRB only. We observed that FN participated to the induced SLEs and its activation was greater after BMI application compared to the 4AP application. Moreover, hippocampal SLEs were significantly shorter after cerebellectomy. Either local BMI injections in FN or arterial CRB perfusion of BMI induced activity in CRB that propagated to the hippocampus. Our results indicate a functional connection between hippocampus and CRB during limbic seizures mediated by FN. CRB capability to generate activity spreading to hippocampus and the shorter SLEs duration, observed after cerebellectomy, suggest a positive CRB modulation role during BMI-induced epileptiform events. In conclusion, IWB in vitro preparation has proven to be suitable to describe the complex cerebro-cerebellar network involved in TLE, in which FN could play an important role.

Brainstem involvement in the development of PTSD

NP 03

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Post-traumatic stress disorder (PTSD) is a neuropsychiatric disorder which Is triggered by exposure to a traumatic experience or intense stress. While the features of the trauma are diverse, indivudals affected by PTSD typically display common symptoms, such as altered cognitive performance, impaired memory and learning, and increased likelihood of secondary neurpsychiatric deficits such as anxiety disorder, depression, and drug abuse. A core symptom of PTSD is excessive arousal (or hyperarousal). Arousal is a complex brain function which coordinates both the central nervous system and peripheral systems in order to trigger behavioral activation. Thus, it is possible that alterations of brain areas linked to arousal regulation could be involved in the development of PTSD symptoms. However, the precise mechanisms and brain circuits which link arousal dysfunction to PTSD are still partially understood.
Here, we focused our attention on the nucleus incertus (NI) a brainstem nucleus which bidirectionally regulates arousal and is highly sensitive to stress. We hypothesized that a traumatic event could trigger a long-lasting change in NI activity and thus lead to hyperarousal. To test this hypothesis, we performed an anatomical mapping of neuronal activation in a mouse model of PTSD, which consisted of a single exposure to foot-shock (FS).
To quantify neuronal activation in the NI, we performed a cFos immunostaining in the NI of FS and control non-FS mice one hour after the shock, and then mapped the number of cFos+ cells to existing anatomical atlases. We found a higher level of cFos+ cells in the NI of FS animals compared to controls. Furthermore, the density of cFos+ cells displayed a gradient across the antero-posterior axis of the NI of FS animals, suggesting subregional activation of NI neurons. These preliminary findings support our hypothesis that a traumatic/stressful event can activate NI neurons in vivo.
We are currently performing behavioral and molecular assessment of the long-term effect of FS on NI activation by quantifying the structural and molecular synaptic remodeling of the NI of FS animals. Our results will shed light on the mechanisms that link arousal dysfunction to PTSD.

Engineering paracellular and transcellular pathways to enable therapeutic delivery across the blood–brain barrier

NP 04

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The blood–brain barrier (BBB) is a highly specialized physiological interface that protects the central nervous system by tightly regulating molecular exchange between the bloodstream and the neural environment. By severely restricting the passage of systemically administered drugs, the BBB represents a major obstacle to the non-invasive treatment of neurological disorders, including conditions arising from genetic dysfunctions of essential endothelial transport systems, such as in the Glucose Transporter Type 1 Deficiency Syndrome (GLUT1DS). To tackle this challenge, our research explores innovative paracellular and transcellular strategies to enhance brain delivery while preserving BBB integrity and function. We investigated precise modulation of paracellular transport through the targeted regulation of tight junctions (TJs), the protein complexes that seal the paracellular space between brain endothelial cells, and developed an integrated in silico–in vitro workflow to identify short peptide modulators capable of transiently and reversibly interfering with Claudin-5 (CLDN5) complex, the dominant TJ isoform in the BBB. By implementing a machine-learning pipeline, we designed a library of short CLDN-5 peptide binders, which were further characterized for CLDN-5 association via all-atom molecular dynamics (MD) simulations and free-energy calculations, followed by experimental validation in a 2D BBB in vitro model. The observed reversible and size-selective modulation of the paracellular space significantly enhanced the transport of small molecules, such as glucose, across the BBB in vitro model. Our complementary approaches leverage both transcellular transport via engineered biomimetic nanocarriers and transient paracellular modulation, providing a versatile technological framework for overcoming the BBB and offering significant opportunities to advance the development of next-generation brain delivery systems.

Development of EIS-miR-25-loaded exosomes as a potential nanotherapeutic strategy for post-stroke neurogenesis

NP 05

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Ischemic stroke remains one of the leading causes of mortality and long-term disability worldwide. Following cerebral ischemia, endogenous neural stem cells (NSCs) located in the subventricular zone (SVZ) and subgranular zone (SGZ) are activated and contribute to tissue repair through proliferation, migration, and differentiation into functional neurons. However, this spontaneous regenerative response is limited and insufficient to fully restore neurological function. Therefore, strategies aimed at enhancing endogenous neurogenesis represent a promising therapeutic approach for post-stroke recovery. Among the molecular regulators involved in this process, microRNAs have emerged as key modulators of neural plasticity and regeneration. In particular, miR-25 has recently attracted attention due to its role in neurogenesis and its increased plasma levels in subacute ischemic stroke patients undergoing high-frequency repetitive transcranial magnetic stimulation (HF-rTMS), suggesting its involvement in endogenous brain repair mechanisms. Based on these observations, we are aiming to develop a system for loading miR-25 into exosomes and using them as delivery vehicles to promote neurogenesis in in vitro models of ischemia. To this end, the mature miR-25 sequence was fused either upstream or downstream of an RNA motif derived from the HIV-1 Gag p17 region, which has previously been reported to promote selective RNA sorting into extracellular vesicles and which we refer to here as Exosome Incorporating Sequences (EIS). The resulting plasmid constructs were amplified in bacterial cells and purified. Subsequent studies will focus on the transfection of HEK293T cells, which will be used as producer cells for the generation and isolation of EIS-miR-25-loaded exosomes. This exosome-based delivery approach may offer a novel method for the targeted delivery of miRNAs and could contribute to innovative therapeutic strategies for post-stroke recovery.

REST-dependent tuning of the synaptic GluN2A/GluN2B balance selectively gates NMDAR-dependent LTP

NP 06

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NMDAR-dependent synaptic plasticity, including LTP and LTD, is a major cellular mechanism implicated in learning and memory. Its induction and expression are shaped by NMDAR signaling properties and by synaptic GluN2A/GluN2B composition, although this relationship is context-dependent and cannot be reduced to a strict GluN2A-LTP/GluN2B-LTD rule. While transcriptional activators of plasticity-related genes are well characterized, the contribution of activity-dependent transcriptional repressors remains less defined. REST/NRSF is an epigenetic repressor of neuronal gene programs and has been shown to repress Grin2b transcription, thereby contributing to the developmental GluN2B-to-GluN2A switch.

Here, we investigated whether REST controls NMDAR-dependent plasticity using chemically induced LTP and LTD combined with electrophysiology, Ca^{2+} imaging and pharmacological dissection of NMDAR subunit contribution. REST inhibition by decoy oligodeoxynucleotides selectively impaired LTP, reducing AMPAR-mediated mEPSC amplitude without affecting event frequency, consistent with a postsynaptic deficit in potentiation, whereas LTD was preserved. REST blockade also reduced evoked NMDA-EPSCs and synaptically driven Ca^{2+} responses, indicating impaired synaptic NMDAR function. Pharmacological analysis showed increased sensitivity to GluN2B-selective antagonism and reduced sensitivity to GluN2A-selective antagonism, consistent with a shift toward GluN2B-dominated synaptic NMDARs, while extrasynaptic NMDAR responses were largely preserved.

These findings identify REST as an epigenetic regulator of synaptic NMDAR composition and support a model in which REST tunes the GluN2A/GluN2B balance to set the synaptic conditions required for LTP expression and metaplastic control of NMDAR-dependent plasticity.

Neuroactive polymer films modulate neuronal activity and synaptic network plasticity

NP 07

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A central challenge in neurophysiology is understanding how neurons integrate bioelectrical and biophysical cues to regulate maturation, synaptic plasticity, and the emergence of functional network dynamics, processes that are profoundly disrupted in neurodegenerative disorders. To investigate whether bioelectronic interfaces can modulate these mechanisms, we explored the effects of a conductive polymer substrate on neuronal identity, synaptic organization, and early network development.

Human SH-SY5Y cells were cultured for 15 days on poly(3-hexylthiophene) (P3HT) thin films in the absence of neuro-inductive stimuli, and cell adhesion, proliferation, biocompatibility, and neuronal differentiation were evaluated. A multi-level characterization was performed by integrating transcriptional profiling, immunocytochemical analysis of neuronal markers (β-III tubulin, MAP2, NF-H), and quantitative neurite morphometry to assess the influence of P3HT on neuronal specification and network-oriented maturation. Gene expression analysis revealed a coordinated upregulation of cytoskeletal and synaptic genes, consisting with the acquisition of a more mature neuronal phenotype. Immunofluorescence analysis confirmed enhanced cytoskeletal organization and compartmentalization, while neurite tracing demonstrated significant increases in elongation and arborization, consistent with the establishment of a denser and more interconnected structural network.

Protein–protein interaction network analysis further confirmed a coordinated transition from stem-like state, characterized by expression of SOX2 and NES, toward neuronal structural maturation (TUBB3, MAP2, NFH) and synaptic specialization (SYP, SYN). These findings indicate that P3HT influences multiple regulatory pathways involved in neuronal development simultaneously. Notably, the enrichment of functional modules associated with cytoskeletal remodeling, synaptic vesicle trafficking, and activity-dependent plasticity suggests that P3HT not only supports neuronal differentiation but actively promotes a cellular state conductive to synaptic integration, increased intrinsic excitability, and early network assembly.

Collectively, these findings support a model in which P3HT acts as a bioelectrical modulator, capable of shaping the molecular and structural landscape required for neuronal maturation and network plasticity. By driving spontaneous neuronal differentiation and fostering the establishment of interconnected neural architectures, P3HT-based substrates emerge as a promising platform for investigating the neurophysiological mechanisms of adaptive plasticity and for the development of next-generation neuroprosthetic and regenerative technologies.

POSTER

2

SESSION

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Academic Stress as a Predictor of Poor Sleep Quality among International Medical Students

CNN 04

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Sleep impairment and stress are common among medical students, but evidence is limited regarding which stress domains most strongly relate to poor sleep in international students. This study examined the association between medical student stress and sleep quality using the Medical Student Stressor Questionnaire (MSSQ) and Pittsburgh Sleep Quality Index (PSQI). A cross-sectional survey was conducted among international medical students. After merging PSQI and MSSQ data by timestamp, 200 students with complete data on sleep quality, stress, age, gender, and study level were included. Study level was categorized as preclinical or clinical. Global PSQI score and poor sleep quality (PSQI ≥ 5) were analyzed in relation to MSSQ domains using group comparisons, Spearman correlations, and regression models. Poor sleep quality was highly prevalent: the mean global PSQI score was 8.44 (SD 3.62), and 83.7% of students met the PSQI criterion for poor sleep. MSSQ domains showed moderate average stress levels. Global PSQI was positively correlated with all MSSQ domains and MSSQ total score. In regression models, demographic variables and study level were not significant predictors of PSQI. When MSSQ domains were added, academic-related stressors emerged as an important independent predictor of worse sleep quality and were associated with higher odds of poor sleep. Poor sleep quality is highly prevalent among international medical students and appears to be more strongly explained by stress exposure than by demographic or study-level characteristics. Academic stressors may represent a key target for interventions aiming to improve sleep and wellbeing in medical education.

CNN 05

Visuospatial grounding of spatial semantics: evidence from transcranial direct-current stimulation modulation of the right posterior parietal cortex during a lexical decision task in virtual reality

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Human brain differently processes the surrounding space as peripersonal (PPS) and extrapersonal (EPS) space. Notably, the use of a tool can remap EPS into PPS. Intriguingly, most languages use spatial terms that dichotomously distinguish between near and far space, and the use of spatial language is affected by handling a tool. The present study investigates the relationship between visuospatial perception and spatial semantics through a lexical decision task. In a virtual reality (VR) environment, 14 healthy subjects performed a task in which they read and pointed to the meaningful term within pairs consisting of a word (Near or Far Semantics) and a pseudoword, presented either in PPS (60 cm) or EPS (120 cm). Vocal response reaction times (RTs) were collected. Two conditions were tested: in the No Tool condition, subjects received visual feedback of their own actions with arms only; in the Tool condition, they held a virtual stick long enough to reach EPS. The task was replicated after applying inhibitory cathodal transcranial direct-current stimulation (tDCS) to the right posterior parietal cortex (rPPC), involved both in visuospatial and spatial semantics processing. Baseline results confirmed our hypotheses of a link between spatial semantics and visuospatial perception. In No Tool condition, RTs for space-related words were faster when the meaning was congruent with presentation distance. In Tool condition, Far Semantics showed increased RTs in EPS, suggesting that EPS is remapped as PPS. In No Tool condition, tDCS abolished the congruency effect for Near Semantics in PPS. In Tool condition, tDCS disrupted tool-induced remapping and its impact on spatial word processing. In conclusion, our study highlights a close connection between spatial semantics and visuospatial perception, and demonstrates that interference with visuospatial functions, whether induced by tool use or by rPPC inhibition, can modulate spatial language processing.

Tracing thoughts: decoding prefrontal circuit dynamics in the MAM model of schizophrenia

CNN 06

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Cognitive impairments are a core feature of schizophrenia and represent a major determinant of long-term functional disability. Particularly, deficits in executive functions, working memory, and cognitive flexibility respond only partially to current treatments. Although increasing evidence implicates prefrontal cortex (PFC) dysfunction in these alterations, the neuronal and circuit-level mechanisms underlying cognitive impairment in schizophrenia remain poorly understood. In this proof-of-concept study, we propose to investigate the neural dynamics underlying executive dysfunction using the methylazoxymethanol acetate (MAM) rat model, which reproduces several behavioral and neurobiological features associated with schizophrenia-related cognitive deficits. We hypothesize that altered neuronal ensemble activity within the PFC contributes to impaired cognitive processing and behavioral performance.

To address this question, we will employ miniature head-mounted microscopes (Miniscopes, UCLA) to perform in vivo calcium imaging of PFC neuronal populations in freely behaving MAM and control rats during radial arm maze (RAM) task execution, allowing longitudinal monitoring of neuronal activity during cognitively demanding behavior. In parallel, calcium imaging will be combined with optogenetic approaches to selectively manipulate neuronal ensembles involved in executive processing and evaluate their potential causal contribution to task performance.

By integrating functional imaging, behavioral analysis, and circuit manipulation, this project aims to characterize PFC network dysfunction associated with schizophrenia-related cognitive impairment. This approach may provide novel insights into the neural basis of executive deficits and support the future development of targeted therapeutic strategies aimed at improving cognitive outcomes in schizophrenia.

Time-resolved aperiodic dynamics during event segmentation in attention-deficit/hyperactivity disorder

CNN 07

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Attention-deficit/hyperactivity disorder (ADHD) is associated with difficulties in organizing behaviour over time, yet the neurophysiological mechanisms underlying these temporal control deficits remain poorly understood. The brain continuously segments ongoing experience into discrete events to guide prediction and adaptive action. In our study, we examined whether adolescents with ADHD differ from neurotypical peers in how they behaviourally and neurally structure continuous experience. A total of 73 adolescents with ADHD and 73 neurotypical (NT) controls viewed a movie while marking perceived event boundaries, with concurrent electroencephalogram (EEG) analysed using time-resolved spectral parameterization to track aperiodic neural activity. Both groups detected meaningful transitions, but NT adolescents segmented more sensitively to situational changes, particularly social and spatial cues. Across the entire recording window, ADHD participants exhibited higher aperiodic exponents, reflecting globally stronger aperiodic exponents. Time-resolved analyses revealed that, around event boundaries, ADHD participants showed exaggerated pre-boundary increases and attenuated post-boundary decreases in the aperiodic exponent – signifying over-stabilization before, and insufficient re-engagement after, event transitions. In ADHD only, behavioural segmentation sensitivity correlated with post-boundary aperiodic modulation. Although counterintuitive given common assumptions about increased neural “noise” in ADHD, this pattern indicates that ADHD is characterized by an imbalance between neural stabilization and re-engagement when parsing continuous experience. Steeper aperiodic activity reflects a restricted neural state space that limits adaptive updating, leading to coarser and less context-sensitive segmentation. These findings link everyday temporal disorganization in ADHD to alterations in aperiodic neural dynamics and impaired temporal cognition in ADHD.

The AQP4 A215T mutation causes a common maturation defect across AQP4 isoforms in megalencephalic leukoencephalopathy with subcortical cysts type 4 (MLC4)

ND 16

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare astrocytopathy affecting brain water homeostasis. A homozygous A215T mutation within the second NPA motif of Aquaporin-4 (AQP4) was recently identified in patients with MLC and associated with impaired AQP4 protein expression, defining a new genetic subtype designated MLC4. AQP4, the main water channel in the central nervous system, is enriched at the astrocyte end-feet and exists as four isoforms: M1, M23, M1ex, and M23ex, which assemble into supramolecular structures known as orthogonal arrays of particles. To determine whether the A215T mutation affects isoforms differently, we mutated and transiently expressed each AQP4 isoform in HEK293 cells. To assess AQP4 biogenesis in a glial context, we then generated stable immortalized astrocyte cell lines (DI TNC1) using constructs that preserved the physiological ratio among the different isoforms. Whereas all WT isoforms reached the plasma membrane, all A215T isoforms accumulated intracellularly and co-localized with an ER marker but not a Golgi marker. Mutant protein expression was markedly reduced but strongly increased following proteasome inhibition, suggesting the involvement of the ER-associated degradation pathway. Interestingly, culturing A215T-expressing cells at 30°C restored plasma membrane localization, consistent with a temperature-sensitive folding defect. To further investigate the functional consequences of the A215T mutation, calcein-based measurements of osmotic water permeability in stable DI TNC1 cells indicate reduced water transport in mutant cells compared with WT cells.
Together, these findings identify A215T as causing a generalized, non-isoform-specific maturation defect that results in a functional null phenotype through the loss of membrane-localized AQP4 and suggest that improving protein folding may represent a therapeutic strategy for MLC4.

Prion detection in the urine of individuals at genetic risk for fatal familial insomnia

ND 17

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Fatal familial insomnia (FFI) is a rare, devastating genetic prion disease caused by the D178N PRNP pathogenic variant, for which no validated biomarker currently exists to detect preclinical pathology or monitor disease progression. Although genetic testing can identify individuals at risk decades before symptom onset, the absence of a reliable, minimally invasive biomarker has limited clinical management and development of preventive or diseasemodifying therapies. In this study, we report the first demonstration of prion seeding activity in urine from D178N carriers using an ultrasensitive protein misfolding cyclic amplification (PMCA) assay optimized with bank vole PrP substrate. We performed a blinded longitudinal analysis of urine samples from 28 carriers (24 asymptomatic and 4 symptomatic) and 37 noncarrier relatives, with followup of five years. Multiple aliquots per collection were tested to maximize analytical sensitivity, and parallel blood samples were analysed when available. Prion seeding activity was detected in urine from 22 of 28 carriers but in none of the noncarrier relatives, yielding 100% specificity within genetically and environmentally matched controls. Detection rates were higher in codon 129 MV carriers than in MM carriers. Once established, urine positivity remained stable across all subsequent collections, including through clinical phenoconversion. All four individuals who developed symptoms during followup had previously tested positive in urine, whereas most urinepositive carriers remained asymptomatic during the observation period. Blood PMCA signals were inconsistent and lacked longitudinal reliability. Kidney function was normal across participants, excluding renal impairment as a confounder. These findings identify urine as the first robust, noninvasive biofluid capable of detecting FFIassociated prion seeding activity from the presymptomatic phase and support its use as a longitudinal biomarker for genetic prion diseases.

Development of new therapeutic approaches for targeting dysfunctional cortico-striatal circuit in HD

ND 18

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The corticostriatal circuit represents a critical pathogenic hotspost in Huntington's disease (HD), a rare genetic neurodegenerative disorder to which no disease modifying therapies are available. Despite the pivotal role of striatal neurodegeneration in HD, cortical dysfunction is recognized as an early driver of pathogenesis, with cortical projection neurons (CPNs) already compromised at prodromal stages. Deciphering the underlying dysfunctional mechanisms is therefore essential to develop novel therapeutics. To address this, we established complementary in vitro and in vivo HD models to investigate circuit-level alterations and evaluate targeted neuroprotective interventions. Utilizing primary neurons from the HD mouse model zQ175DN partitioned within compartmentalized microfluidic chambers, we successfully reconstituted the corticostriatal circuit in vitro. zQ175DN heterozygous knock-in mouse model closely mimics the human condition with the insertion of the allele encoding the human HTT exon 1 sequence with a CAG repeat stretch of ~190. This platform allows to identify authentic corticostriatal synapses for the precise characterization of synaptic connectivity via immunocytochemistry, detailed neuronal morphological analysis, and the tracking of Brain Derived Neurotrophic Factor (BDNF) vesicle transport using super-resolution microscopy. This system was further leveraged to selectively assess the efficacy of novel neuroprotective pharmacological strategies on individual circuit components. Concurrently, we are developing a platform that combines a knock-in HD mouse model with viral-based targeting strategies to comprehensively dissect corticostriatal circuit dynamics in the intact brain.

Niclosamide improves neuroprotective pathways in a C9orf72 mouse model of ALS/FTD

ND 19

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease characterized by progressive motor neuron degeneration, leading to loss of motor function and vital abilities. The most common genetic cause of ALS and frontotemporal dementia (FTD) is a G4C2 hexanucleotide repeat expansion in the non-coding region of C9orf72, which contributes to disease pathogenesis through both loss- and gain-of-function mechanisms. Currently, effective therapies for ALS/FTD are limited, and drug development is hindered by disease heterogeneity and high failure rates of de novo approaches. Drug repurposing offers a promising strategy to accelerate translation by leveraging compounds with known safety and pharmacokinetics, reducing development time and risk. Niclosamide, an FDA-approved anthelmintic drug, has recently emerged as a potential therapeutic candidate due to its anti-inflammatory, anti-fibrotic, and autophagy-modulating properties. Previous studies in ALS models have shown that niclosamide reduces neuroinflammation and exerts beneficial effects. In light of these findings, we evaluated the therapeutic efficacy of niclosamide in a (G4C2)149 repeat expansion C9orf72 mouse model of ALS/FTD. Preliminary results show that a 4-week intraperitoneal treatment reduces neurofilament light chain (NfL) levels, a biomarker of neuroaxonal damage, and restores the postsynaptic marker PSD95, indicating improved synaptic integrity and suggesting reduced neuroaxonal damage and a potential neuroprotective effect. Furthermore, niclosamide decreases astrocytic and microglial activation in the motor cortex and increases expression of the microglial homeostatic marker P2RY12. Finally, it modulates autophagy-related pathways and extracellular matrix components, supporting a broad neuroprotective and disease-modifying profile. Overall, these findings support niclosamide as a promising multi-target therapeutic candidate for C9orf72-associated ALS/FTD, providing a strong translational rationale for its advancement toward clinical evaluation.

ND 20

Development of an in vitro human brain-based 3D system to study the modulation of neuroinflammatory mechanisms and carnosine neuroprotective activity

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Abstract: Recent evidence suggests that crosstalk between neurons and glial cells contributes to neuroinflammation and the progression of neurodegenerative disorders, including Alzheimer's disease (AD). Studying intercellular communication is crucial to understand the mechanisms driving neuroinflammation and neurodegeneration in AD as well as the activity of potential therapeutics such as carnosine. This dipeptide possesses antioxidant, anti-inflammatory, and anti-aggregating properties, and modulates both neural and immune cells, making it a promising candidate for AD. This work proposes to develop an in vitro human brain-based 3D system comprised of neurons (SH-SY5Y-derived), astrocytes (HA), and microglia (HMC3) sharing the same extracellular environment while remaining physically separated through a multi-step seeding strategy, allowing to investigate intracellular communication that is reproducible and accessible. After 3D model set-up refinement and optimization, immunofluorescence was employed to characterize cell populations individually as well as part of the triculture system. The 3D system was then treated with Aβ oligomers (oAβ) and the neuroprotective activity of carnosine tested. Neuronal ROS production at single-cell level, measured by using an automated cell counter/microchip electrophoresis and plotted through Matlab 2023R, increased following oAβ challenge. Interestingly, neuronal monocultures were more susceptible to oAβ compared to neurons part of the 3D system, suggesting a modulatory and potentially protective role exerted by glial cells. Moreover, carnosine pre-treatment effectively reduced oAβ-induced ROS production in all cell populations, although distinct responses were observed between mono- and tricultures. Overall, these findings demonstrate the proposed in vitro human brain-based 3D system versatility to investigate neuroinflammatory mechanisms associated with AD, also underlining the efficacy of carnosine as a potential therapeutic agent.

Astrocyte-derived extracellular vesicles in Parkinson's disease: multi-omics profiling of neuroprotective pathways and post-translational modifications

ND 21

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Parkinson's disease (PD) is characterized by the progressive loss of dopaminergic (DAergic) neurons in the ventral midbrain (VMB) and the consequent depletion of dopamine in the striatum (STR). Since current therapies only manage symptoms, discovering novel neuroprotective mechanisms to halt this degenerative process is a critical priority. In this context, astrocytes (AS) are crucial for maintaining DAergic neuron homeostasis, communicating also via extracellular vesicles (EVs). These cell-derived lipid nanoparticles transport bioactive molecules (e.g., proteins, DNAs, RNAs) capable of modulating target cell fate.

In previous work, we demonstrated that EVs secreted by primary mouse AS from the VMB and STR protect neuronal cells from MPP+-induced toxicity, with only VMB-AS-EVs fully restoring ATP production. To decipher the molecular drivers of this neuroprotection, we characterized the transcriptomic profile of donor AS and the protein cargo of AS-EVs through high-resolution Mass Spectrometry. The integrated multi-omics analysis revealed that AS-EVs carry molecules linked to nervous system development, cell-to-cell communication, axonal guidance, and synapse formation. Notably, VMB-AS-EVs exhibited a distinct enrichment in mitochondrial proteins, including the voltage-dependent anion channel isoforms 1 and 2 (VDAC1/2), which are key regulators of mitochondrial activity and apoptosis.

Furthermore, we identified region-specific protein signatures with distinct post-translational modification (PTM) patterns, including phosphorylation, oxidation, and deamidation, highlighting a sophisticated regulatory landscape within the EV cargo.

Overall, our findings support the existence of a region-specific neuroprotective signature in AS-EVs, pointing to VDACs and mitochondrial pathways as potential mediators of the rescue observed in vitro. These data lay the groundwork for future studies aimed at elucidating how specific PTMs modulate AS-EV-mediated neuroprotection in PD.

Novel GRK5 variants in Parkinson's disease: identification and functional characterization of their effects on protein dynamics and stress response

ND 22

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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. Here, 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 analysed to identify and prioritize candidate variants based on allele frequency, predicted deleteriousness, and genetic burden. Among 44 identified common GRK5 variants (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 ($p = 0.004$). eQTL analysis from GTEx revealed a significant correlation between the p.Leu41 risk allele and increased GRK5 mRNA expression in the human substantia nigra. 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 the variants exert destabilizing effects on GRK5 structure and dynamics. GRK5-GFP plasmids carrying the identified variants were generated by site-directed mutagenesis and transfected into SH-SY5Y and HeLa cells. Subcellular localization analysis, under basal and metabolic stress conditions, revealed altered intracellular distribution associated with specific variants. Moreover, expression analyses showed dysregulation of SQSTM1 and mTOR transcript levels under stress, suggesting impaired autophagic response and altered cellular homeostasis. 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 PD-derived dopaminergic neurons and investigate the interplay between GRK5 variants and BCL2 exploring the autophagy-apoptosis axis.

Unraveling myelin abnormalities in synucleinopathies

ND 23

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Synucleinopathies (Parkinson's disease – PD, Dementia with Lewy bodies – DLB and Multiple system atrophy – MSA) are neurodegenerative diseases characterized by the presence of alpha-synuclein (aSyn) aggregates in the brain and peripheral tissues such as the skin. The diagnosis of synucleinopathies at early stages is challenging, leading to delayed disease management and the need of additional reliable biomarkers. By using correlative light and electron microscopy (CLEM), we previously reported that PD donors show higher myelin damage in the skin compared to non-neurological control and MSA groups, suggesting a different involvement of the peripheral myelination among synucleinopathies and non-neurological controls, which can be used as a disease classifier. We next established a cellular co-culture model based on iPSC-derived sensory neurons and rat primary Schwann cells to study the interaction of peripheral myelin and aSyn in synucleinopathies and to understand if the accumulation of misfolded aSyn drives an increase in myelin damage in the presence of brain-derived aSyn fibrils. CLEM is used to assess the level of structural and morphological preservation of the myelin sheaths in the myelinating cellular model. Four damage categories (none, mild, moderate and severe damage) are used to categorize and quantify myelin fragmentation. To date, the cause of peripheral myelin damage previously observed in the skin of synucleinopathy donors remains unknown. Therefore, the results from the myelinating cellular model help establish a causative and temporal association between aSyn and myelin abnormalities. The discovery and validation of a biomarker that is effective in diagnosing and differentiating between synucleinopathies would have significant implications for early detection, disease monitoring and treatment.

Effect of discontinuous immunization with 12A12 Monoclonal Antibody on AD phenotype of aged 3xTg Mice

ND 24

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Alzheimer's Disease (AD), the leading cause of dementia worldwide, is a complex neurodegenerative disorder characterized by extracellular accumulation of parenchymal amyloid-β (Aβ) into senile plaques and intraneuronal tau-laden neurofibrillary tangles. Clinical and experimental evidence demonstrates that Aβ and tau interact reciprocally to drive cognitive decline. Therefore, "candidate" therapeutic antibodies intercepting the co-pathogenetic link existing between Ab and tau, especially in the preclinical phase of AD, are more likely to achieve higher clinical benefit in contrasting the neuropathological lesions and related cognitive impairment. We have previously showed that discontinuous immunization with the monoclonal antibody (mAb) 12A12, targeting the 20-22kDa N-terminal tau fragments (NH_2htau), effectively mitigates memory and learning deficits, as well as neurochemical and histological alterations, in 9-month-old Tg2576 mouse model which overexpress a mutant form of APP (isoform 695) with the Swedish mutation (KM670/671NL) leading to an age-dependent Aβ accumulation/aggregation and plaque deposition. Given the synergistic toxicity of Aβ and tau, the present study is aimed at evaluating the efficacy of 12A12 mAb immunization in 3xTg mice, a model characterized by the accumulation of both Aβ plaques and tau tangles owing to the presence of three mutations associated with familial AD (APP Swedish, MAPT P301L, and PSEN1 M146V). Following intravenous administration of the antibody under the same immunization regimen, behavioral assessments along with Western blotting and immunofluorescence analyses on the hippocampus have been carried out. Preliminary results demonstrate that a positive trend in improvement of cognitive deficits, as evaluated by Novel Object Recognition (NOR) test, along with relevant changes in biochemical and morphological readouts are detected in 12A12 mAb-immunized transgenic mice in comparison to their saline-treated counterpart.

Amyloid-beta 1–42 aggregation states alter mitochondrial bioenergetics and copper homeostasis in SH-SY5Y cells

ND 25

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Alzheimer's disease (AD) is now considered not only a proteinopathy characterized by the accumulation of beta-amyloid (Aβ) and tau, but also a pathological condition associated with alterations in cellular bioenergetics, mitochondrial vulnerability, oxidative stress, and metal dyshomeostasis. In this context, Aβ1–42 is particularly relevant because of its high propensity to aggregate into oligomeric and fibrillar species, which are respectively associated with early neuronal dysfunction and advanced amyloid deposition. Moreover, the presence of metals such as copper, zinc, and iron within amyloid plaques may contribute to cellular damage; in particular, the interaction between copper and Aβ may promote redox alterations and the production of reactive oxygen species (ROS), thereby impairing mitochondrial function. In the present study, SH-SY5Y cells differentiated with all-trans retinoic acid were exposed to Aβ1–42 preparations pre-incubated for 24 hours at either 4°C or 37°C, in order to compare aggregates enriched, respectively, in oligomeric and fibrillar species. This model was used to evaluate cell viability, mitochondrial and glycolytic metabolism, mRNA-level transcriptional remodeling, and selected markers of copper homeostasis, including voltage-dependent anion channel (VDAC), antioxidant 1 copper chaperone (ATOX1), and solute carrier family 31 member 1 (SLC31A1). The integration of bioenergetic analysis using Seahorse technology, mRNA expression profiling, and protein marker assessment revealed an early alteration of the mitochondria–copper axis in response to Aβ1–42. Overall, the data suggest a general bioenergetic perturbation compared with control cells, rather than a clear functional distinction between the two amyloid preparations.

Prevalence of antibodies against neuronal antigens and serum biomarker profile in patients with cognitive impairment of recent onset

ND 26

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Autoimmune encephalitis (AE) is characterized by a pathological immune response against self-antigens expressed in the central nervous system. Cognitive impairment is a core feature of AE, but not all patients with neuronal antibodies fulfill its diagnostic criteria, and their significance in broader cognitive impairment syndromes remains unclear as they may represent epiphenomena of neurodegeneration or directly modify the disease course. Since neuronal autoantibodies can also be found in healthy subjects, concomitant neuroinflammation and/or blood-brain barrier disruption may be required to enable their pathogenic role. We prospectively enrolled patients with new-onset neurocognitive disorders (onset within 24 months, no seizures within 4 weeks) and tested serum and CSF for antibodies against AE-associated neuronal antigens by in-house cell-based assays. Serum S100B and CXCL13 were measured by ELISA and MAGPIX respectively. S100B values were analyzed by Fisher's exact test. CXCL13 was log-transformed and compared by Wilcoxon rank-sum test. Among 55 patients (50.9% F; median age 70 years), autoantibodies were detected in 11/55 (20%), targeting LGI1 (n=2), IgLON5 (n=3), NMDAR, CASPR2, GABABR, GlyR, CV2, and mGluR1 (n=1 each). Antibody-positive patients were predominantly male (81.8% vs 40.9%). Serum S100B was quantifiable in 17/40 tested patients, with a trend toward a higher frequency of detectable levels in antibody-positive compared with antibody-negative patients, although the difference was not statistically significant (p=0.456). Serum CXCL13 (available in 52/55; median 95.8 pg/mL) did not differ between groups (p=0.717). Neuronal antibodies are detectable in one in five patients with cognitive impairment of recent onset, with IgLON5 and LGI1 as the most frequent targets and a striking male predominance in seropositive patients. These preliminary findings support systematic antibody screening in early cognitive impairment.

Early microglial dysfunction is associated with altered cerebellar synaptic development in Friedreich's Ataxia

ND 27

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Friedreich's ataxia (FRDA) is a rare inherited neurodegenerative disorder caused by mutations in the FXN gene, leading to reduced frataxin expression, mitochondrial dysfunction, and progressive impairment of the nervous system. The cerebellum is one of the most affected brain regions, and its degeneration contributes to severe deficits in motor coordination. Although the neuronal mechanisms underlying FRDA have been extensively investigated, increasing evidence suggests that non-neuronal cells, including microglia, may contribute to disease progression. In particular, microglia play a crucial role in synaptic maturation and circuit refinement during postnatal cerebellar development, although their involvement in FRDA remains poorly understood. In this study, we investigated in vivo cerebellar microglia during early postnatal development in KIKO mice, a murine model of FRDA, at postnatal days (P) 7, 15, 21, and 30
. At P7, KIKO microglia displayed early alterations in morphological maturation, characterized by hyper-ramification and increased branching complexity, together with early synaptic abnormalities, related to VGLUT2-positive excitatory synapses and VGAT-positive inhibitory terminals. These alterations became more pronounced at P21, when KIKO microglia exhibited further exacerbation of the hyper-ramified phenotype observed at P7, together with reduced Iba1 immunoreactivity and decreased expression of homeostatic markers, including P2Y12 and Cd68. These changes were accompanied by an imbalance between excitatory and inhibitory synaptic markers, reduced C1q deposition, and dysregulation of pathways associated with neurodevelopment and gliogenesis. Finally, although several microglial markers partially normalized at P30, synaptic alterations persisted. Overall, our findings support a contribution of microglial dysfunction and altered synaptic remodeling to FRDA pathogenesis during early cerebellar development.

Optimizing neurorehabilitation in retinal degeneration: coupling subretinal P3HT nanoparticles with sensory stimulation

ND 28

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While poly(3-hexylthiophene) (P3HT) nanoparticles demonstrated to be 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 subretinal P3HT nanoparticles microinjection, rd10 mice underwent a two-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), and immunohistochemistry.
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 visuo-motor 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 conjugated polymer subretinal prosthetic strategy with sensory stimulation can effectively optimize neurorehabilitation in retinal dystrophy caused by photoreceptor degeneration.

Ryanodine receptor-mediated calcium release regulates presynaptic function and is altered in Alzheimer's Disease models

ND 29

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Altered Ca^{2+} homeostasis is a hallmark of the early stages of Alzheimer's disease (AD). Intracellular Ca^{2+} release plays a fundamental role in regulating essential neuronal functions, including neurotransmitter release, synaptic transmission, and gene expression. Ryanodine receptors (RyRs), Ca^{2+}-permeable channels located on the endoplasmic reticulum membrane, are key contributors to intracellular Ca^{2+} dynamics and homeostasis. However, their precise role in Ca^{2+}-dependent mechanisms underlying synaptic transmission remains incompletely understood. In the present study, we investigated the contribution of RyR-mediated Ca^{2+} release in excitatory synaptic transmission and examined how this mechanism is altered under AD-like conditions. Pharmacological inhibition of RyRs with dantrolene did not affect the average amplitude of evoked excitatory postsynaptic currents (eEPSCs), but significantly reduced the paired-pulse ratio and attenuated eEPSC amplitudes during high-frequency stimulation. Conversely, in AD-like in-vitro and ex-vivo models the paired-pulse facilitation is potentiated and the eEPSCs amplitudes during high-frequency stimulation is more sustained as compared to the control conditions. Dantrolene administration to Aβ-42-treated cells and 3xTg-mice derived slices restore the paired-pulse ratio to control conditions. Taken together, our findings indicate that RyRs play a key role in sustaining glutamate release and supporting vesicle recycling during periods of intense synaptic activity in hippocampal neurons and suggest that RyRs dysregulation may contribute to early synaptic dysfunction associated with AD.

Precision prevention of neurodegenerative diseases: lifestyle, natural bioactives and artificial intelligence across the life course

ND 41

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Neurodegenerative diseases develop over long preclinical and prodromal phases in which biological vulnerability, lifestyle-related exposures, environmental determinants and reduced brain resilience progressively interact. This work presents an integrated prevention-oriented framework for the early management of neurodegenerative diseases, with particular attention to dementia and Alzheimer's disease. Current evidence supports a shift from late-stage symptomatic care toward earlier identification of at-risk individuals and multidomain interventions acting across the life course. Physical activity, healthy dietary patterns, cognitive and social engagement, sensory health, vascular and metabolic control, and reduction of environmental exposures represent key modifiable targets. Natural bioactives and nutraceuticals may contribute to neuroprotection through antioxidant, anti-inflammatory, mitochondrial, vascular, synaptic and gut–brain axis mechanisms; however, their clinical translation requires improved standardization, bioavailability, blood–brain barrier penetration, pharmacokinetic characterization and biomarker-informed trials. Artificial intelligence may strengthen this framework by integrating neuroimaging, fluid biomarkers, digital phenotyping, speech analysis and clinical data to support risk stratification, monitoring and personalized intervention. It may also accelerate the prioritization of neuroprotective natural compounds and improve trial enrichment. Nevertheless, responsible implementation requires external validation, transparency, data protection, equity of access and human-supervised clinical decision-making. Overall, combining lifestyle prevention, natural medicines and artificial intelligence may support a more anticipatory, personalized and sustainable model of brain health, aimed at delaying pathological trajectories before irreversible cognitive and functional decline occurs.

Early butyrate rescues mucus barrier dysfunction in a preclinical model of autism spectrum disorder

NDEV 07

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by deficits in social communication, repetitive behaviors, and cognitive inflexibility. Moreover, children with ASD often exhibit gastrointestinal symptoms. While current research has focused on inflammatory biomarkers and microbiota-derived signatures, intestinal barrier dysfunction may also occur without overt inflammation and may involve altered neurosecretory regulation. The present study aimed to investigate mucus barrier integrity and enteric neuroglial pathways in a preclinical model of ASD, and to evaluate the therapeutic potential of early butyrate supplementation.
The BTBR mouse model of ASD was compared with the neurotypical C57BL/6J strain. Colonic tissues were analyzed by histology, immunofluorescence, and targeted transcriptomic approaches to assess mucus layer organization, goblet cell function, inflammatory status, and enteric neuroglial markers. Sodium butyrate was administered in drinking water (0.23 mg/ml) for 4 weeks after weaning.
BTBR mice showed reduced colonic mucus production and altered crypt-associated mucus folding, associated with impaired goblet cell secretion and reduced antimicrobial defense, even in the absence of significant inflammation or disruption of epithelial junctional integrity. Molecular profiling identified dysregulated enteric pathways involved in mucus regulation, including cholinergic and VIPergic signaling, with altered glial support signatures. Early post-weaning sodium butyrate treatment significantly restored mucus architecture, improved markers of goblet cell activity, and promoted broad normalization of the associated neuroglial profile.
These findings indicate that gut barrier dysfunction in ASD may extend beyond classical inflammatory mechanisms and involve impaired neuroglial control of the mucus layer. Early butyrate supplementation emerged as a promising pharmacological strategy to preserve intestinal homeostasis and barrier function.

Tracking therapeutic efficacy in Angelman syndrome: a novel neuroimaging biomarker

NDEV 08

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Loss of maternal UBE3A expression in the brain causes Angelman syndrome (AS), a neurodevelopmental disorder characterized by cognitive impairments, behavioral alterations, seizures, and motor dysfunctions. No disease-modifying therapies are available for AS, and clinical management of patients primarily relies on palliative approaches to control the most invalidating symptoms. While promising therapeutic strategies have emerged in recent years, the development of quantitative, reliable, and unbiased biomarkers of brain function has lagged, hampering the assessment of therapeutic efficacy. Here, we investigated the use of visually evoked responses measured with intrinsic optical signal (IOS) imaging, a non-invasive neuroimaging tool of high translational value, as a functional biomarker for monitoring therapeutic efficacy in this disorder. Recordings of visual IOS (V-IOS) in juvenile (P45) and adult (P90) Ube3a-deficient (AS) and wild-type (WT) animals revealed a significant increase in the amplitude of V-IOS responses elicited by contralateral eye stimulation in AS mice compared to controls, demonstrating the ability of V-IOS to differentiate mutant subjects and controls. Moreover, we found that higher V-IOS responses were associated with poorer outcomes in marble-burying and rotarod tests in P90 AS mice, suggesting a great ability of the V-IOS biomarker to predict disease severity in adult mutant animals. Finally, we demonstrated that V-IOS responses are sensitive to UBE3A reinstatement therapies. Neonatal administration of either an AAV vector expressing human UBE3A or Ube3a-specific ASOs significantly increased UBE3A expression in the cortex of AS mice and reverted alterations in V-IOS amplitude. Notably, the extent of V-IOS recovery correlated with both the UBE3A cortical protein levels and the magnitude of motor improvements in the rotarod test. Taken together, these findings suggest that V-IOS could serve as a promising biomarker of brain function for AS.

Molecular and functional characterization of adult microglia in NRG1 TM HET mice, a mouse model of neurodevelopmental disorder

NDEV 09

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Neuregulin-1 (Nrg1) is a key neurotrophin that plays critical roles in brain development, regulating neuronal differentiation, migration, synapse formation, myelination and neuron-glia communication. In addition, Nrg1 also exerts neuroprotective and anti-inflammatory 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 evidence indicate the involvement of microglia, the tissue-resident immune cells of the central nervous system, 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.

Integrating micro-Raman Spectroscopy and Atomic Force Microscopy for biological longitudinal characterization of human Cortical Brain Organoids

NDEV 10

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Cortical brain organoids (cORGs) derived from human induced pluripotent stem cells (hiPSCs) represent one of the most promising models for studying human brain development in vitro. Their maturation is commonly assessed using well-established conventional methods, such as gene expression analysis, immunofluorescence staining, and histological characterization, which often require sample fixation and processing. While these approaches provide valuable molecular and structural information, they are inherently endpoint analyses and are limited to the investigation of pre-selected molecular markers. In this context, the integration of micro-Raman Spectroscopy (µ-RS) and Atomic Force Microscopy (AFM) into the analytical workflow offers a non-destructive approach for characterizing organoid maturation. µ-RS measures the inelastic scattering of light by molecular vibrational modes, providing information on the biochemical composition of the organoid, including lipids, proteins and nucleic acids, without the need for staining or fixation. In addition to µ-RS, AFM provides quantitative information on morphological and nanomechanical properties, such as surface topography, stiffness and viscoelastic behavior, under near-physiological conditions. Together, µ-RS and AFM enable the assessment of biochemical and biophysical features associated with organoid development, allowing a more comprehensive characterization of the maturation process. Within this framework, we are conducting preliminary studies on cORGs derived from three independent healthy control hiPSC lines. Organoids are characterized at multiple maturation stages using both conventional biological methods and the complementary physical techniques described above. By integrating molecular, biochemical and nanomechanical information, we aim to establish a reference profile of organoid maturation that will support future studies on disease-related in vitro models.

Modulation of nicotinic receptors in inhibitory prefrontal interneurons and their effect on behavior in ASD mouse models

NDEV 11

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The cholinergic system regulates neuronal excitability and network dynamics, underlying processes such as attention,learning and behavioral flexibility with nicotinic acetylcholine receptors (nAChRs) as a central component of this system. Disruptions in cholinergic signaling have been implicated in autism spectrum disorder (ASD), a neurodevelopmental condition characterized by deficits in social interaction and cognitive flexibility. These behavioral features are closely associated with an imbalance between excitatory/inhibitory signaling, involving specific interneuron subtypes such as vasoactive intestinal peptide (VIP)-interneurons and parvalbumin positive Chandelier cells (ChC PV+). This study investigates the expression and functional role of nAChRs in the prefrontal cortex (PFC) of the BTBR mouse model, and evaluates whether targeted genetic manipulation of alpha7 and beta2 subunits rescues circuit function and behavioral outcomes. To characterize nAChR expression in VIP and ChCs PV+ interneurons, we combined viral labeling with RNAscope and immunostaining in control C57BL/6 mice. VIP comprise approximately 2% of the neuronal population, with significant expression of alpha7(35%) and beta2(41.8%) subunits, and 20% co-expression. In addition, 77% of cells transduced by the ChC-targeting virus are PV+ interneurons, representing 1.45% of total PFC cells. Baseline behavioral screening revealed that BTBR mice exhibit robust hyperactivity, altered anxiety-like responses, and impaired cognitive flexibility in Open Field, Elevated Plus Maze, and Y-maze tests compared to controls. To move beyond traditional assays, we are using Live Mouse Tracker (LMT) for high-resolution automated analysis of spontaneous social dynamics. Together, these findings aim to define how nicotinic receptor modulation using CRISPRa and shRNA in specific interneuron populations shapes prefrontal circuit dynamics and contributes to behavioral phenotypes relevant to ASD.

Neuronal activity control by products of Foxg1-responsive, Tdark Rnf genes

NDEV 12

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The transcription factor FOXG1 masters telencephalic development and tunes maturation and activity of neocortical neurons. Distinct heterozygous mutations within this gene lead to FOXG1 Syndrome, a rare disorder with ASD symptoms, epilepsy and severe neurocognitive impairment. Among Tdark genes, few are Rnfs encoding for presumptive E3 ubiquitin ligases. Namely, 13 Rnfs respond to FOXG1 manipulation and neuronal stimulation by extracellular K+, while 2 Rnfs encode for putative FOXG1 interactors. Protein products of such Rnf genes interact with transcription factors, ion channels, neurotransmitter receptors and others. Therefore, we predicted they contribute to mediate the documented interplay between Foxg1 and neuronal activity.
To test this prediction, we performed either knock-down and overexpression of the 15 genes in primary cultures of murine pallial precursors, followed by proteomic profiling using the SomaScan platform. Remarkably, several proteins affected in these preparations are related to neuronal activity.
The functional impact of selected Tdark genes and in particular the KD of specific Rnfs (Rnf4, 44, 149, 214, 217, 219) on electrical activity was assessed by profiling calcium transients in primary pallial cultures transduced by the pSyn-GCaMP6 Ca2+ sensor. In parallel, neurons were engineered to express pSyn-hM3D and, by combining synapses blockade with CNO administration, we dissected cell-autonomous and network-level mechanisms triggered by these Rnfs.
Finally, to elucidate molecular events driven by Tdark proteins within the Foxg1-neuronal activity interplay, we are evaluating their impact on the half-life of selected presumptive targets of their action, including FOXG1.
We expect that this study will support the emerging scenario of a transcription factor (FOXG1) exerting a temporally structured, multilevel control on a number of activity-related genes and suggest novel targets for pharmacological mitigation of FOXG1 syndrome.

EBV infection drives neuroinflammation in MS brain through mechanisms of viral immune evasion

NI 09

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system whose development depends on the interaction of genetic and environmental factors. Among these, Epstein–Barr virus (EBV) is considered a prerequisite to the onset of MS, but the pathological mechanisms linking EBV to neuroinflammation are still largely unclear. Studies on 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, indicating a chronic, yet ineffective, antiviral immune response in advanced stages of the disease. This suggests that EBV may exploit intracerebral immune evasion strategies. The programmed cell death protein 1 (PD-1) pathway represents a potential candidate, as expression of its ligand PD-L1 can be induced by EBV latent proteins in EBV-associated tumors. We investigated the involvement of the PD-1/PD-L1 axis in MS brain by performing immunohistochemical analyses on highly inflamed post-mortem brain tissue from donors with progressive MS. Our findings revealed that EBV induces the upregulation of PD-L1 on brain-infiltrating infected B cells, both during latent infection and during the early stages of the lytic cycle. PD-1-positive T lymphocytes were frequently observed in close proximity to PD-L1-expressing EBV+ B cells, mainly within meningeal inflammatory infiltrates. These findings support a model in which EBV exploits the PD-1/PD-L1 checkpoint to evade immune surveillance and to maintain viral persistence in the CNS which may drive chronic inflammation through a continuous antigenic stimulation. To understand the interactions between EBV infection and local antiviral immune responses may facilitate the development of therapeutic strategies aimed at restoring antiviral immunity and limiting EBV-driven inflammatory processes in progressive MS.

Hematopoietic and innate immune cells as novel targets of AgRP neuropeptide signaling

NI 10

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The central nervous system plays a pivotal role in the regulation of peripheral immunity and hematopoiesis. We recently demonstrated that hypothalamic circuits modulate bone marrow activity through sympathetic nervous system signaling, influencing hematopoietic stem and progenitor cell mobilization and differentiation. In addition, we showed that Agouti-related peptide (AgRP) neurons regulate hematopoiesis through modulation of hypothalamic paraventricular nucleus activity and downstream noradrenergic signaling. Moreover, impaired AgRP-dependent regulation contributes to altered hematopoietic responses during neuroinflammatory conditions such as experimental autoimmune encephalomyelitis. Beyond their neuronal activity, AgRP release the AgRP neuropeptide into the cerebrospinal fluid and circulation. Notably, we showed increased circulating AgRP levels in persons with multiple sclerosis, suggesting a wider role for this peptide in neuroimmune communication. To investigate whether AgRP may directly influence immune and hematopoietic cells, we assessed the expression of melanocortin receptor 3 (MC3R), a major AgRP target receptor, across different immune populations. Flow cytometric analyses revealed MC3R expression in multiple HSPC subsets, including Lin−, Sca1+ and LSK populations. Among mature immune cells, receptor expression was detected in macrophages, monocytes, neutrophils, natural killer cells and dendritic cells, whereas B and T lymphocytes showed minimal or undetectable expression. Furthermore, receptor expression was confirmed in microglial cell line and validated by immunofluorescence approaches. These findings identify a broad repertoire of hematopoietic and innate immune cells potentially responsive to AgRP signaling. Collectively, our data support the existence of a previously unrecognized neuropeptide-mediated pathway linking hypothalamic activity to immune regulation and hematopoietic homeostasis.

Microglia-to-astrocyte connexin 43 transfer potentially contributes to the chronification of pain hypersensitivity after astroglial connexin 43 deletion

NI 11

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Neuropathic pain arises from maladaptive plasticity within the somatosensory system after nerve injury. Reactive glial cells contribute to pain chronification through neuroinflammatory signaling and altered intercellular communication. Connexin 43 (Cx43), the main astrocytic gap junction protein, is upregulated in neuropathic pain and implicated in inflammatory signal propagation, yet the mechanisms regulating astroglial Cx43 remain unclear. To investigate Cx43-mediated glial communication, we first explored microglia–astrocyte crosstalk in vitro. Public RNA-seq datasets identified GJA1/Cx43 as highly induced in LPS-stimulated microglia. In HMC3 microglia and CCF-STTG1 astrocyte-like co-cultures, siRNA-mediated Cx43 knockdown showed that astrocytic Cx43 depletion was rescued upon direct interaction with microglia. Using Cx43-Flag constructs, we demonstrated unidirectional Cx43 transfer from microglia to astrocytes, abolished in transwell conditions. Functionally, recipient astrocytes showed increased migratory capacity, suggesting a reactive astroglial phenotype. To assess its relevance in vivo, we used an inducible conditional Cx43 knockout mouse model targeting Slc1a3-positive astrocytes in the spared nerve injury (SNI) model. Astroglial Cx43 deletion attenuated mechanical allodynia during the early post-injury phase. However, this improvement was transient, as hypersensitivity progressively returned toward wild-type SNI levels. Histological analyses of spinal cord sensory laminae suggested that Cx43 deletion modulates glial responses, including microglial morphology and Cx43 organization. Collectively, our data showed that glial networks may adapt to astrocytic Cx43 loss, potentially limiting the beneficial long-term effect of astroglial Cx43 deletion on mechanical allodynia. Thus, Cx43 shuttling may represent an unrecognized form of glial plasticity involved in the maintenance of neuropathic pain.

Inhibition of NLRP3 through MCC950 reduces neuroinflammation and increases BDNF signaling in a mouse model of Huntington's disease

NI 12

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by a mutation in the HTT gene and characterized by progressive cognitive and motor decline. Mutant huntingtin (mHTT) forms intranuclear inclusions (NIIs), promotes microglial activation, and impairs cortico-striatal Brain-Derived Neurotrophic Factor (BDNF) transport. BDNF is crucial for the survival of striatal Medium Spiny Neurons (MSNs), and its depletion triggers progressive neuronal loss, driving chronic neuroinflammation. Recently, NOD- LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome has emerged as a key mediator of neuroinflammation and pyroptosis in HD. However, effective in vivo strategies to counteract its activation remain to be fully explored. Therefore, we hypothesized that the selective inhibition of NLRP3 by MCC950, by preventing its assembly, could exert neuroprotective effects in R6/2 mice. To test this, WT and pre-symptomatic R6/2 mice were intraperitoneally injected with either vehicle (0.9% saline solution) or MCC950 (10 mg/kg daily) from 4 weeks of age until their natural death. Motor performance was assessed weekly using Open Field and Rotarod tests, while body weight and survival rate were also monitored. Following sacrifice, immunofluorescence was performed to evaluate NLRP3 inhibition, microglial activation and BDNF modulation. Additionally, Golgi-Cox staining was used to analyze the effects of MCC950 on MSNs' dendritic spine density in the dorsal striatum. Notably, R6/2 mice treated with MCC950 showed a significant extension in lifespan compared to R6/2 control littermates. Furthermore, MCC950 reduced NLRP3 inflammasome and microglial activation, restored BDNF levels, and preserved MSNs dendritic spine density. Behavioral analyses are currently ongoing. These results demonstrate that MCC950 mitigates NLRP3-mediated neuroinflammation and exerts neurotrophic effects on R6/2 mouse model, suggesting it as a promising therapeutic candidate for HD.

EBI2-mediated signaling in glia and its implications for remyelination in organotypic cerebellar cultures

NI 13

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The Epstein–Barr virus-induced gene 2 receptor (EBI2/GPR183) regulates immune cell migration and glial function within the central nervous system (CNS). We previously demonstrated that the selective EBI2 agonist CF3-7α,25-dihydroxycholesterol (CF3-7α,25-OHC) promotes remyelination in experimental models; however, the downstream molecular mechanisms remain poorly understood. Here, we investigated whether activation of EBI2 influences the broader oxysterol signaling network in organotypic cerebellar slice cultures from wild-type and EBI2 knockout mice following demyelination. Treatment with CF3-7α,25-OHC altered the expression of genes involved in oxysterol metabolism and cholesterol homeostasis, including liver X receptors LXRa/β, ABCA1, SREBP1c, CH25H, and HSD3B7. Notably, changes in LXRβ and its downstream target genes were also observed in EBI2-deficient tissue, despite neither CF3-7α,25-OHC nor its endogenous counterpart 7α,25-OHC directly activating LXRs. These findings suggest that exogenous EBI2 agonist administration reshapes endogenous oxysterol metabolism, resulting in secondary modulation of LXR-regulated pathways rather than direct LXR activation. Together, these results reveal previously unrecognized crosstalk between EBI2 signaling and the oxysterol metabolic network in the CNS, providing new insight into how pharmacological EBI2 activation may influence cholesterol homeostasis, neuroinflammation, and myelin repair.

Immunomodulatory and remyelinating properties of the oxysterol analogue CF3-7α,25OHC in mouse models of multiple sclerosis

NI 14

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INTRODUCTION: Epstein-Barr virus-induced gene 2 (EBI2) regulates immune cell migration and is implicated in multiple sclerosis (MS) pathophysiology, where its deficiency impairs remyelination. Because natural oxysterols have a short half-life, we evaluated a biostable synthetic agonist, CF3-7α,25-OHC, to assess its neuroimmunomodulatory and regenerative potential.
AIM: To evaluate the capacity of CF3-7α,25-OHC to accelerate remyelination and modulate autoimmune responses in mouse models of MS.
METHODS: In the cuprizone model, male C57BL/6 mice were fed a 0.2% cuprizone diet for 9 weeks, followed by 2 weeks of CF3-7α,25-OHC treatment (20 mg/kg). To address the autoimmune component of MS, ongoing studies utilize the Experimental Autoimmune Encephalomyelitis (EAE) model, conducted exclusively in EBI2 knock-out (KO) mice. Evaluation includes daily clinical scoring (0–4.5), Luxol Fast Blue (LFB) staining, qPCR, and blood counts.
RESULTS: In the cuprizone model, CF3-7α,25-OHC significantly accelerated remyelination in the corpus callosum compared to controls. Treatment led to a pronounced reduction in peripheral white blood cells, decreasing lymphocytes by 51% and monocytes by 66%. The analogue also upregulated Ebi2 brain expression and increased the synthesis of 15 lipid classes. Ongoing EAE experiments in EBI2 KO mice are currently evaluating the receptor's role during peak autoimmune neuroinflammation.
CONCLUSIONS: CF3-7α,25-OHC exerts dual actions by promoting myelin repair and inducing potent systemic immunomodulatory effects, validating the EBI2/oxysterol axis as a promising therapeutic target for MS.

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SKYBONE: Viral lineage tracing of skull bone marrow-derived immune cells in a murine model of multiple sclerosis

NI 15

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The skull bone marrow (SkBM) is an emerging hematopoietic niche directly connected to the meninges through vascular channels, but the contribution of skull-resident hematopoietic stem cells (HSCs) to central nervous system (CNS) inflammation remains unclear. In multiple sclerosis (MS), meningeal tertiary lymphoid structures (TLS) are associated with severe and progressive disease and support local B cell responses. SKYBONE aims to determine whether SkBM-derived HSC progeny, particularly B cells, contribute to meningeal inflammation and TLS formation in a mouse model of MS. To address this question, we are developing an in vivo viral lineage-tracing platform based on lentiviral vectors carrying HSC-oriented synthetic promoters upstream of an EGFP-Cre cassette.

Candidate promoter elements were selected through integrative analysis of RNA-seq and ATAC-seq datasets, followed by transcription factor binding site enrichment and synthetic promoter design. The resulting vectors will be tested in vitro for specificity and efficiency and then applied in IgHMOG x tdTomato reporter mice after experimental autoimmune encephalomyelitis (EAE) induction, allowing stable labelling and tracking of SkBM-derived immune cells in meninges and CNS tissues. Preliminary work identified candidate regulatory elements enriched in HSC-associated genes and generated an in-house EGFP-Cre lentiviral backbone, validating the feasibility of this strategy. By linking skull hematopoiesis to CNS border immunity, this project may clarify the origin of meningeal B cells in chronic neuroinflammation and provide new insight into mechanisms underlying progressive MS.

Aspects of neuroinflammation in treatment-resistant schizophrenia

NI 16

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Introduction: In recent years, the use of hematological indices as diagnostic or prognostic biomarkers in various inflammatory diseases has been widely discussed.
Objective: to analyze the relationship between psychopathological manifestations of treatment-resistant schizophrenia (TRS) and hematological markers of inflammation.
Materials and Methods: the group included 36 patients with TRS, which were characterized using the Calgary Depression Scale (CDSS), Daily Social Functioning (PSP), and Positive and Negative Symptom Scale (PANSS). This group were analyzed using routine hematological parameters and inflammatory indices (LMR, PIV, SIRI, PLR, NLR, SII) calculated based on the peripheral blood count.
Results: The obtained hematological parameters allowed to divide the sample into 4 groups: group 1 (LMR>30) (n=7), group 2 (LMR 8-30) (n=11), group 4 (LMR<8, SIRI>1.1) (n=6), group 3 (LMR<8, SIRI<1.1) (n=12). Group 1 is characterized by monocytopenia (0.046±0.014, p=0.0001), group 3 by a relative decrease in platelets (203.25±29.29, p=0.0069). Group 4, in addition to the highest SIRI index (>1.1), has the maximum PIV value (331.304±75.339, p=0.0001) and a relative increase in monocytes (0.59±0.19, p=0.007). Group 2 represents a transitional link between the groups.
Group 4 (inflammatory phenotype) was characterized by the most severe clinical picture (PANSS=103.66±8.68, p=0.0075) with a predominance of disorganization domains, including delusions (p=0.04), hallucinations (p=0.04), thought disorders (p=0.04), mannerisms and posturing (p=0.01) in comparison with the rest of the sample.
Conclusions: The obtained data highlight the variability of TRS mechanisms and the possible role of inflammation.

Investigating blood-brain barrier interactions of Gadolinium-based contrast agents using an in vitro human model

NIM 01

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Gadolinium-based contrast agents (GBCAs) are routinely used in magnetic resonance imaging (MRI). Although macrocyclic GBCAs were initially considered safe and rapidly eliminated from the organism, it is now well established that a fraction of administered gadolinium (Gd) can be retained for prolonged periods in tissues such as the brain, bone, and skin. Toxicity of free Gd+3 ions is of particular concern in patients who undergo repeated MRI examinations, as cumulative exposure may increase the burden of retained Gd. Despite growing evidence of Gd retention, the mechanisms by which GBCAs cross the blood-brain barrier (BBB) remain poorly understood. Since GBCAs are administered intravenously, their first cellular interactions occur within the bloodstream. Therefore, this study aimed to investigate the mechanisms governing GBCA distribution to brain tissue, with a particular focus on the potential role of white blood cells as mediators of Gd transport across the BBB.

The interaction of the macrocyclic GBCA gadoterate meglumine (Dotarem), one of the most widely used contrast agents in clinical practice due to its high thermodynamic and kinetic stability, was investigated with an in vitro model of the human BBB. To address this question, we employed the human brain microvascular endothelial cell line hCMEC/D3 cultured in Transwell inserts to generate a tight BBB-like barrier. Cellular uptake of Dotarem was assessed by quantifying intracellular Gd using both single-cell inductively coupled plasma mass spectrometry (SC-ICP-MS) and ICP-MS. Our results indicate that hCMEC/D3 cells take up Dotarem in a time- and concentration-dependent manner. Furthermore, experiments performed using the BBB model suggest that GBCAs interact with the endothelial barrier and potentially influence the transport across the BBB. These findings provide new insights into the early cellular events that may contribute to Gd biodistribution and retention within the central nervous system.

Keywords: MRI, Gadolinium based contrast agents, GBCA, Gadoterate meglumine, Blood-Brain Barrier, White Blood cells, K562, Extracellular vesicles.

The role of cognitive reserve in Amyotrophic Lateral Sclerosis with C9ORF72 expansion: a 2-[18F]FDG-PET study

NIM 02

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Objectives. We aimed at assessing whether education (a proxy of cognitive reserve, CR) may enhance resilience to cognitive impairment in ALS, even in the presence of C9ORF72 expansion, which is a strong genetic risk factor for cognitive impairment.
Materials. We included 76 C9ORF72-ALS patients (C9-ALS) and 40 healthy controls (HC). We stratified the C9-ALS cohort into high-education (HE) and low-education (LE) subgroups according to the cohort median. The HE and LE groups were matched for sex, age at PET, site of onset, ASL-FRS-R total score, and cognitive status (n=18 each).
Methods. We compared brain 2-[18F]FDG-PET metabolism of HE and LE C9-ALS patients with HC using the two-sample t-test model of SPM12. Subsequently, we compared the HE and LE groups using the same tool. The metabolic cluster showing a significant difference between HE and LE was used as seed region in an interregional correlation analysis (IRCA) to evaluate brain connectivity with the multiple regression tool of SPM12.
Results. Both C9-ALS groups (compared to HC) showed a relative hypometabolism of the caudate and medial thalamus. HE patients showed an additional cortical cluster of relative hypometabolism (not detected in LE), consistent with a more extensive brain damage, involving frontal cortex. Furthermore, a more extensive relative hypermetabolism in the cerebellum was found in HE. The comparison between HE and LE identified a relative hypermetabolism in HE in frontal and temporal regions, amygdala, and putamen. In the IRCA in the HE group, we found a negative correlation with the cuneus and precuneus. IRCA in the LE group showed broader negative correlations in occipito-parietal cortex, precentral and postcentral gyri and the middle temporal gyrus.
Discussion. Compared to HC, HE showed more widespread disease-related metabolic changes than LE with overlapping cognitive status. IRCA in HE patients revealed reduced connectivity within posterior nodes of the Default Mode Network (DMN), possibly suggesting a higher disease burden compared to LE with the same cognitive status.
Conclusions. Our results point out that CR may play a protective role even in the presence of an unfavorable genetic background due to the presence of the C9ORF72 hexanucleotide expansion.

NIM 03

Monitoring disease activity through GFAP and Neurofilament Light Chain longitudinal changes in SOD1-related ALS patients receiving antisense oligonucleotide therapy

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Amyotrophic Lateral Sclerosis (ALS) is a fatal adult-onset motor neuron disease, with approximately 2% of cases caused by mutations in the SOD1 gene. Tofersen, an antisense oligonucleotide (ASO) recently approved by Food and Drug Administration and European Medecines Agency, is developed to reduce SOD1 mRNA levels. To monitor disease progression, Glial Fibrillary Acidic Protein (GFAP) serves as an indicator of reactive astrogliosis, while Neurofilament Light Chain (NfL) is a robust marker of axonal injury. This study investigates the dynamics of GFAP and NfL concentrations in cerebrospinal fluid (CSF) of ALS patients with different SOD1 mutations treated with Tofersen, aiming to assess the interplay between astrocytic neuroinflammation and ongoing neurodegeneration. We included patients carrying SOD1 mutations treated with Tofersen in Turin ALS Center within the compassionate use program. GFAP and NfL levels are measured longitudinally until month 12 after the first infusion using the Single Molecule Array (Simoa®) technique. The cohort analyzed includes two known pathogenic variants (p.L145F; p.V32A), one novel variant (p.A90del), and a patient carrying both SOD1 p.N20S substitution, considered a Variant of Uncertain Significance, and a pathological GGGGCC expansion in the first intron of C9orf72 gene. GFAP levels show a significant upward trend during 12 months from basal infusion across the entire cohort. On the other hand Nfl levels exhibit a significant decrease during the same period, except for the dual-mutation patient. This preliminary longitudinal evaluation observes an astrocytic reactive response following prolonged intrathecal treatment, although the drug counteracts neurodegeneration by lowering NfL levels. Results confirm recent published data on a potential immune response triggered by ASO infusion. The dual-mutation case shows the worst response to Tofersen, pointing C9orf72 as primary pathogenic driver.

Dual-targeted peptide nanofibers for selective co-delivery of temozolomide and 3-bromopyruvate in glioblastoma

NO 08

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Combination therapy represents a powerful strategy to counter the aggressiveness of glioblastoma (GBM) by simultaneously disrupting multiple oncogenic pathways while potentially limiting systemic side effects. Here, we developed an innovative tumor-targeted nanoplatform based on self-assembling peptide nanofibers engineered for the co-delivery of two synergistic agents: the glycolysis inhibitor 1,3-bromopyruvate (BrP) and the alkylating chemotherapeutic temozolomide (TMZ). To achieve controlled, on-demand drug release within the tumor microenvironment, the therapeutics were conjugated via a matrix metalloproteinase-9 (MMP-9)-responsive linker. Furthermore, the nanofiber architecture was functionalized with a dual-peptide targeting system to maximize selectivity and delivery efficiency: the falGea peptide, which specifically binds to the overexpressed EGFRvIII receptor on GBM cells, and gH625, a cell-penetrating peptide designed to mediate transport across the blood-brain barrier (BBB). The therapeutic efficacy of the platform was then evaluated in U-87 MG glioblastoma cells cultured in an advanced 3D system. Finally, transport assays performed in a dynamic 3D in vitro BBB model demonstrated that the incorporation of gH625 significantly enhanced the translocation of the nanostructure across the endothelial barrier. Overall, these findings highlight the potential of this multifunctional peptide nanofiber as a highly precise, dual-targeted platform capable of overcoming major delivery obstacles and offering an effective tool for glioblastoma treatment.

Photoglioblaster: dual-triggered phage therapy for precision targeting of glioblastoma

NO 09

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Introduction: Glioblastoma (GBM) is one of the most aggressive and lethal solid tumors, characterized by poor prognosis and limited therapeutic options. Photodynamic and sonodynamic therapy (PDT and SDT) are minimally invasive strategies based on the activation of sensitizer molecules that generate reactive oxygen species (ROS), leading to tumor cell death. Despite their potential, the lack of selective delivery systems limits their clinical application. This study aimed to develop and validate a targeted bacteriophage-based platform for the selective delivery of a dual photo/sono-activable sensitizer to EGFRvIII-glioma cells.
Material and Method:
The M13 bacteriophage was genetically engineered to display an EGFRvIII-specific scFv on the minor coat protein pIII, enabling receptor-selective targeting. The sensitizer Rose Bengal (RB), activatable by both light and ultrasound, was chemically conjugated to the major coat protein pVIII. A murine glioblastoma model expressing EGFRvIII (mGBM-vIII) was established. Targeting specificity was assessed by immunofluorescence comparing mGBM-vIII and parental EGFRvIII-negative cells upon phage incubation. Phage internalization was evaluated by confocal microscopy. In vitro PDT and SDT were performed on mGBM-vIII cells, and viability was measured 24 h post-treatment by MTT assay. Intracellular ROS levels were quantified after treatment. The project subsequently advanced to in vivo evaluation of phage performance in tumor-bearing mice. Both intravenous administration and intratumoral delivery were investigated. Quantitative biodistribution studies by qPCR are currently ongoing.
Result and Discussion:
Engineered phage constructs selectively recognized mGBM-vIII cells, while no signal was detected in parental controls, confirming high targeting specificity. Confocal imaging demonstrated not only surface binding but also efficient intracellular localization of retargeted phages, a critical feature for enhancing intracellular ROS generation. RB-conjugated phages induced significant tumor cell death 24 h after PDT or SDT compared to RB alone. Consistently, marked ROS production was observed following both laser and ultrasound activation. In vivo, phage-derived signal was detected within the tumor mass following both intratumoral and intravenous administration, demonstrating effective tumor targeting with either delivery route. Ongoing qPCR biodistribution analyses will provide quantitative comparison of phage accumulation in brain versus peripheral organs.
Conclusion:
This study demonstrates that EGFRvIII-targeted, RB-conjugated M13 phages enable selective tumor targeting, intracellular localization, and effective photo/sono-induced cytotoxicity in preclinical GBM models, while showing a favorable safety profile. These findings provide strong proof-of-concept for a minimally invasive and translationally relevant therapeutic platform.

Radiation therapy triggers a reshaping of transcriptional landscape of healthy microglia cell line analyzed through long reads RNA sequencing

NO 10

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Glioblastoma is a highly aggressive brain tumor, and microglia plays a key role in its microenvironment. Although radiation therapy is a standard treatment for tumor control, its effects on the microenvironment—specifically on healthy microglial cells—remain unclear. This project investigates the impact of radiotherapy on the transcriptome of healthy microglia. To identify potential transcriptomic rearrangements, we extracted total RNA from healthy microglial cell lines exposed to 15 Gray of radiation and from untreated controls. We performed Long-Read RNA sequencing using Oxford Nanopore PromethION. We conducted differential expression analysis on 153,484 annotated transcripts and examined differential isoform usage to identify isoform switching between conditions. We performed pathway enrichment analysis on both differentially expressed genes and those undergoing isoform switching to determine the most significantly affected pathways.
We identified 13,448 differentially expressed transcripts, involving 8,913 genes. Gene ontology analysis indicated that ncRNA processing and ribosome biogenesis were the most affected biological processes, while pathway enrichment analysis highlighted translation as the primary impacted pathway. Isoform usage was quantified as isoform fraction (IF), the relative abundance of each transcript within its parent gene, while differential usage was expressed as dIF, the change in IF between the two conditions. Isoform switching analysis revealed a distinct expression landscape between treatment and control groups, identifying 827 significant switches across 420 genes. Translation was the most significantly affected pathway, followed by cell cycle regulation. Overall, we observed a shift toward non-coding transcript variants in irradiated microglia.
These preliminary findings provide new insights into gene expression regulation, contributing to a better understanding of the glioblastoma microenvironment and the impact of radiation therapy.

Unraveling STAG2-driven neuro-oncogenesis in medulloblastoma for new therapeutic targeting

NO 11

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Medulloblastoma (MB) is a cerebellar tumor of embryonal origin, likely due to impaired hindbrain-specific neural progenitor differentiation, ultimately leading to oncogenesis; it is the most common malignant brain tumor in children and remains associated with significant mortality and severe long-term therapy-related sequelae. Increasing evidence suggests that alterations in the cohesin complex, a key regulator of genome organization, transcription, and DNA repair, contribute to cancer development. In particular, the cohesin subunit STAG2 has emerged as a tumor suppressor gene. Our recent works identified deleterious STAG2 variants in a subset of pediatric brain tumor patients and demonstrated that STAG2 loss of function promotes oncogenic processes in experimental models. However, the mechanisms by which STAG2 deficiency contributes to MB development remain largely unknown. Thus, to study the role of STAG2 in neuro-oncogenesis and the effects of its downregulation on neural differentiation and cerebellar development, we silenced STAG2 in neural precursor cells (NPCs) and cerebellar organoids (hCOs) using short-hairpin RNA. Specifically, we identified in NPCs a significant upregulation of oncogenes known as STAG2 downstream targets, and in hCO, defects in the expression of key genes involved in cerebellar differentiation and altered regulation of related oncogenic targets, thus suggesting a role for STAG2 in the pathogenesis of MB. These neural models also aim to set the basis to test a patient-tailored treatment by using PARP inhibitors (PARPi). This group of drugs has been implemented in trials for tumors carrying cohesin variants, and, notably, we recently demonstrated partial phenotype rescue upon PARPi treatment in in vivo STAG2-silenced fly models, characterized by increased DNA damage and impaired neural differentiation, paving the way for PARPi as a worthwhile, successful therapeutic approach in MB.

Gut-brain axis in brain tumor development: exploring the impact of bile acids on glioma

NO 12

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Gliomas are the most common malignant tumors of the Central Nervous System (CNS) and are characterized by high recurrence rates, aggressive invasiveness, and strong immune evasion, all of which contribute to poor patient prognosis. Despite standard treatment, survival remains limited. Increasing evidence indicates that gut microbiota and its metabolome play a crucial role in regulating host and brain physiology and biological processes. In fact, alterations in microbial composition and metabolite production have been linked to several pathological conditions, including glioma. Among microbial metabolites, bile acids (BAs) are gaining particular interest, since the dysregulation of their metabolism has been implicated in several neurodegenerative diseases, in which their administration has been shown to alleviate neurological symptoms, acting on brain cells. Using a syngeneic mouse model established with murine glioma cell line GL261, we found that glioma development modifies the gut metabolome by reducing the level of several metabolites, including BAs, suggesting that their administration may influence glioma progression. Among the reduced BAs, Tauroursodeoxycholic acid (TUDCA) was selected because of its favorable safety and tolerability profile shown after administration in several clinical trials for neurological disorders. Moreover, we investigated the BAs receptor TGR5, for which TUDCA has a high affinity; in particular, we focused on its role as a mediator of TUDCA effects on GL261 cells (in vitro), and on glioma tumor development (in vivo). In vitro, we assessed the modulation of GL261 proliferation and migration, evaluating the effect of TUDCA and INT-777, a specific agonist of TGR5 and the effects of TUDCA on BV2 microglia cells phenotype in a GL261 microenvironment; in vivo, we performed preliminary experiments on the effect of INT-777 intranasal administration on tumor development in GL261 bearing mice.

Assessing the in vitro and in vivo effects of monoclonal antibodies against Glioblastoma

NO 13

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Glioblastoma (GBM) is the most aggressive brain tumor, most commonly arising in the frontal and temporal lobes. Despite multimodal treatment approaches, GBM remains associated with rapid progression, high recurrence rates and poor overall survival, making its management a significant therapeutic challenge. Monoclonal antibodies, owing to their high specificity and sensitivity toward biological targets, have been widely investigated as a therapeutic agent in several cancers, including GBM. Our study aimed to assess the ability of an anti-HER3 receptor monoclonal antibody (anti-HER3 mAb) to cross the blood-brain barrier (BBB) and to evaluate its therapeutic potential in GBM mouse models. HER3 is a tyrosine kinases receptor involved in many cellular processes such as proliferation, differentiation, cell survival, migration and invasion. It is frequently overexpressed in GBM tissues. Upon binding to its ligand, Neuregulin-1 (NRG1), HER3 activates the Phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT) signaling pathway, promoting tumor cell proliferation, survival, and metabolic reprogramming. According to the aim, we used an in vitro BBB model composed of murine endothelial cells (bEnd.3) and murine primary astrocytes. This co-culture system created a barrier, as revealed by the Trans-Endothelial Electrical Resistance (TEER) measurement, that was efficiently crossed by anti-HER3 mAb. We evaluated also the direct effects of anti-HER3 mAb treatment on several glioma murine cell lines, GL-261, SB-28 and CT-2A, observing impairment of survival, proliferation and invasion. Then, glioma-bearing mice were treated intraperitoneally with the anti-HER3 mAb. The treatment reduced the tumor volume and the percentage of proliferating cells (Ki-67^{+}) within the tumor mass. Overall, these findings suggest that anti-HER3 mAb may represent a promising therapeutic strategy in GBM mouse model, supporting further investigation of its translational applicability.

In vitro and in vivo models to assess the impact of ionizing radiation on calcium dynamics in Glioblastoma

NO 14

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Glioblastoma (GB) is the most aggressive primary brain tumor and is characterized by massive infiltration of the surrounding parenchyma. Gliomas establish functional networks that rely on calcium (Ca^{2+}) signaling to promote proliferation, invasion and therapeutic resistance, highlighting Ca^{2+} dynamics as a potential therapeutic target. FLASH radiotherapy (FLASH-RT), which delivers radiation at ultra-high dose rates (UHDR), has emerged as a promising approach for brain malignancies, capable of limiting the damage to healthy tissues (FLASH effect), compared to Conventional Radiotherapy (CONV-RT). Ionizing radiation is known to perturb Ca^{2+} activity, yet the in vivo effects on Ca^{2+} dynamics remain poorly understood, particularly in vivo. In this study, we employed an electron LINear ACcelerator (LINAC), able of switching between UHDR and CONV-RT. Murine GL261 and CT2A glioma cells expressing the GCaMP6s calcium sensor are orthotopically implanted in mice, and Ca^{2+} activity is longitudinally monitored by Two-Photon imaging before and after RT. Clonogenic assays revealed different dose-dependent responses, with GCaMP6s-GL261 cells being more sensitivity to FLASH-RT. Preliminary in vivo experiments showed that both irradiation modes significantly delayed tumor growth compared to non-irradiated mice, with similar anti tumor-efficacy. Furthermore, GCaMP6s-GL261 cells appears undergoing apoptotic pathways, reflecting the technical challenges in acquiring in vivo calcium dynamics post RT. This is the first study that compares tumor-associated Ca^{2+} responses in in vivo GB models following CONV-, FLASH- and Minibeam-RT. I aim to investigate in real time the role of Ca^{2+} signaling in radiation response and evaluate whether spatially fractionated irradiation may further improve healthy tissue sparing while maintaining therapeutic efficacy, thus resulting in a safer clinical alternative for cancer treatment, particularly in sensitive anatomical sites like the brain.

Cancer-neuronal crosstalk in glioblastoma: neuronal-dependent mechanisms in tumor progression

NO 15

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Glioblastoma (GBM) remains the most aggressive primary brain tumor in adults, with a dismal prognosis despite multimodal therapy. Traditional tumor-centric approaches have failed to fully explain GBM progression and recurrence, underscoring the importance of the tumor microenvironment and, more recently, of neurons as active players in cancer biology. We established an in vitro neuro-tumor unit (NTU) by co-culturing patient-derived glioblastoma stem-like cells (GSCs) with murine neurons and astrocytes. GSC proliferation was enhanced by neurons and neuronal activity, but not by astrocytes alone. Across 11 heterogeneous GSC lines, most showed increased proliferation in response to neuronal signals ('sensitive' GSCs, senGSCs), whereas a minority remained refractory ('refractory' GSCs, refGSCs). In addition, neurons induced an apoptotic response in refGSCs, detected at both transcriptional and functional levels. These divergent responses were associated with distinct metabolic adaptations: refGSCs relied almost exclusively on glycolysis in coculture, while senGSCs also activated de novo OXPHOS to sustain their proliferative boost. Collectively, our findings highlight the multimodal role of neurons and their activity in promoting GSC proliferation and metabolic reprogramming. This work strengthens the emerging field of cancer neuroscience and opens new therapeutic opportunities aimed at disrupting neuron–GBM crosstalk while preserving brain function.

P2X7R blockade restrains glioblastoma development and regulates tumor–microglia interactions

NO 20

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P2X7R Blockade Restrains Glioblastoma Development and Regulates Tumor–Microglia Interactions Glioblastoma is the most aggressive primary brain tumor in adults and is characterized by high invasiveness, therapeutic resistance, and a strongly immunosuppressive microenvironment. Increasing evidence suggests that P2X7 receptor signaling plays a key role in glioblastoma progression by regulating tumor proliferation, neuroinflammation, and microglia activation. The aim of this study was to investigate the effects of anti-P2X7R treatment in in vitro and in vivo glioblastoma models. Human U87 and murine GL261 glioblastoma cell lines were treated with anti-P2X7R compounds in both 2D and 3D culture systems. In vitro cytotoxicity assays were performed at 24, 48, and 72 h in biological triplicates and demonstrated significant antitumoral activity in both cell lines, with comparable effects observed in spheroid models generated in U-bottom 384-well plates. To evaluate tumor–microglia interactions, co-culture systems using U87 cells and CHME human microglial cells were established in transwell plates. Confocal microscopy analyses showed that anti-P2X7R treatment modulated microglial infiltration into glioblastoma spheroids, suggesting a role for P2X7R signaling in regulating tumor-associated neuroinflammation and tumor–microglia crosstalk. In vivo experiments were performed in CX3CR1 heterozygous mice bearing fluorescent microglia orthotopically injected with GL261 mCherry-LUC cells. Preventive administration of anti-P2X7R treatment, initiated 6 hours post-inoculation, significantly slowed down tumor volume growth as calculated after 14 days of therapy, supporting the involvement of P2X7R during the early phases of glioblastoma establishment. Regarding the tumor microenvironment, animals treated with anti-P2X7R either 6 hours or 5 days post-implantation exhibited an increased recruitment of microglial cells around the tumor, with a corresponding decrease of microglia in the peritumoral area. In parallel, ongoing digital droplet PCR analyses on samples derived from both 2D cultures and co-culture systems are being performed to investigate the molecular pathways involved in P2X7R-mediated neuroinflammation and microglial phenotypic modulation. Overall, these findings support P2X7R as a promising therapeutic target in glioblastoma.

PEDOT:PSS functionalized with halloysite nanotubes promotes neuronal maturation and early network-oriented organization

NP 08

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Neurodegenerative diseases represent one of the leading causes of physical and cognitive disability worldwide, currently affecting nearly 15% of the global population. Despite significant advances in basic and clinical neuroscience, effective therapeutic strategies capable of preventing or reversing neuronal loss remain limited. In this context, regenerative medicine and neuroprosthetic technologies are emerging as promising approaches to restore neural function through implantable interfaces designed to interact with damaged circuits and support tissue repair. Among these, conductive polymer-based systems have gained considerable attention for their ability to mimic the electrochemical properties of neural tissue and deliver controlled electrical and structural cues that guide neuronal and glial differentiation, opening new avenues for next-generation neural regenerative therapies. To investigate how engineered bioelectronic interfaces may influence neuronal maturation and early circuit formation, we examined the neuroactive behaviour of a PEDOT:PSS substrate functionalized with Halloysite Nanotubes, a natural aluminosilicate nanomaterial whose tubular morphology enhances surface interactions and ionic–electronic coupling. Human SH-SY5Y cells were cultured for 15 days on PEDOT:PSS–Halloysite substrates in the absence of neuro-inductive stimuli. To assess biocompatibility and cellular responses to the hybrid material, we performed MTT assays and DAPI staining, confirming robust viability, adhesion, and proliferation. The neuro-inductive potential of the substrate was evaluated through gene expression analyses and immunofluorescence using late neuronal markers (MAP2, NF-H), which revealed a progressive reinforcement of neuronal identity and cytoskeletal organization. To further characterize the spontaneous neuronal differentiation induced by the conductive composite, we quantified neurite morphology using NeuronJ-based tracing, demonstrating marked increases in neurite elongation, branching complexity, and the formation of interconnected cellular clusters indicative of early network-oriented structural organization. Halloysite Nanotubes significantly strengthened the neuroactive properties of PEDOT:PSS, likely by providing nanotopographical and electroactive cues that favour cytoskeletal stabilization and synaptic differentiation. Although these findings highlight a clear neuroactive influence of PEDOT:PSS functionalized with Halloysite Nanotubes, they derive from preliminary structural and molecular analyses. Functional electrophysiological assessments were not performed in this study and will be essential in future work to determine whether the observed morphological and differentiation-related changes translate into measurable alterations in neuronal excitability, synaptic function, and network-level plasticity. Together, these preliminary results support a model in which PEDOT:PSS functionalized with Halloysite Nanotubes acts as an active bioelectronic interface capable of tuning the molecular and structural determinants of neuronal differentiation and early network organization. This hybrid platform provides a solid foundation for future investigations aimed at dissecting the neurophysiological mechanisms of adaptive plasticity and developing next-generation bioelectronic tools for neural repair and modulation.

Adult hippocampal neurogenesis shapes ideomotor action control in mice

NP 09

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Humans select and learn their goal-directed actions through the anticipation of the outcomes. While this “ideomotor principle” is well supported in humans, its biological instantiation and relevance for the organization of naturalistic behavior have been difficult to examine. This includes the underlying neurobiological mechanisms, for example, hippocampal plasticity. We leverage high-resolution, continuous behavioral tracking in mice over a month-long series of automated learning tasks to test whether ideomotor principles operate as general mechanisms of action organization and how they relate to individualized levels of experience-dependent adult hippocampal neurogenesis. We monitored action–outcome formation in a large cohort of mice living in the same enriched environment that provided a sequence of learning tasks, capturing self-initiated decisions at a resolution not achievable in humans. We quantified how animals form action–outcome associations and how flexibly previously learned associations are reused or updated. These measures revealed a systematic and stable structure underlying how action–outcome relations develop and are managed, even when both genetic background and the shared environment are identical. Inter-individual differences in how actions and their consequences were organized correlated to levels of neurogenesis. Higher levels of neurogenesis were associated with broader and more variable exploration and with greater flexibility in updating action–outcome relations, whereas lower neurogenesis was linked to more rigid, strongly bound action repertoires. These findings demonstrate that general principles of goal-directed action, conceptualized from experiments in humans, operate in a naturalistic animal setting and correlate to adult hippocampal neurogenesis. Our work thus links cognitive theory to a specific cellular plasticity mechanism and indicates hippocampal neurogenesis as a key substrate supporting flexible behavioral organization.

Molecular biomarkers of unmyelinated peripheral nerve regeneration: a systematic immunofluorescence screening in human skin biopsies

NP 10

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While the mechanisms of peripheral nerve degeneration are well characterized, the molecular events governing regeneration and reinnervation of unmyelinated fibers remain poorly understood, representing a fundamental gap in peripheral neuroscience. The epidermis is innervated exclusively by the free endings of unmyelinated C fibers and poorly myelinated Aδ fibers, which mediate thermoreception, nociception and itching. These small-calibre fibers cross the dermo-epidermal junction and branch into the epidermal layers; their density, quantified as intraepidermal nerve fibre density (IENFD), is the main morphological parameter in skin biopsy analysis. Predominantly unmyelinated autonomic fibers further inform on the integrity of the peripheral autonomic nervous system, making skin biopsy a tool for simultaneous evaluation of sensory, autonomic and mechanoreceptive innervation. Through a systematic review of the literature, a panel of candidate molecules was identified: GAP-43, SCG10, SPRR1A, ATF3 and semaphorins. The present study aims to identify a marker capable of selectively detecting reinnervating intraepidermal nerve fibers, distinguishing them from mature ones. Skin punch biopsies (3 mm) were fixed in Zamboni and processed by indirect immunofluorescence combining each candidate with anti-PGP9.5 antibody. GAP-43 co-localized with all PGP9.5-positive fibers without selective subpopulation, consistent with its role as a broadly expressed growth-competence marker. SCG10, tested as the next candidate, on initial assessment, showed a markedly different pattern, with the signal restricted to a subpopulation of PGP9.5+ fibers. Since SCG10 is a microtubule-destabilizing protein enriched in growth cones and elongating axons, this selectivity suggests labelling of structurally dynamic rather than mature stabilized axonal endings, positioning SCG10 as a promising candidate. Validation across further candidates and conditions of known denervation and reinnervation is ongoing.

Gene supplementation therapy restores motor deficits in Gnao1[C215Y] mice and proves broad efficacy in C. elegans model

NP 11

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The heterotrimeric G proteins are ubiquitous membrane-bound complexes specialized in the transduction of receptor-mediated extracellular signals into intracellular responses. Mutations in the G protein subunit alpha O1 (Gαo), encoded by GNAO1, have been associated with neurodevelopmental disorders characterized by prominent movement disorder with or without epilepsy. The Gnao1[C215Y]/+ mouse model recapitulates key features of the human disease, with a relatively mild phenotype, normal viability, and late onset movement abnormalities, detectable in specific motor tasks. Here we report functional alterations in cortical layer V pyramidal neurons from Gnao1[C215Y]/+ mice, with reduction of the inward currents (Ih) and impairment of the GABAB-mediated outward current. We propose an adeno-associated virus (AAV)-based gene therapy aimed at overexpressing the wild-type form of Gαo by intracerebroventricular injection in newborn heterozygous Gnao1[C215Y]/ + mice. Our results demonstrate that overexpression of the wild-type protein mitigates behavioral abnormalities and restores functional neuronal deficits in young adult mice, thereby supporting the development of AAV-mediated gene augmentation strategies to counteract the effects of GNAO1 variants in patients. The potential of gene supplementation therapy is further supported by data from genetically modified C. elegans strains carrying not only the relatively mild C215Y variant but also a panel of goa-1/GNAO1 mutations associated with more severe phenotypes, suggesting a broader potential of this therapeutic strategy.

Cognition at the core of metabolic syndrome: linking neurometabolic load to cognitive and affective vulnerability in high-fat diet rat model.

NP 12

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Metabolic Syndrome (MetS) is defined by peripheral metabolic abnormalities impacting brain physiology, whose underlying mechanisms remain elusive. Reframing cognitive impairment as a core feature of MetS is supported by emerging evidence indicating how cognitive alterations may represent an early and mechanistically relevant feature of MetS progression than a mere sequelae of metabolic dysfunction. To this aim, we employed a longitudinal high-fat diet (HFD) rat model over 20 weeks, allowing us to characterize the temporal evolution of MetS and identify neuro-metabolic markers delineating impaired cognitive and affective dimensions. Rats were subjected to behavioral testing assessing reactivity, anxiety-like behavior, and declarative memory, alongside longitudinal profiling of systemic metabolic and redox markers. Prolonged HFD exposure induced a progressive deterioration of anxiety-related behavior and memory performance that temporally paralleled metabolic dysregulation. Our findings reveal a progressively shifting MetS phenotype, characterized by an early predominance of neuro-metabolic alterations followed by convergence with cognitive and affective dysfunctions. Multivariate analyses showed coordinated neurometabolic coupling, whereby cognitive dysfunction co-varied with altered metabolic burden, oxidative stress, leptin signaling, and ketone body levels. Importantly, causal modelling identified distinct neurometabolic pathways underlying domain-specific vulnerability. In particular, systemic leptin signalling emerged as an integrative signal linking metabolic load and neuroendocrine dysregulation with affective dimension, whereas memory impairment was preferentially linked to redox imbalance. Overall, these results reconceptualize that cognitive impairment is structurally embedded within the progression of MetS. Rather than arising as a secondary epiphenomenon, cognitive dysfunction contributes to the organization and expansion of the neuro-metabolic phenotype.

REST supports GABAergic control of gamma-like network activity

NP 13

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Gamma oscillations arise from fast reciprocal interactions between principal neurons and GABAergic interneurons, with GABAA receptor-mediated inhibition providing the temporal precision required for synchronization yet their functions and mechanisms remain a matter of debate. Here we review the cellular and synaptic mechanisms underlying gamma oscillations and outline empirical questions and controversial conceptual issues. Our main points are as follows: First, gamma-band rhythmogenesis is inextricably tied to perisomatic inhibition. Second, gamma oscillations are short-lived and typically emerge from the coordinated interaction of excitation and inhibition, which can be detected as local field potentials. Third, gamma rhythm typically concurs with irregular firing of single neurons, and the network frequency of gamma oscillations varies extensively depending on the underlying mechanism. To document gamma oscillations, efforts should be made to distinguish them from mere increases of gamma-band power and/or increased spiking activity. Fourth, the magnitude of gamma oscillation is modulated by slower rhythms. Such cross-frequency coupling may serve to couple active patches of cortical circuits. Because of their ubiquitous nature and strong correlation with the “operational modes” of local circuits, gamma oscillations continue to provide important clues about neuronal population dynamics in health and disease. REST/NRSF is an activity-sensitive transcriptional repressor involved in neuronal homeostasis and in the control of excitability, synaptic strength and perisomatic GABAergic inhibition thus contributing to neuronal homeostasis. However, whether GABAergic transmission is also implicated in the homeostatic action of REST/NRSF is unknown. Here, we show that hyperactivity-induced REST/NRSF activation, triggers a homeostatic rearrangement of GABAergic inhibition, with increased frequency of miniature inhibitory postsynaptic currents (IPSCs). We tested whether REST contributes to the inhibitory network state required for gamma-like activity in vitro.

Primary cortical neurons grown on multiwell microelectrode arrays were stimulated with carbachol (CCh), a muscarinic agonist classically used to induce gamma oscillations in hippocampal slices. CCh increased gamma-frequency activity and gamma-related bursting, whereas atropine abolished this response, confirming muscarinic dependence. GABAA receptor blockade eliminated gamma-frequency activity, supporting the requirement for inhibitory transmission. To probe interneuron recruitment, we used IEM-1460, an open-channel blocker preferentially targeting GluA2-lacking, Ca^{2+}-permeable AMPA receptors, which are enriched in fast-spiking interneurons and support rapid excitatory drive onto these cells yet the underlying cellular mechanisms remain poorly understood. Gamma oscillations (30 to 80 hertz). IEM-1460 suppressed gamma-related bursting, consistent with reduced excitatory recruitment of inhibitory interneurons rather than direct GABAA receptor blockade.

REST inhibition by antisense oligodeoxynucleotides significantly reduced CCh-induced gamma-like activity, indicating that REST supports the inhibitory network configuration required for rhythm generation. We are now testing whether gamma-like activity reciprocally modulates REST expression. These findings support a functional link between REST-dependent regulation of GABAergic inhibition and gamma-like network dynamics.

Lysolecithin injection in the hippocampus of guinea-pigs: focal demyelination induced in vivo

NP 14

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Demyelination occurs after traumatic injury, infections or demyelinating diseases and in post-surgical human tissue and animal models with focal epilepsy. We hypothesized that demyelination affecting the axonal impulse propagation, may also alter the extracellular potassium buffering, leading to hyperexcitability and recurrent spontaneous seizures. Depth electrodes were bilaterally implanted in dorsal hippocampi of guinea-pigs. One week after surgery, 24h/day video-EEG monitoring started and unilateral injection of lysolecithin (LL; 1-2 µl; 20 mg/ml) or NaCl 0,9% in the right CA1 was performed. Five days post-injection, the brains were extracted and sliced for immunoistochemical analysis or electrophysiological in vitro recordings. Field potentials and extracellular K+ concentration ([K+]o) shifts were simultaneously recorded close and far from the LL injection site in parasagittal slices (400 µm thick). High K+ (10 mM) was either locally or bath applied; epileptiform activity was induced by bath administration of the K+ channel blocker 4-aminopyridine (4AP; 100 µM) and 0 Mg2+. Quantitative analyses demonstrated local demyelination, astrogliosis, activated microglia, increased of OLIG2+ cells and partial neuronal loss in the injected area, that were not seen in NaCl-injected animals. In 7 out of 11 LL-injected hippocampi, in vivo video-EEG recordings demonstrated the presence of interictal epileptiform events. In vitro recordings (18 slices from 8 LL-injected animals) showed no spontaneous activities and no epileptiform discharges after high K+ applications; 4AP-0 Mg2+ perfusion induced series of spikes (14 slices; 7 animals) associated with higher [K+]o changes in sites adjacent and remote from LL injection site (respectively 3,77±0,20 mM vs 3,65±0,05 mM, 5 slices from 3 animals). LL injected in CA1 region induced demyelination that correlated with sporadic spikes in vivo and higher [K+]o increases during in vitro epileptiform activity.

POSTER

3

SESSION

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BBB-on-a-Flow: Fabrication of blood-brain barrier vessel-like structures within a millifluidic bioreactor

CNN 08

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3D bioprinting technology enables the development of functional three-dimensional constructs through the controlled, layer-by-layer deposition of cells and biomaterials, with the aim of replicating physiological tissue-like microenvironments. This capability led to widespread adoption in tissue engineering and in the bio-fabrication of in vitro tissue and organ models used to investigate physiological and pathophysiological mechanisms, as well as for drug screening applications. Despite significant advances in both technologies and biomaterials, the fabrication of complex specialized barriers remains challenging. In particular, the development of a functional and perfusable blood-brain barrier (BBB) model is critical to ensure the proper transport of nutrients, oxygen, and metabolic waste, thereby maintaining cell viability throughout the entire three-dimensional construct. Our aim is focused on the development of vessel-like structures capable of recapitulating both the physiological and morphological features of brain vessels, using 3D bioprinting model composed of GelMA Fibrin and bEnd.3 cells (murine brain endothelial cells), cultured within a fluid-dynamic bioreactor inserted in a fluid-dynamic bioreactor. PrestoBlue viability assays demonstrated a progressive increase in cell viability up to 21 days of culture. Similarly, ROS assays revealed a gradual reduction in oxidative stress conditions over time, while LDH cytotoxicity assays showed a decrease in cytotoxicity levels. The maintenance of the endothelial phenotype was confirmed by positive immunofluorescence staining for CD31. By leveraging this approach, we successfully fabricated a perfusable, vessel-like structure integrated within a millifluidic bioreactor, under continuous flow conditions.

CNN 09

Extra hand finger augmentation in congenital oligodactyly: design rationale and transcranial magnetic stimulation-based neuroplasticity assessment

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Supernumerary fingers have been proposed as augmentative tools for improving grasping ability, particularly in upper-limb impairment. Previous studies have shown the feasibility of EMG-based control interfaces for robotic extra-fingers. In parallel, non-invasive brain stimulation techniques, including Transcranial Magnetic Stimulation, have been used to investigate motor cortex excitability, intracortical inhibition, and plasticity mechanisms.
This project aims to develop a EMG-controlled supernumerary finger for a participant with congenital upper-limb malformation, characterized by the absence of the fourth and fifth fingers. The device will be based on an open-source 3D-printable project adapted through CAD redesign. The system integrates a wearable forearm-mounted actuation unit, tendon-based transmission, and surface EMG acquisition from the flexor carpi ulnaris, selected as a voluntary control source for finger flexion–extension.
The main objective focuses on the use of the robotic finger as an experimental platform to investigate motor adaptation and cortical reorganization. The participant will perform structured gross-grasping tasks before and after a training protocol. Task performances will be evaluated together with neurophysiological markers obtained through TMS, including motor threshold, cortical representation of the target muscle, and cortical silent period. The working hypothesis is that repeated voluntary control of the additional robotic effector may induce measurable changes in corticospinal excitability and intracortical inhibition, supporting the integration of the device into the user's motor repertoire. This project proposes a translational framework for studying embodiment and neuroplasticity in assistive technologies. The work may contribute to the design of adaptive neuroprosthetic systems and to the development of experimental protocols for evaluating human–robot integration in individuals with congenital or acquired hand differences.

Social reactivity and socio-sensory functioning in autism: a multimodal study integrating eye-tracking, autonomic measures and EEG

CNN 10

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Autism spectrum disorder is characterized by marked heterogeneity in social attention, sensory responsivity, and physiological regulation. Social reactivity can be conceptualized as the dynamic interaction between orienting to socially salient stimuli, visual engagement, and autonomic and neurophysiological modulation during social information processing.
This study aims to investigate social reactivity and socio-sensory functioning in autistic children through a multimodal protocol integrating eye-tracking, autonomic measures, caregiver-report questionnaires, neuropsychological assessment, and exploratory electroencephalography/event-related potentials (EEG/ERP). Children aged 7–12 years with autism spectrum disorder and typically developing peers will be enrolled in an observational comparative design.
The primary eye-tracking outcome will be the Eye-to-Face proportion, defined as fixation time on the eyes/upper-face region relative to total fixation time on the face during standardized facial stimuli presentation. Secondary outcomes will include face fixation probability, dwell proportion, latency to first fixation, mean fixation duration, and gaze transitions between areas of interest. During the task, heart rate variability and galvanic skin response will be recorded as indices of autonomic regulation and arousal. Caregiver-report and neuropsychological measures will assess sensory profile, sleep, adaptive functioning, visual attention, emotion comprehension, and theory of mind. In an exploratory subgroup, EEG/ERP responses will be examined, focusing on N170, P300, and Late Positive Potential components.
By integrating attentional, autonomic, sensory, adaptive, and neurophysiological measures, the study aims to characterize individual differences in socio-sensory reactivity and identify multimodal profiles of social functioning in autism.

Stress, sleep quality, and educational migration context among international medical students

CNN 11

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International medical students may experience sleep problems not only because of academic workload but also due to stressors related to educational migration. This study examined sleep quality and stress among international medical students, emphasizing how migration-related context may shape interpretation of Pittsburgh Sleep Quality Index (PSQI) and Medical Student Stressor Questionnaire (MSSQ) findings. Cross-sectional PSQI and MSSQ data were analyzed from 200 students with complete information on sleep quality, stress, age, gender, and study level. Study level was classified as preclinical or clinical. Descriptive statistics, group comparisons, correlation analyses, regression models, and mediation/moderation frameworks were used to examine relationships between study levels, stress, and sleep quality. Poor sleep was highly prevalent, with a mean global PSQI score of 8.44 (SD 3.62) and 83.7% classified as poor sleepers. Stress was moderate across all MSSQ domains. Female students reported higher stress across several domains, but global PSQI did not significantly differ by gender. Preclinical and clinical students did not differ significantly in global PSQI. Stress was consistently associated with poorer sleep quality, while study level, age, and gender were not significant predictors. Mediation analysis did not support an indirect pathway from study level to PSQI through stress, because study level did not meaningfully predict MSSQ total. Poor sleep quality was widespread and closely linked to stress. The absence of strong study-level effects suggests that sleep impairment may reflect a broader vulnerability shared across training stages. Educational migration factors, including language barriers, adaptation demands, social separation, and financial pressures, should be measured in future studies to clarify their contribution to stress-related sleep impairment.

Epigenetic regulation in Krabbe disease: microRNAs as putative biomarkers and therapeutic targets

ND 30

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Krabbe disease (KD) is a rare autosomal recessive leukodystrophy classified among lysosomal storage disorders. Mutations in the GALC gene lead to deficient activity of galactocerebrosidase, a lysosomal enzyme involved in sphingolipid metabolism. Consequently, psychosine accumulates in both the peripheral and central nervous systems (PNS and CNS), exerting cytotoxic effects that promote demyelination, astrogliosis, and axonal degeneration. To date, no curative therapy is available for KD. Hematopoietic stem cell transplantation may slow disease progression only in presymptomatic patients, but diagnosis frequently occurs too late. Recently, microRNAs (miRNAs), a class of small non-coding RNAs involved in post-transcriptional gene regulation, have emerged as promising biomarkers and therapeutic targets in several neurodegenerative disorders.

The aim of this study was to identify miRNA-based biomarkers and therapeutic targets in KD through bioinformatic analysis of the miRNA–mRNA regulatory network. Candidate target genes were selected through literature mining and a computational approach. Gene Ontology and pathway enrichment analyses identified biological processes associated with CNS myelination, oligodendrocyte differentiation, and neuronal ensheathment. miRNA–mRNA interactions were predicted using miRTarBase and miRDB,and revealed several CNS-expressed miRNAs involved in pathways related to neurodegeneration, apoptosis, lysosomal dysfunction, inflammasome activation, and lipid/sphingolipid metabolism. Network analysis identified GALC, hsa-let-7a, and APLNR as major regulatory hubs. Furthermore, several miRNAs predicted to regulate GALC have also been associated with glioma and glioblastoma. Overall, these findings support the role of miRNAs as potential epigenetic regulators and candidate biomarkers in KD, providing new insights into disease monitoring and targeted therapeutic strategies.

Targeting VDAC1 to prevent amyloid-β-mediated mitochondrial dysfunction in neurodegenerative disorders

ND 31

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Voltage-Dependent Anion-selective Channel 1 (VDAC1) is the most abundant pore-forming protein of the outer mitochondrial membrane (OMM), where it mediates the voltage-dependent exchange of ions (Mg, Ca, Cl) and small metabolites (ATP/ADP, Krebs' cycle intermediates) between the cytosol and mitochondria, showing a marked preference for negatively charged species under physiological conditions. Several studies have identified VDAC1 as the principal mitochondrial binding site for misfolded proteins in neurodegenerative diseases, including intracellular amyloid-β (Aβ) aggregates in Alzheimer's disease (AD). This interaction contributes to mitochondrial dysfunction by enhancing oxidative stress and promoting apoptotic pathways. Here, we demonstrate that Aβ oligomers profoundly alter both VDAC1 electrophysiological properties and mitochondrial function through a combined approach based on Planar Lipid Bilayer recordings and High-Resolution Respirometry. Specifically, Aβ oligomers modify VDAC1 conductance in its open state, reduce channel voltage dependence, and markedly alter ion selectivity by promoting the emergence of a cation-selective state. Consistent with these findings, exposure to Aβ oligomers impaired mitochondrial respiration in neuroblastoma cells and in primary cortical neurons derived from rat embryos, selectively affecting oxygen consumption associated with ADP phosphorylation. Notably, the toxic effects induced by Aβ oligomers were strongly counteracted by NHK1, a small VDAC1-targeting peptide derived from Hexokinase 1 (HK1), which binds VDAC1 and modulates channel activity. Similar results have been observed in the presence of fibroblast from Amyotrophic lateral sclerosis mice. Collectively, these findings identify VDAC1 as a key mediator of mitochondrial dysfunction in AD and support its potential as a therapeutic target for the development of innovative strategies against this neurodegenerative disorder.

Exploring the vault complex in Alzheimer's disease: first characterization of MVP, VPARP, TEP1 and vtRNA expression in Aβ1-42-treated differentiated SH-SY5Y cells

ND 32

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder driven by amyloid-beta (Aβ) accumulation and neuronal dysfunction. Vault ribonucleoprotein particles consist of three proteins - major vault protein (MVP), vault poly(ADP-ribose) polymerase (VPARP), and telomerase-associated protein 1 (TEP1) - and small non-coding vault RNAs (vtRNAs). Vault components regulate cellular stress responses, autophagy, and RNA stability; notably, MVP modulates apoptotic resistance and oxidative stress through B-cell lymphoma 2 (Bcl-2)/c-Jun N-terminal kinase (JNK) and phosphoinositide 3-kinase (PI3K)/Akt signalling. However, their expression remains poorly defined in Aβ-driven neuronal injury. We hypothesize that Aβ1-42 alters vault complex expression as an early cellular response to amyloid-induced neuronal damage. Therefore, this study aims to characterize vault complex expression alongside markers of oxidative stress, apoptosis, and autophagy in a neuronal model of AD. Human SH-SY5Y neuroblastoma cells will be differentiated with all-trans retinoic acid and exposed for 24 hours to 2 µM or 5 µM Aβ1-42 preparations pre-incubated at 4°C (oligomeric) or 37°C (fibrillar). MVP, VPARP, and TEP1 protein levels will be assessed by Western blot; vtRNA1-1, vtRNA1-2, vtRNA1-3, and vtRNA2-1 expression by reverse transcription quantitative PCR (RT-qPCR); oxidative stress by reactive oxygen species (ROS), lipid peroxidation, protein oxidation, superoxide dismutase (SOD)/catalase activity, and reduced/oxidised glutathione (GSH/GSSG) ratio; apoptosis by cleaved vs. uncleaved caspase-3 and Bcl-2 levels; and autophagy by LC3 (light chain 3) and sequestosome-1 (p62) levels, vs. vehicle-treated controls. To our knowledge, this is the first evaluation of vault complex expression under distinct Aβ1-42 assembly conditions in a neuronal model, potentially uncovering novel AD mechanisms and identifying vault components as candidate biomarkers or therapeutic targets.

How could the sensory dysfunction be restored in the 3xTg-AD mouse model of Alzheimer's Disease?

ND 33

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Alzheimer's disease (AD) is the fifth leading cause of death worldwide and a progressive neurodegenerative disorder primarily characterized by cognitive deficits and memory loss. However, emerging evidence suggests that sensory systems are affected long before the onset of hallmark clinical symptoms. Among these, visual and olfactory impairments have been identified as potential early biomarkers of AD progression, reflecting underlying neuropathology within the brain's sensory circuits. Its distinctive pathological features include beta-amyloid (Aß) plaques and intracellular neurofibrillary tangles of hyperphosphorylated tau protein.
Here, we aim to dissect the mechanisms that drive neurodegeneration in the individual sensory systems, such as the visual and olfactory systems in the triple-transgenic mouse model of Alzheimer's disease, that exhibits both Aβ and tau-pathology.
Initially, behavioral characterization, including fear-conditioning, odor discrimination, and multi-sensory tests, are performed on control mice at ages corresponding to the neurodegenerative stages of the 3xTg-AD mice, in which the accumulation of Aβ has been observed.
A decline in associative and working memory was found to correlate with sensory impairments in aged animals compared to young controls. Then, we will evaluate the sensory integration together with cognitive impairment in 3xTg-AD mice performing different behavioral tests in response to different stimuli (e.g visual, olfactory). Indeed, 7-tesla magnetic resonance and in vivo electrophysiology, will be performed to study the brain morphology and neural activities in controls compared to 3xTg-AD mice.
Subsequently, to further dissect the sensory system impairments, we compared the retinal layers atrophy observed in AD patients using Optical Coherence Tomography, together with odor discrimination tests, with the data obtained from 3xTg-AD.

Transcriptional Master Regulators and novel genetic determinants of Parkinson's disease identified by multi-omics network analysis

ND 34

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The multifactorial nature of Parkinson's Disease (PD), whose genetic basis remains not fully understood, makes this complex neurodegenerative disorder an ideal candidate for multi-omics integration studies. In this context, by integrating transcriptomic, proteomic and genomic data, we reconstructed the Gene Regulatory Network of the PD Substantia Nigra (ARACNe3). The analysis of transcription factor (TF)-associated subnetworks (NaRnEA), identified key Master Regulators (MRs) that drive gene expression deregulation in the brain region most affected in PD pathogenesis. To biologically validate these bioinformatic results, we confirmed MR cellular expression by analyzing their protein abundance through a proteomic dataset obtained from dopaminergic neurons differentiated from iPSCs of five healthy subject (HS) and six PD patients. Several MRs displayed significantly altered protein abundance between groups, supporting the network-based bioinformatic predictions. Furthermore, to evaluate whether our approach could detect novel PD-associated genes, we selected a panel of 123 genes, including MRs and most connected TFs from the ARACNe3 network, from Whole Exome Sequencing (WES) data of 804 PD patients and 664 HS samples. We identified and tested 1049 common variants (MAF > 0.01) through association analysis, revealing 14 novel genetic variants in 10 genes significantly associated with PD risk onset. Notably, several of these genes, including RORC, YY1 and SATB2, have previously been implicated in PD-related mechanisms. Pathway-level analysis (PROGENy) revealed dysregulation of Hypoxia, VEGF, and Estrogen signaling when comparing PD vs HS. Lastly, the MR-pathway correlation analysis identified significant rewiring of MR-pathway interactions between conditions, suggesting altered transcriptional coordination in PD. Overall, this multi-omics integration approach proved effective in identifying novel genetic determinants and regulatory mechanisms underlying PD pathogenesis.

ND 35

Nrf2/NOX2 pathway dysregulation and oxidative stress biomarkers in Gaucher disease–associated parkinsonism: insights into a potential therapeutic target

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Parkinson's disease (PD) is the second most prevalent neurodegenerative disorder, yet its underlying genetic and molecular mechanisms remain incompletely understood. Variants in the Gamma-Aminobutyric Acid (GBA) gene, encoding the lysosomal enzyme glucocerebrosidase, are not only responsible for Gaucher disease (GD) but also represent a significant genetic risk factor for PD, contributing to lysosomal dysfunction, oxidative stress and autophagy impairment. Among the key regulators of redox homeostasis, the Nrf2/NOX2 signalling axis has emerged as a pivotal pathway in the modulation of neuroinflammation and neurodegeneration. This study aims to explore the pathogenic link between GBA mutations and PD, focusing on the redox imbalance and the role of Nrf2 signalling in an in vivo Gba D409V knock-in (KI) mouse model, compared to wild-type (WT) C57BL/6J controls. Animals 8-weeks old were evaluated over a 3-month period, with tissue and behavioural assessments conducted at 7, 14, 30, 60 and 90 days. Early timepoints (7 and 14 days) did not reveal significant changes in behavioural performance, expression of PD-related markers (Tyrosine hydroxylase, Dopamine Transporter, α-synuclein), or oxidative stress indicators, including Nuclear factor erythroid 2-related factor 2 (Nrf2), NADPH oxidase 2 (NOX2), malondialdehyde (MDA) and nitrate/nitrite levels. However, at 30, 60 and especially 90 days, significant alterations emerged, particularly a disrupted Nrf2/NOX2 balance, accompanied by molecular and biochemical signatures of oxidative stress. These findings suggest a time-dependent progression of oxidative alterations in this GD model and support the role of GBA variants in promoting neurodegenerative processes. Unravelling these mechanisms is essential for the identification of early biomarkers and may offer new therapeutic insights for GBA1-associated PD.

Effects of ATM alteration on microglial responses in Ataxia Telangectasia

ND 36

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Ataxia-telangiectasia (A-T) is an autosomal recessive neurodegenerative disorder characterized by loss-of function of the ATM gene, a regulator of the DNA damage response and cellular redox homeostasis. ATM deficiency has been also correlated with neuroinflammation and progressive cerebellar degeneration. In addition, alterations in both cortex and hippocampus have also been described and glial cells, particularly microglia, are suggested to actively contribute to neuronal dysfunction. We first analyzed the murine A-T model to identify microglial activation, neuronal integrity, and astrocytic responses in brain tissue from young mice (12 days-old pups). By Western blot and immunohistochemistry analyses, in both hippocampus and cortex of A-T animals, protein levels of GFAP, a marker of astrocytes, and the deacetylase SIRT1 were reduced, whereas synaptophysin, used as a neuronal marker, was not modified. In parallel, in MC3 human microglial cells, H_2O_2 and UVC, used to induce DNA damage, significantly increased pCHK2 nuclear levels, a key cyclin kinase in the ATM pathway, an effect that was prevented in the presence of ATM kinase inhibition by KU 55933 (10 µM for 1h). However, KU-55933-induced inhibition of ATM did not alter the increase of ROS in response to 200 µM H_2O_2, up to 30 min, while increasing the presence of micronuclei, suggestive of DNA damage. In parallel, SIRT1 nuclear translocation was reduced by KU-55933. Finally, preliminary data suggest that treatment with drugs able to act on the ATM downstream signalling, identified by a bioinformatics analysis, are able to partially rescue the impaired ATM function. In conclusion, we have established an in vitro model to study ATM dysfunction in microglia to investigate therapeutic interventions downstream of the lost DNA repairing activity of ATM.

Modeling riboflavin transporter deficiency using patient-derived iPSC motor neurons reveals mitophagy defects

ND 37

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Riboflavin Transporter Deficiency (RTD) is a rare, neurological disorder characterized by hearing loss and sensory ataxia associated with spinal motor neuron (MN) degeneration. The disease is caused by loss of function mutations in SLC52A2 or SLC52A3 genes, respectively encoding riboflavin transporters hRFT2 and hRFT3. As Riboflavin (RF) is the precursor of the coenzymes FMN and FAD, their abnormally low levels result in defective functionality of flavoproteins, which are involved in cellular bioenergetics and cell survival processes, namely autophagy. Given the inadequacy of in vivo models recapitulating RTD phenotype and considering the difficulty to access the tissue of interest, we proposed induced pluripotent stem cells (iPSCs) as a human in vitro model useful to discover pathogenetic mechanisms of RTD cells. More specifically we perform combined confocal and immunoblotting analyses aimed at characterizing the patho-mechanisms associated to RTD. Confocal analyses and WB experiments demonstrate a reduced activation of PINK1/Parkin mediated mitophagy in RTD iPSCs and derived MNs, accompanied by changes in mitochondrial morphology in response to mitophagy initiation. Indeed, Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) reveal an increased number of rounded mitochondria in RTD cells. Consistent with these results, we explored the distribution and levels of MFN2 and pDRP1 demonstrating higher rates of mitochondrial fission in RTD cells, suggesting an imbalance in mitochondrial dynamics. Furthermore, Seahorse analyses revealed impaired mitochondrial function and alterated bioenergetics in RTD patient-derived cells. Additionally, we demonstrated an abnormal activation of the autophagic process by WB experiments for LC3 and p62. Overall, our work contributes to expanding the knowledge on multiple cellular features associated to RTD phenotype, supporting the role played by mitophagy in its pathogenesis.

Dysregulation of pro-regenerative program emerges as a candidate mechanism of small fiber neuropathy

ND 38

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Aδ and C fibers, collectively referred as small nerve fibers, are the main peripheral neurons responsible for thermal and mechanical sensation, nociception, and autonomic responses. By interacting with skin-resident cells and sensory structures, they initiate and propagate sensory stimuli and pain. Although these fibers exhibit marked plasticity throughout life, they are susceptible to degeneration, which can result in Small Fiber Neuropathy (SFN), a complex disorder characterized by sensory impairment, allodynia and hyperalgesia. Despite its huge impact on patients' life quality, disease-modifying treatments for SFN are still lacking, and the molecular mechanisms behind SFN pathogenesis are poorly understood. To investigate the pathological changes in a human-based model, we combined morphological and molecular analyses of skin biopsies and iPSC-derived sensory neurons (iSNs) obtained from SFN patients and age- and sex-matched healthy controls. Confocal microscopy coupled with 3D-reconstruction of small nerve fibers in patients' skin biopsies revealed a reduction in Acetylated microtubule (AcMT)-positive fibers. AcMTs are a specialized subset of microtubules that supports axonal transport and regeneration by enhancing resistance to mechanical breakdown. In addition, we observed a significant decrease in phosphorylated MAP1B, a microtubule-associated protein involved in pro-regenerative programs. These findings were recapitulated in patient-iSN, which also shown a significantly higher number of axons with varicosities, an early sign of axonal degeneration often linked to impaired intracellular organelle transport. Consistently, live-cell imaging demonstrated that mitochondria in patient-derived SNs were less motile and smaller than in control cells. This study provides new insights into SFN pathophysiology and a translational link between patient tissue and in vitro model, strengthening relevance of iSNs for mechanistic studies and development of tailored therapy.

Plasma and CSF biomarker profiling in cerebral amyloid angiopathy: correlations with clinical and neuroradiological features

ND 39

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Cerebral amyloid angiopathy (CAA) is a cerebral small vessel disease (CSVD) characterized by β-amyloid deposition in cortical and leptomeningeal arterioles, leading to vessel fragility, lobar intracerebral haemorrhage, and cognitive decline. Differentiating CAA from other CSVD, such as deep perforating arteriopathy (DPA), remains challenging, and reliable fluid biomarkers reflecting disease burden, neurovascular damage, and prognosis are still lacking. Using Single Molecule Array (Simoa), we analyzed plasma and cerebrospinal fluid (CSF) samples from patients with CAA, DPA, and healthy controls, quantifying Aβ40, Aβ42, neurofilament light chain (NfL), and glial fibrillary acidic protein (GFAP). Biomarker levels were correlated with clinical and neuroradiological variables at baseline (T0), as well as with clinical history. Significant associations emerged between circulating biomarkers and disease burden indicators in CAA, including white matter hyperintensities (WMHs), modified Rankin Scale (mRS), and MoCA score. Plasma NfL correlated with prior clinical events, supporting its role as a marker of disease severity, while CSF Aβ40 correlated with future clinical events, suggesting prognostic value for disease progression and recurrence risk. Plasma and CSF biomarker profiles showed a moderate but significant correlation, indicating that peripheral measures may reflect central pathological processes. Furthermore, Aβ40 and Aβ42 significantly distinguished CAA from both healthy controls and DPA, supporting their diagnostic utility in the differential characterization of CSVD. Overall, these findings support the clinical relevance of plasma and CSF biomarkers in CAA and highlight Simoa-based assays as promising tools for disease stratification, monitoring, and differential diagnosis between amyloid and non-amyloid angiopathies.

Biochemical and functional characterization of a β4GalNT1 knock-out murine neuroblastoma cell model

ND 40

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The ganglioside GM1 is a crucial component of neuronal membranes involved in the regulation of intracellular signaling pathways through its oligosaccharide moiety. GM1 deficiency has been linked to neurodegenerative conditions, including sporadic Parkinson's disease. Reduced GM1 availability at the plasma membrane has been associated with impaired neurotrophic signaling, mitochondrial dysfunction, oxidative stress and excitotoxicity, ultimately promoting neuronal loss. Here, we characterized a homozygous β4GalNAcT1-KO Neuro2a (N2a) murine neuroblastoma cell line to investigate the consequences of GM1 depletion. Lipid analysis confirmed complete loss of GM1 and complex gangliosides, accompanied by accumulation of biosynthetic precursors, mainly GM3, indicating membrane lipid remodeling. Morphological assessment revealed a more differentiated phenotype in KO cells compared to wild-type (WT) controls. Consistently, KO cells displayed enhanced hyperactivation of the TrkA/MAPK signaling pathway. Metabolic analyses showed upregulation of mitochondrial respiratory chain components and increased ATP production. Comparable levels of the mitochondrial marker TOM20 between WT and KO cells indicate increased mitochondrial activity rather than mitochondrial biogenesis. Increased metabolic activity was associated with mitochondrial oxidative stress and activation of p38 kinase. Overall, β4GalNAcT1 ablation and GM1 deficiency alter membrane lipid composition, mitochondrial function and redox balance, supporting a role for GM1 in neuronal homeostasis and neurodegeneration.

Interleukin-9 induces calretinin expression in spinal neurons and may restore its downregulation in amyotrophic lateral sclerosis

ND 42

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Amyotrophic lateral sclerosis (ALS) is a relentless neurodegenerative disease. Neuroinflammation plays a role in ALS pathogenesis and anti-inflammatory molecules may therefore provide beneficial effects. Interleukin-9 (IL-9) is a cytokine with protective properties in multiple sclerosis. On this basis, we hypothesize that IL-9 may also exert a beneficial role in ALS. First, IL-9 levels were measured in the cerebrospinal fluid (CSF) of ALS patients and high levels are associated with less severe neurodegeneration and slower disease progression. Then, to investigate the underlying mechanisms, the expression of the IL-9 receptor (IL-9R) was examined in the spinal cord, the primary site of motor neuron degeneration in ALS. Both human and mouse spinal cord tissues contain IL-9-responsive cells (IL-9R+), predominantly neurons. In primary spinal cord cultures, IL-9 activates STAT and AKT signalling pathways and transcriptomic analysis reveals that IL-9 upregulates genes involved in synaptic function, including Calb2, encoding calretinin (CR), a calcium-binding protein that regulates neuronal excitability. The relevance of these findings was assessed in the SOD1-G93A ALS mouse model, where both Calb2 and CR expression are reduced in spinal interneurons compared with healthy littermates. In parallel, ALS patients show higher concentrations of soluble CR in the CSF than individuals with other neurodegenerative diseases. Moreover, soluble CR levels positively correlate with neurofilament concentrations, a marker of neurodegeneration. These findings identify a previously unrecognized role of IL-9 in spinal neurons through the regulation of Calb2 and CR expression. The reduced expression of these molecules in ALS interneurons suggests their involvement in disease pathophysiology, while increased soluble CR levels support its potential as a novel ALS biomarker. Furthermore, the ability of IL-9 to restore Calb2 and CR expression highlights its potential as a therapeutic target.

Membrane and mitochondrial defects drive sensory neuron dysfunction in FLVCR1 related neuropathies

ND 43

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FLVCR1-related sensory neuropathies are rare childhood-onset disorders characterized by progressive degeneration of sensory neurons and photoreceptors, leading to sensory ataxia, pain insensitivity, and retinitis pigmentosa. Feline Leukemia Virus Subgroup C Receptor 1 (FLVCR1) encodes a transporter involved in choline and ethanolamine transport and regulation of mitochondria-associated membranes (MAMs). However, mechanisms linking FLVCR1 dysfunction to selective sensory neuron vulnerability remain unclear. To investigate disease mechanisms, we generated induced pluripotent stem cells (iPSCs) from patient fibroblasts carrying pathogenic FLVCR1 variants and differentiated them into sensory neurons (SNs). In parallel, we generated a genetically engineered FLVCR1 mutant iPSC line. Analyses included biochemical assays, Western blotting, mitochondrial functional assays, and confocal imaging. Patient-derived cells showed reduced intracellular choline (−40% vs controls), altered membrane lipids, including reduced phosphatidylcholine and ganglioside GM1, and impaired membrane fluidity. These defects were associated with mitochondrial dysfunction, including ~50% reduction in electron transport chain activity and ATP production. Consistently, sensory neurons showed impaired neurotrophic signaling, reduced neurite outgrowth, and altered network organization. The engineered mutant recapitulated the same phenotype. Importantly, choline supplementation rescued these abnormalities, restoring membrane composition, improving mitochondrial function, and enhancing neurite outgrowth and neurotrophic signaling, with full pathway recovery. Overall, our findings identify a dual pathogenic mechanism in which FLVCR1 mutations disrupt membrane homeostasis and mitochondrial bioenergetics, compromising sensory neuron integrity. Our model recapitulates key metabolic and structural features of FLVCR1 neuropathy and highlights choline supplementation as a promising therapeutic strategy.

CYFIP1 haploinsufficiency drives altered contactomics, reactive phenotype and metabolic reprogramming of microglia

NDEV 13

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Microglia are resident immune cells that maintain brain homeostasis throughout life. Dysregulation of microglial function alters synaptic pruning, brain circuitry, contributing to Autism Spectrum Disorder (ASD), a multifactorial neurodevelopmental disorder (NDD). The Cytoplasmic FMR1-Interacting Protein 1 (CYFIP1) gene, linked to ASD, is highly expressed in microglia and regulates phagocytosis and migration. Recently, microglial contactomics with neuronal somatic and synaptic compartments has emerged as a novel mechanism controlling neuronal activity, although its role in neurodevelopment remains unclear. To investigate CYFIP1 function in microglia, we analyzed microglial density, morphology, and contactomics in the cortex of young Cyfip1 heterozygous (Cyfip1+/−) mice under basal conditions and after an immune challenge. Image analysis showed increased microglial density and arborization in Cyfip1+/− cortices, along with reduced neuronal somatic contacts compared to controls. Moreover, a 24-hour lipopolysaccharide (LPS) treatment reproduced Cyfip1+/−-induced microgliosis in WT mice, further reduced neuronal somatic contacts in a genotype-dependent manner, and restored Cyfip1+/− microglial morphology to a WT-like state. To obtain a comprehensive microglial profile, we performed a proteomic analysis of magnetically isolated cortical microglia. Cyfip1+/− microglia displayed a reactive/chronically activated profile, characterized by upregulation of inflammatory and immune-related proteins overlapping with those in LPS-treated WT microglia. Several upregulated proteins were also linked to cytoskeletal remodeling and cell proliferation, consistent with the observed changes in density and morphology. Finally, Cyfip1+/− microglia showed alterations in lipid metabolism, potentially associated with peroxisomal dysfunction and plasmalogen biosynthesis, a poorly explored pathway linking microglial biology to NDDs and thus a putative molecular target.

Caenorhabditis elegans as an integrated in vivo platform for dissecting neuronal function and dysfunction and enabling therapeutic discovery in neuroscience

NDEV 14

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Caenorhabditis elegans is a powerful 3R-compliant model to dissect molecular mechanisms underlying neurological diseases. Its low cost, short life cycle, genetic tractability, and suitability for large-scale studies make it a valuable tool for translational neuroscience. Despite its simplicity, it shares conserved molecular pathways with mammals, and ~70% of human disease genes have worm orthologues. Its nervous system, composed of only 302 neurons, is fully mapped and retains key features of mammalian synapses.

In our laboratory, we generated transgenic and CRISPR/Cas9-edited models to investigate the pathogenic mechanisms underlying neurodevelopmental disorders, including developmental and epileptic encephalopathy and movement disorders, and explore therapeutic strategies. We functionally dissected synaptic transmission using levamisole (a nicotinic acetylcholine receptor agonist) and aldicarb (an acetylcholinesterase inhibitor), enabling discrimination between postsynaptic and presynaptic defects. An automated tracking system was used to assess the locomotor behavior, while learning assays were performed following conditioning with high doses of benzaldehyde. Neuronal activity was recorded in vivo at single-cell resolution in head ganglia neurons using a custom microfluidic device in pan-neuronally calcium indicator–expressing models. In parallel, we established a FRET-based imaging platform to quantify cAMP in pharyngeal neurons. Finally, we applied these platforms to drug screening and recently identified caffeine as a potential modulator of GNAO1-related dyskinesia, laying the basis for future clinical investigation.

Overall, our findings highlight C. elegans as a suitable in vivo platform for modelling neurological diseases, dissecting disease mechanisms, and enabling therapeutic discovery through integrated functional and behavioral assays, providing a complementary approach that reduces the need for vertebrate animal models in neuroscience research.

Ion channel repertoire shapes early spontaneous activity and subtype identity in developing cortical neurons

NDEV 15

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Cortical development emerges from coordinated interactions between gene expression programs, environmental cues, and neuronal activity. While spontaneous activity is known to support neurogenesis and circuit assembly, how molecular features of developing neurons relate to emerging activity patterns remains poorly understood.
Here, we explore whether excitatory projection neuron subtypes exhibit distinct activity-related gene expression during development, and how these molecular configurations evolve over time. Using longitudinal single-cell transcriptomic analyses, we identify dynamic and subtype-associated trajectories of ion channel expression across developing projection neuron populations. Our data demonstrate that specific ion channel repertoires are associated with distinctive intrinsic activity properties from their earliest developmental stages. Integrating genetic, pharmacological, two-photon imaging, and transcriptomic approaches, we investigate the role of the neuronal “channellome” in functional maturation and structural differentiation of selected neuronal subtypes, identifying a potential molecular link between cortical neuronal identity and early activity patterns.

Influence of microenvironmental factors on hippocampal neural stem cell fate in vitro

NDEV 16

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Neural stem cells (NSCs) have been studied for over three decades to elucidate their molecular and biological properties, as well as their therapeutic potential. However, their ability to generate in vitro functional replicas of either the developing or adult brain remains limited compared to in vivo neurogenesis. This discrepancy is largely attributed to the difficulty of reproducing the native niche microenvironment in vitro, which comprises paracrine signaling, extracellular matrix (ECM) interactions, and cell–cell contacts that play a crucial role in regulating NSC fate. The present study investigates the ability of rat embryonic hippocampal NSCs to differentiate in vitro into neurons and glial cells, with the aim of generating a functional hippocampal tissue model. To this end, three key aspects of the microenvironment are explored: culture dimensionality, temporal regulation of growth factor exposure, and extracellular matrix composition. Specifically, a differentiation protocol is designed to sequentially deliver major morphogens involved in embryonic neural development, following a defined temporal order. This approach is compared to a standard BDNF-based differentiation protocol in both 2D NSC cultures and 3D NSC spheroids, the latter further supplemented with an alginate-based biomimetic ECM. Biomarker expression is assessed via immunohistochemistry and RT-PCR, while neuronal functionality is evaluated using calcium imaging and microelectrode array electrophysiology. Our results indicate that the proposed protocol promotes neuronal differentiation, functional maturation, and the emergence of hippocampal signatures, while the inclusion of a third dimension and exogenous ECM significantly influences tissue morphogenesis. Overall, the integration of multiple microenvironmental cues appears critical for effectively modulating NSC fate and for developing in vitro models of brain development and disease.

Listening to neurons: TREM2-dependent microglial support of neuronal fitness

NDEV 17

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Despite the traditional view of the brain as an immunologically privileged organ, continuous crosstalk between microglia — the resident immune cells of the central nervous system — and neurons is essential for the development and maintenance of neuronal circuits. Disruptions in this bidirectional communication have been linked to several neurodevelopmental disorders, including autism and schizophrenia. Among microglial receptors, the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) has emerged as a key regulator of microglial function, influencing synaptic refinement and neuronal metabolic homeostasis. However, the mechanisms through which microglia sense neuronal status and adapt their supportive functions remain poorly understood. In this project, we investigate how microglia detect the physiological and metabolic state of neurons and how they respond to sustain neuronal health and maturation. Using trans-well systems, microglial conditioned media, released factors and microglia–neuron co-culture models, we explored the contribution of soluble factors and direct cell-to-cell interactions during early neuronal development. In parallel, we aim to identify the downstream neuronal pathways activated in response to microglial signalling. Our findings show that wild-type microglia promote neuronal maturation and metabolic fitness, whereas TREM2-deficient microglia display an impaired ability to sense and support neuronal needs. Moreover, metabolically impaired TREM2 knockout neurons partially recover their phenotype when exposed to a supportive extracellular environment, highlighting the critical role of microglial-derived cues in neuronal resilience. Overall, this study provides new insights into microglia–neuron communication and identifies TREM2-dependent mechanisms as potential therapeutic targets for neurological disorders associated with microglial dysfunction.

A tri-polymer nanoplastic mixture triggers gut-brain axis dysfunction: focus on inflammation and synaptic plasticity

NI 17

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Nanoplastics (NPs) represent an emerging threat to human health due to their nanoscale dimensions, which enable them to cross biological barriers and accumulate ubiquitously in tissues and organs. Their widespread presence in the human body has recently led to the concept of “Homo plasticus”, highlighting the pervasive nature of chronic plastic exposure in daily life. Here, we investigated the sex-related biological effects of short-term oral exposure to a mixture of three polymeric NPs, which are primarily responsible for plastic pollution in the environment. Male and female C57BL/6J mice received by oral gavage a mixture of 50 nm polystyrene, polypropylene, and polyethylene nanoparticles (1:1:1; 2.5 mg/kg) daily for 7 days to assess the impact of subacute exposure on the gut–brain axis. Colon and hippocampal tissues were collected for RT-qPCR and Western blot analyses. NP exposure modulated both intestinal and central barrier-related parameters, as shown by changes in colonic tight junctions and blood-brain barrier markers; in particular, mRNA and protein expression of occludin were altered in the hippocampus of both sexes. At the same time, NPs promoted inflammatory responses in the colon and CNS. Notably, sex-dependent differences emerged, with TNF-α upregulation predominantly observed in females, while IL-1β expression was mainly increased in males, both peripherally and centrally. Furthermore, NPs impaired synaptic plasticity, altering BDNF-TrkB pathway and reducing post-synaptic marker PSD-95 in hippocampus of both sexes.
In conclusion, a short exposure of a low-dosage mixture of three polymeric NPs acts as an early trigger of gut-brain axis dysfunction, leading to peripheral and central inflammation, impairing blood-brain barrier integrity and synaptic plasticity. Importantly, our results reveal early sex-specific differences, underscoring the need for further studies to elucidate mechanisms underlying NP-induced neurotoxicity in a sex-dependent manner.

Crosstalk between APOE ε4 genotype, PPARγ, and astrocyte reactivity in Alzheimer's disease progression

NI 18

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The Peroxisome Proliferator-Activated Receptor γ (PPARγ) is a promising therapeutic target for Alzheimer's disease (AD) due to its anti-inflammatory and neuroprotective functions. Although PPARγ agonists have shown promising effects in preclinical models, clinical trials revealed limited efficacy for Apolipoprotein E ε4 (APOE4) allele carriers. As the principal genetic risk factor for sporadic AD, APOE4 is associated with profound alterations in astrocytes, the main APOE producers in the brain, including the acquisition of a reactive and pro-inflammatory phenotype and contributing to AD pathology. The molecular mechanisms underlying this phenotype remain poorly understood. In our work, we employed three experimental models: i) isogenic APOE3 and APOE4 human-induced Pluripotent Stem Cells (hiPSCs)-derived astrocytes. ii) immortalized APOE3 and APOE4 mouse astrocytes. iii) ex vivo human temporal cortices from APOE3 (ε3/ε3) or APOE4 (ε3/ε4) AD donors. Throughout all models, qRT-PCR and Western blotting showed an outstanding lower level of PPARγ in APOE4 astrocytes, compared to APOE3 ones. Treatment of hiPSC-derived astrocytes with a pro-inflammatory cytokine mix, known to induce an inflammatory reaction, induced the release of a variety of inflammatory mediators, with different responses between APOE3 and APOE4 astrocytes. Given the established role of PPARγ in alleviating inflammation, we hypothesize that APOE4 drives astrocyte reactivity by reducing PPARγ expression. Further experiments are, however, needed to elucidate the mechanisms through which APOE4 effects PPARγ expression and how, in turn, PPARγ promotes astrocyte reactivity. Moreover, a more comprehensive proteomic analysis on additional 106 post-mortem human temporal cortices from controls and AD donors, both APOE3 and APOE4, is ongoing to give more insights on APOE4-induced AD pathology and neuroinflammation.

SPMs system imbalance contributes to neuroinflammation in Alzheimer's disease

NI 19

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Resolution of inflammation is a physiological process that limits acute inflammation and prevents its progression to chronicity1. It is driven mostly by innate immune cells — including macrophages — which clear the inflamed environment of dead cells and tissue debris and both produce and are targeted by endogenous immunomodulatory bioactive lipids, notably Specialized Pro-resolving Mediators (SPMs): lipoxins, resolvins, protectins and neuroprotectins, maresins2,3, the main effectors of the resolution process. Alzheimer's disease (AD) features significant unresolved neuroinflammation induced by dysfunctional microglia4. Therefore, we investigated SPM system modulation in AD. We recruited 101 participants: 31 healthy controls (HC) and 70 cognitively impaired (CI) subjects, divided according to amyloid PET (amy-PET) in 25 amyloid-negative (A-) and 45 amyloid-positive (A+)5. In concordance with amy-PET results, plasma levels of phosphorylated tau (P-p-tau217) were increased by 200% in the A+ group compared to A- and HC. Plasma cytokine levels showed an upward trend in A- and A+ groups compared to HC, confirming chronic neuroinflammation in neurodegenerative diseases. Using flow cytometry, we analyzed the protein expression of key enzymatic markers (5-,12-,15-LOX,15-PGDH) and receptors (GPR18, GPR32, FPR2, ChemR23) in monocyte-derived macrophages. Principal component analysis (PCA) revealed a clear separation between the HC and A+. Individual analysis showed a significant increase in GPR18 in A+, a decrease in ChemR23 in both A+ and A-, and a reduction of 15-LOX in A-. These alterations were accompanied by lower SPM levels, particularly Resolvins E1 and D series, in plasma and macrophage supernatants of CI subjects. Overall, our findings indicate dysregulation of resolution pathways in AD and support a link between chronic neuroinflammation and impaired inflammatory resolution, highlighting the importance of resolution processes and their key mediators, SPMs lipids.

NGAL and VCAM-1 are upregulated in cervical arterial dissection: a plasma biomarker study

NI 20

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Spontaneous Cervical Artery Dissections (CAD) are a relatively rare but clinically significant cause of stroke in young adults, but circulating biomarkers reflecting vascular and clinical phenotypes remain poorly characterized. We quantified plasma levels of TREM-2, ICAM-1, VCAM-1, NGAL, and TIMP-1 by ELLA platform in 59 CAD patients and 37 age/sex matched healthy donors (HD). Group differences were assessed and a Spearman correlation matrix was generated between the 5 proteins and 78 demographic, clinical, and neuroimaging variables. To capture the multisystemic nature and heterogeneous presentation of CAD, we examined whether protein expression associates to connective tissue markers or neuroimaging features of arteriopathy.

NGAL ($p<0.0001$) and VCAM-1 ($p=0.0165$) were significantly upregulated in CAD patients versus HD, suggesting enhanced vascular inflammation and endothelial activation. TIMP-1 showed significant inverse correlations with ischemic event at dissection onset ($r\approx0.48$, $p=0.0003$), gingival recession ($r\approx-0.38$, $p=0.018$), and scoliosis/kyphoscoliosis ($r\approx-0.33$, $p=0.037$). NGAL correlated with neck pain at dissection onset ($r\approx-0.83$, $p=0.015$) and discharge mRS ($r\approx-0.40$, $p=0.004$), linking to initial symptomatology and functional outcome. TREM-2 correlated with headache at dissection onset ($r\approx-0.38$, $p=0.0068$), indicating a potential role in neuroinflammatory pain pathways. These associations pointed to a complex interplay between inflammatory, endothelial, and matrix-remodeling processes, which may underlie vascular fragility and support the role of connective tissue alterations of CAD.

Our findings support NGAL, VCAM-1, TIMP-1, and TREM-2 as candidate biomarkers reflecting vascular, skeletal, and clinical manifestations in CAD. Beyond their potential diagnostic role, these markers might also help to refine risk stratification, identify subgroups with prominent connective tissue features, and guide future studies in CAD patients.

Longitudinal characterization of visual pathway involvement in two novel OSP- and MOBP-induced experimental autoimmune encephalomyelitis models

NI 21

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Experimental autoimmune encephalomyelitis(EAE) induced by myelin oligodendrocyte glycoprotein(MOG) is widely used to model multiple sclerosis(MS), but it does not fully reflect the MS heterogeneity. To explore alternative models that may capture additional aspects of MS, we longitudinally characterized visual system evolvement in EAE induced by oligodendrocyte-specific protein(OSP) and myelin oligodendrocyte basic protein(MOBP). OSP- and MOBP-EAE were induced in C57BL/6 mice. Motor symptoms were assessed daily while visual-evoked potential(VEP), electroretinography(ERG), and optical coherence tomography(OCT) were acquired at baseline and every two weeks from week 4. No animals developed overt motor symptoms during the 169-day follow-up, EAE mice showed loss of the N1 component on VEP at different time points, followed variable degree of recovery. Therefore, data were realigned according to N1 loss and classified into N1 absence, post-absence, and recovery. Visual readouts were compared across phases. All EAE mice showed at least one episode of N1 absence by 126 day post-immunization, with an average of 2.1 episodes per mouse. Following N1 absence, the post-absence phase showed by prolonged VEP latency, thickening of retinal nerve fiber layer(RNFL) and thinning of inner plexiform layer thinning(IPL) on OCT, and reduced amplitude of photopic negative response(PhNR) on ERG. During recovery, VEP latency and PhNR amplitude partially improved, accompanied by increased IPL thickness. Histology showed reduced retinal ganglion cell counts in EAE mice, which positively correlated with IPL thickness. OSP- and MOBP-EAE showed recurrent VEP N1 absence, suggesting a relapsing-remitting-like pattern of visual pathway involvement without overt motor symptoms. Longitudinal in-vivo assessments suggested transient functional impairment, retinal structural changes, and partial recovery followed by the N1 absence. Further retinal and optic nerve histology will validate the findings.

Exploring the role of ATM signalling in HSV-1-infected monocytes and its potential contribution to neuroinflammation

NI 22

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Herpes simplex virus type 1 (HSV-1) is a widespread neurotropic virus that has increasingly been associated with neuroinflammatory processes implicated in several neurological disorders. Monocytes are considered important mediators of systemic inflammation and may contribute to the dissemination of both viral particles and inflammatory signals. Previously, our studies have shown that HSV-1 infection of THP-1 monocytic cells induces the expression of inflammatory cytokines and chemokines, including CXCL10. In parallel, accumulating evidence indicates that the DNA damage response (DDR) is activated during HSV-1 infection and may contribute to virus-host interactions beyond those mediated by genome maintenance mechanisms. Among the key regulators of the DDR, ATM (Ataxia Telangiectasia Mutated) kinase plays a central role in coordinating cellular responses to virus-induced stress. In the present study, we investigated the activation of ATM- and AKT-dependent signalling pathways following HSV-1 infection in various cellular models, including THP-1 monocytic cells, SH-SY5Y neuronal cells, and HEp-2 epithelial cells. Cells were infected with HSV-1, and protein extracts were collected at different hours post-infection. The expression levels of total ATM, phosphorylated ATM (p-ATM), total AKT, and phosphorylated AKT (p-AKT) were analyzed by western blotting. A time-dependent increase in both p-ATM and p-AKT levels was observed in all cell models analyzed. Ultimately, while we verified ATM activation across multiple cell lines, the specific findings in THP-1 monocytic cells strongly encourage further investigation into the interplay between HSV-1 and ATM in this model. Given the pivotal role of monocytes in orchestrating systemic immune responses, exploring this pathway in THP-1 cells could provide crucial insights into how HSV-1-induced ATM activation serves as a key trigger for downstream neuroinflammatory processes.

An innervated and immunocompetent human skin equivalent of psoriasis

NI 23

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Psoriasis is a common immune-mediated inflammatory skin disease affecting 2-3% of the world's population. This skin disease is characterized by an altered crosstalk between keratinocytes and T cells leading to abnormal epidermal hyperplasia and dysfunctional immune response such as acute leukocyte infiltration in the dermis and epidermis. Current state-of-the-art in vitro models have successfully transitioned towards 3D immunocompetent platforms to study the crosstalk between keratinocytes and lymphocytes. However, while these models effectively incorporate immune components to mimic psoriatic inflammation, they consistently lack sensory innervation. This absence of a nervous component represents a significant gap, as it fails to account for the complex neuro-immune interactions that characterize the native skin microenvironment and contribute to the disease's pathophysiology.
To overcome the limitations of traditional skin models, an innervated human skin equivalent (HSE) was previously developed featuring a dermal compartment composed of endogenous extracellular matrix (ECM), thereby avoiding exogenous scaffolds. While this platform has been validated for its functional and morphological similarity to native skin, this study aims to advance its complexity by inducing a psoriasis-like phenotype via a cytokine cocktail (IL-17A, IL-22, IL-1α, TNF-α). The platform was further evolved into an immunocompetent system through the incorporation of T cells, enabling the investigation of neuro-immune interactions. This innovation allows us to observe how sensory innervation modulates immune-driven inflammatory responses in a diseased state. By stimulating the psoriatic HSE with capsaicin, we investigated the differential response of sensory fibers compared to healthy controls, aiming to elucidate the specific role of neuroinflammation in modulating the psoriatic response.This integrated system represents more than a specialized tool for psoriasis; indeed, it could serve also as a strong platform for investigating the pathophysiology of other inflammatory skin conditions, such as Atopic Dermatitis (AD). Furthermore, this model holds significant potential as a high-fidelity tool for future drug delivery testing and pharmacological validation, offering a more predictive alternative to traditional platforms.

Targeting GLT-1 and PPAR-α: dual therapy with ceftriaxone and PEA alleviates neuropathic pain and neuroinflammation in SNI mice

NI 24

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Background: Neuropathic pain is a chronic and debilitating condition characterized not only by sensory abnormalities but also by affective, cognitive, and motor impairments. Increasing evidence implicates neuroinflammation and dysregulation of glutamatergic signaling in its pathogenesis. The astrocytic glutamate transporter-1 (GLT-1), a major regulator of extracellular glutamate homeostasis, is altered following nerve injury, contributing to excitotoxicity, central sensitization, and neuronal dysfunction. This study investigated whether ceftriaxone (CEF), a GLT-1 activator, and ultramicronized palmitoylethanolamide (umPEA), a PPAR-α agonist with anti-inflammatory properties, exert complementary therapeutic effects in a mouse model of spared nerve injury (SNI)-induced neuropathic pain.
Methods: Male mice (7–8 weeks old, 22–25 g) underwent SNI surgery and received daily intraperitoneal administration of umPEA (5 or 10 mg/kg), CEF (100 or 200 mg/kg), or a combination of subeffective doses (umPEA 5 mg/kg + CEF 100 mg/kg) for 28 days. Mechanical allodynia, thermal hyperalgesia, anxiety-like behavior, depression-like behavior, motor coordination, gait performance, and recognition memory were assessed. Neurochemical and molecular analyses included in vivo microdialysis for extracellular glutamate and GABA quantification in the CA1 and CA3 region of hippocampus , Western blotting, ELISA, immunohistochemistry, and double immunofluorescence to evaluate GLT-1 expression, glial activation, BDNF levels, and spinal and hippocampal alterations.
Results: Low-dose PEA at 5 mg/kg and ceftriaxone at 100 mg/kg, when administered individually, did not significantly reduce SNI-induced mechanical allodynia, nor did they alleviate motor and cognitive impairments or anxiety- and depression-like behaviors. However, the combination of PEA (5 mg/kg) and ceftriaxone (100 mg/kg) resulted in a significant improvement in pain thresholds. Furthermore, this combination enhanced behavioral outcomes related to depression, motor coordination, and memory. Biochemically, the combination therapy notably affects amino acid balance, particularly in glutamate and GABA levels, GLT-1 expression, and reduced astrocytic activation.
Conclusion: Combined administration of subeffective doses of umPEA and ceftriaxone exerts synergistic neuroprotective and analgesic effects in SNI-induced neuropathic pain. These benefits are associated with restoration of glutamatergic homeostasis, modulation of glial reactivity, and improvement of sensory, affective, cognitive, and motor dysfunctions. Targeting both neuroinflammatory and glutamatergic pathways may therefore represent a promising multimodal therapeutic strategy for neuropathic pain and its associated comorbidities.

Neuroinflammatory remodeling and inflammasome activation in the superior colliculus of an MPTP-induced parkinsonian rat model

NI 25

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Parkinson's disease (PD) is increasingly recognized as a multisystem disorder characterized not only by nigrostriatal degeneration but also by widespread neuroinflammatory processes involving extra-nigral brain regions. The superior colliculus (SC), a midbrain structure involved in sensorimotor integration and closely connected with dopaminergic nuclei, has received limited attention in this context. The present study investigated neurodegenerative and neuroinflammatory alterations in the SC of an MPTP-induced parkinsonian rat model. Adult male Sprague-Dawley rats were treated with MPTP and SC sections were analyzed by histological and immunohistochemical approaches. MPTP administration produced the expected dopaminergic degeneration in the substantia nigra and was associated with marked alterations in the SC. Nissl and NeuN staining revealed reduced neuronal density and disruption of the normal laminar organization. These changes were accompanied by pronounced astrocytic and microglial activation, as demonstrated by increased GFAP and Iba1 immunoreactivity. Furthermore, MPTP-treated animals exhibited enhanced expression of the inflammasome-related markers NLRP3, Caspase-1 and IL-1β, indicating activation of pro-inflammatory signaling pathways within the SC. Collectively, these findings demonstrate that the superior colliculus undergoes significant neurodegenerative and neuroinflammatory remodeling following dopaminergic injury. The observed glial activation and inflammasome signaling suggest that this structure may contribute to the extra-nigral pathology of PD and represent a potential target for future therapeutic strategies.

Functional network reorganization following VIM-MRgFUS for Parkinson's disease

NIM 04

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Thalamotomy reduces tremor in Parkinson's disease (PD) and has experienced a renaissance with the advent of magnetic resonance-guided focused ultrasound (MRgFUS). However, the mechanisms underlying its therapeutic effects remain unclear. Tremor generation in PD is increasingly conceptualized as a network-level dysfunction, with disrupted topological properties reported. Investigating the effects of MRgFUS at the network level may therefore provide insight into its mechanisms. We applied graph theory analysis to examine changes in small-world properties and modular architecture in PD patients six months after unilateral MRgFUS targeting the thalamic ventral intermediate (VIM) nucleus. Twenty-one PD patients (mean age 61.76 ± 11.85 years) underwent unilateral VIM-MRgFUS and clinical and MRI assessments at baseline and 6 months. Tremor severity was assessed using the Fahn-Tolosa-Marin Tremor Rating Scale (FTM) and Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III. MRI data were preprocessed with the CONN toolbox. Functional connectivity matrices were constructed from the Human Brainnetome Atlas. Graph theory analysis was performed using the Graph Theoretical Network Analysis toolbox. Modular structure was identified using a modified greedy optimization algorithm, and inter- and intra-modular connectivity were calculated. Changes in network metrics were assessed with paired t-tests or Wilcoxon tests, and associations examined with Spearman correlation. For subgroup analyses, 19 patients with complete data were classified as responders (n = 11, ≥30% tremor improvement) or relapsers (n = 8, < 30% tremor improvement). Tremor scores decreased significantly after MRgFUS contralateral to the lesioned thalamus. ($p < 0.05$). Small-worldness and normalized clustering coefficient increased significantly after MRgFUS ($p < 0.05$), whereas clustering coefficient, characteristic path length, normalized characteristic path length, global efficiency, local efficiency, and modularity showed no significant changes. Community detection revealed six functional modules. Trend-level increases in inter-modular connectivity were observed between cortical-basal ganglia and thalamic modules, and between cerebellar and temporal-hippocampal modules. In responders, small-worldness, normalized clustering coefficient, and global efficiency increased significantly, while characteristic path length decreased significantly after MRgFUS ($p < 0.05$). Changes in global efficiency positively correlated with improvements in tremor subscales FTM-A/B ($p < 0.05$) and FTM-C ($p < 0.001$)(measuring impairments in activities of daily living), whereas changes in characteristic path length negatively correlated with FTM-C improvement ($p < 0.001$). No significant changes were observed in relapsers. These findings suggest that MRgFUS thalamotomy for TD induced partial reorganization of small-world properties, indicating a shift towards a more integrated network topology, especially in responders. Changes correlated with tremor reduction and improved functional disability, highlighting that effective treatment impacts on global network integration.

Network-level genetic organization of human cortical folding

NIM 05

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Cortical folding is a heritable feature and provides a reliable anatomical landmark for brain function, but whether shared genetic influences on sulcal morphology follow large-scale functional brain networks remains unclear. We examined genetic correlations among cortical sulci across >40,000 participants from the UK Biobank. Sulci were mapped to functional networks using the Schaefer 1000-parcel with Yeo's 7-network atlas as the main framework, and the results were validated across other Schaefer, Power and Gordon functional atlases. Pairwise genetic correlations were estimated for four sulcal shape descriptors, width, mean depth, surface area, and length, with sulcal width showing the strongest genetic correlation among the examined metrics. Network-level analyses showed that genetic correlations were stronger within than between functional networks, indicating modular genetic influences on cortical folding. The highest within-network correlations were observed in the frontoparietal control, dorsal attention, and visual networks, suggesting coordinated genetic architecture. We further examined network-wise genetic correlations between sulcal metrics and 21 clinical, cognitive, and behavioral states. The strongest profiles involved psychiatric phenotypes, anxiety-related traits and schizophrenia, and neurological disorders, Parkinson's disease and epilepsy, suggesting that genetic influences on cortical folding may show disorder-specific alignment with structural and functional systems. Together, these findings indicate that the genetic architecture of cortical folding mirrors functional brain network organization and extends beyond individual sulcal features to a validated network-level framework. Clinically, this approach may help identify imaging-genetic endophenotypes that link inherited variation in brain morphology to functional vulnerability and disease risk, supporting future stratification and mechanistic studies in neurological and psychiatric disorders.

Early cladribine administration prevents connectomic and molecular changes in experimental multiple sclerosis

NIM 06

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Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) characterized by inflammation, demyelination, and neuronal loss. Pathological changes within the CNS disrupt structural and functional connectivity across brain networks, leading to clinical manifestations including motor and cognitive deficits. However, the link between network disruption and disease severity remains unclear, and no therapies specifically preserve the connectome. Functional connectivity (FC) changes observed in patients are inconsistent, likely reflecting the complex interplay between structural and functional damage and heterogeneity in disease stage, clinical expression, patient populations, and treatment. To address these limitations, we used the Experimental Autoimmune Encephalomyelitis (EAE) mouse model of MS to identify FC signatures with resting-state fMRI and test whether progressive clinical changes are associated with specific patterns of network reorganization. Connectivity alterations were examined in relation to structural, metabolic, transcriptomic, and molecular measures obtained from the same animals within a multidimensional framework. In parallel, we tested whether Cladribine, an MS-approved molecule modulating both immunity and synaptic transmission, could prevent EAE-related pathological changes across scales, from molecular to network-level alterations. Our findings show region- and stage-specific FC alterations in EAE, mainly within sensory-motor, thalamic, and hypothalamic networks. These alterations are accompanied by transcriptomic changes primarily involving immune and inflammatory markers. Cortical regions show concurrent changes in regional volume and glial activation markers. Importantly, Cladribine exerts a protective effect on cortical networks, likely mediated by its impact on structural organization and transcriptomic regulation, highlighting the relevance of broad pharmacological targets in supporting cognitive and sensorimotor functions.

MEOX2 as a putative driver of therapy resistance in glioblastoma stem cells

NO 16

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Glioblastoma (GBM) is the most aggressive and lethal primary brain tumor in adults, characterized by rapid progression, extensive heterogeneity, and poor patient prognosis. Despite the current standard of care, consisting of maximal surgical resection followed by radiotherapy and temozolomide (TMZ)-based chemotherapy, tumor recurrence remains almost inevitable due to the marked resistance of GBM cells to conventional therapies.
The transcription factor Mesenchyme Homeobox 2 (MEOX2) is overexpressed across glioma subtypes and correlates with poor patient survival, particularly in radioresistant tumors. Previous studies, together with our findings, have demonstrated that MEOX2 is highly enriched in glioblastoma stem cells (GSCs), a subpopulation critically involved in tumor maintenance, recurrence, and therapy resistance. MEOX2 knock-down (KD) markedly impaired GSC survival and self-renewal capacity while increasing sensitivity to TMZ treatment, suggesting that MEOX2 targeting may enhance the efficacy of current therapeutic approaches. To further assess the therapeutic relevance of MEOX2, we screened a panel of FDA-approved compounds, identifying candidate strategies capable of potentiating anti-tumor effects in GSCs and overcoming treatment resistance.
To elucidate the molecular mechanisms underlying MEOX2 activity, we performed ATAC-sequencing (Assay for Transposase-Accessible Chromatin sequencing), a genome-wide approach used to map chromatin accessibility and identify regulatory elements involved in transcriptional control. This analysis enabled the characterization of chromatin remodelling events and transcriptional programs associated with MEOX2-dependent therapy resistance in GSCs.

Combining TMS and Action Observation to probe the fronto-parietal network integrity in glioma patients: a proof-of-concept study

NO 18

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The observation of others' actions recruits the same fronto-parietal network involved in action execution. This phenomenon, known as the 'mirror mechanism', can be assessed in humans indirectly via transcranial magnetic stimulation (TMS) over the primary motor cortex, by measuring action observation (AO)-induced facilitation of corticospinal excitability. Clinically, the mirror mechanism has been applied in motor rehabilitation, where AO promotes cortical plasticity following peripheral damages or central lesions. A well-established literature has demonstrated that AO engages widespread sensorimotor areas, offering a tool to probe the functional integrity of patients with motor networks lesions. In this framework, patients with fronto-parietal glioblastoma represent a highly informative model. Here, we present a proof-of-concept study aimed at introducing AO combined with TMS into the presurgical assessment of glioma patients. Thirty patients will be recruited preoperatively. Single-pulse TMS is delivered over the ipsilesional and controlesional primary motor cortices, while electromyography records motor-evoked potentials (MEPs) from three hand muscles involved in grasping. Fifteen MEPs are collected at rest and during the observation of reach-to-grasp actions (AO). Preliminary findings indicate that the affected hemisphere, unlike the healthy one, doesn't show AO-induced facilitation, suggesting a tumor-related impairment of fronto-parietal network. Furthermore, lesion-symptom mapping, diffusion tensor imaging, and histological features will clarify structural and functional connectivity underlying AO-induced CSE modulations. Combining TMS and AO may provide an indirect proxy of motor network integrity, paving the way for future diagnostic approaches. Finally, longitudinal post-operative subministration of AO during rehabilitative TMS, may index motor regions responsiveness and representing a biomarker of individual sensitivity to neuromodulatory interventions.

Functional targeting of MEX3A through RNA-based therapeutic strategies in glioblastoma

NO 19

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Glioblastoma (GBM) is the most common and aggressive brain tumor in adults. Its rapid progression, diffuse infiltration, and molecular heterogeneity are associated with poor clinical outcome, with a median survival of approximately 15 months. Although standard and emerging targeted- and immune-therapies have improved patient management, their efficacy remains limited due to extensive intra-tumoral variability and a highly immunosuppressive microenvironment. These challenges highlight the need to identify novel molecular mechanisms driving GBM aggressiveness. In this context, we identified the RNA-binding protein and E3 ubiquitin ligase Muscle Excess 3A (MEX3A) as a promising therapeutic target for GBM. MEX3A is highly overexpressed in our GBM patient cohort. Its high levels correlate with aggressive clinicopathological features and reduced overall and progression-free survival in our patient cohort, supporting the role of MEX3A as both a diagnostic and independent prognostic biomarker. Investigating MEX3A function in GBM tumorigenesis, we demonstrated that it promotes proteasome-dependent degradation of the Retinoic acid-Inducible Gene (RIG-I), a key regulator of type I interferon response and apoptosis, thereby supporting tumor cell survival and immune evasion. Based on these findings, we are evaluating combined MEX3A targeting and RIG-I activation in preclinical models, including patient-derived primary cells, organoids, and immunocompetent syngeneic mouse models. Our data show significant synergistic anti-tumor effects. In parallel, we are exploiting MEX3A RNA-binding activity as an additional therapeutic vulnerability by developing RNA-based aptamers targeting its RNA-binding domains to interfere with its oncogenic functions and assessing their efficacy in the same models. Overall, these findings identify MEX3A as a promising therapeutic target and support innovative RNA-based strategies for GBM treatment.

Mechanistic insights into Nucleotide Excision Repair in Glioma Stem Cells

NO 21

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High-grade gliomas are the most aggressive and lethal primary brain tumors in adults, characterized by rapid growth, extensive infiltration into healthy tissue, genetic heterogeneity, and strong resistance to conventional therapies. Increasing evidence highlights the role of glioma stem cells (GSCs) in tumor progression, recurrence, and therapeutic resistance. GSCs exhibit enhanced self-renewal, plasticity, and an increased ability to respond to DNA damage.
Here, we investigate the Nucleotide Excision DNA Repair (NER) pathway in GSCs compared with their differentiated counterparts, on patient-derived cells. Using functional assays, including lesion detection, transcription-recovery assay and DNA synthesis assays, we evaluate the ability of GSCs to recognize and repair UV-induced DNA lesions. In addition, through a CRISPR-Cas9-based gene editing approach, we generate XPC knockout cell lines. Since XPC is a key factor of the Global Genome NER (GG-NER) pathway, this model enables the selective investigation of Transcription-Coupled NER (TC-NER). Furthermore, an XPC-GFP fusion construct has been developed to study XPC in vivo dynamics during DNA repair.
We demonstrate that differentiated cells retained UV-induced DNA damage for a longer time after irradiation, while stem-like cells reveal a more rapid damage resolution. Through transcription-recovery assay, we validate that GSCs resume transcription more efficiently after the repair of UV-induced DNA damage than differentiated cells. Measurements of DNA synthesis during repair reveal a significantly different trend in repair-associated DNA synthesis between stem-like and differentiated cells. Moreover, the same experiments in XPC knockout cells (TC-NER-dependent repair) show higher DNA synthesis levels in GSCs, while no gap filling emerges in differentiated cells. Overall, these findings indicate that GSCs possess a robust transcription-coupled repair activity, potentially contributing to their resistance to therapies.

Targeting glioblastoma stem cells with biomimetic vesicles for boron neutron capture therapy

NO 22

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Glioblastoma (GBM) is the most aggressive primary brain tumor, with a median survival of approximately 15 months. Standard treatment combining radiotherapy and temozolomide provides limited benefit, and tumor recurrence remains a major clinical challenge, underscoring the urgent need for novel therapeutic strategies. Boron neutron capture therapy (BNCT) is a cellular-level radiotherapy based on the nuclear reaction between thermal neutrons and boron-containing compound into malignant cells to selectively destroy cancer cells while sparing adjacent healthy tissue, mainly used in refractory cancer as GBM. However, efficient and selective boron delivery to GBM cells, including glioblastoma stem cells (GSCs), remains a critical unmet need. Cell membrane derived vesicles (CMVs) retain the homing properties of the cell membrane of origin, while overcoming limitations of naturally secreted extracellular vesicles, such as low yield and genetic material contamination. CMVs were prepared from patient-derived GSC homogenates by differential centrifugation and serial extrusion, and characterized for size, zeta potential, particle concentration, absence of nucleic acid contamination, and proteomic profiling, confirming retention of GSC-specific membrane proteins, including annexin A2, α3-β1 integrins and CD44. CMVs loaded with sodium borocaptate (BSH) exhibited selective and concentration-dependent internalization in GBM cells, with limited uptake in normal brain cells, and demonstrated blood-brain barrier crossing ability in vitro. Upon neutron irradiation, BSH-loaded CMVs emitted alpha particles, validating their BNCT relevant functionality. Cell viability and spherogenesis assays, performed with and without neutron irradiation, confirmed selective GSC-killing activity. Future in vivo studies will assess biodistribution and therapeutic efficacy in animal models. Overall, BSH-loaded CMVs represent a promising biomimetic nanoplatform for targeted boron delivery and BNCT of GBM.

Alterations of GABAergic transmission in models of neurotoxicity and neurodevelopmental disorders: molecular, synaptic, and functional mechanisms

NP 15

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GABAergic inhibition is not a stable circuit property, but the integrated outcome of presynaptic and postsynaptic mechanisms that are rapidly remodeled. Its strength depends on target identity, release-site number, vesicle-pool organization, synchronous/asynchronous release balance, and postsynaptic GABAA receptor availability. Even modest changes in these variables may disrupt excitation/inhibition (E/I) balance and circuit stability. Here, I combined electrophysiology, Ca2+ imaging, and confocal microscopy of immunolabelled synapses to examine three neuropathological models. In Cdkl5 loss, a model of CDKL5 deficiency disorder, GABAergic transmission was enhanced, including inhibitory drive and release efficacy in PV and CCK interneurons (INs). Paradoxically, increased inhibition shifted S1 microcircuits toward excitation, because potentiated autaptic and IN-to-IN inhibition reduced unitary PV IN output onto pyramidal neurons. In an in vitro Alzheimer's disease model based on Aβ42 oligomers, inhibitory efficacy was modified by ryanodine receptor (RyR) dysfunction, with more release sites, expansion of the synchronous releasable pool (RRP), and a shift toward synchronous release. Stabilizing the RyR–calstabin interaction with S107 rescued these alterations, supporting a causal role for RyR dysfunction. Finally, acute exposure to non-toxic glyphosate weakened hippocampal GABAergic synapses through combined pre- and post-synaptic effects: fewer release sites, a smaller synchronous RRP, and reduced GABAA receptor availability, while release probability was unchanged. Increased asynchronous release indicated redistribution of resources rather than exocytosis blockade. Overall, these findings identify inhibitory synapses as plastic substrates of pathology and show that defining altered components is essential to understand maladaptive E/I imbalance and circuit dysfunction.

Smoothened modulation affects disease progression in SOD1G93A mouse model of amyotrophic lateral sclerosis

NP 16

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Amyotrophic lateral sclerosis (ALS) is a fatal motor neuron (MN) disease for which no effective disease-modifying treatment exists. Identifying neuroprotective pathways that can be targeted before irreversible MN loss occurs remains a critical priority. The Sonic Hedgehog/Smoothened (Shh/Smo) axis is a conserved signalling pathway with established roles in neuronal survival, glial homeostasis, and synaptic plasticity in the adult central nervous system. While preliminary evidence has suggested its involvement in ALS, whether it represents a pharmacologically accessible target in progressive MN degeneration remains largely unexplored. Using a transgenic mouse model of ALS carrying the G93A mutation of the gene encoding the superoxide dismutase 1 (SOD1-G93A), we show that key Shh pathway components are intrinsically downregulated during disease progression, revealing a disease-driven suppression of endogenous neuroprotective signalling. Pharmacological Smo modulation from the presymptomatic stage produced strikingly divergent outcomes. Smo inhibition with vismodegib accelerated MN loss, worsened motor performance, exacerbated muscle atrophy and markedly increased cytoplasmic mislocalisation of TAR DNA-binding protein 43 (TDP-43) in spinal MNs. Smo activation with clobetasol partially preserved MN integrity, enhanced downstream pathway activation, and restored expression of genes critical for MN identity and survival. Transcriptomic profiling uncovered treatment-specific molecular signatures, revealing that Smo modulation engages distinct transcriptional programmes involving cilium organisation, microtubule dynamics, neuroinflammation, and oxidative stress, pathways not previously associated with Shh signalling in the context of ALS. These findings provide evidence that Shh signalling may play an important role in ALS pathogenesis and support the development of more selective Smo agonists as a future therapeutic strategy.

Cortisol-driven dysregulation in human hippocampal progenitor cells: insights into the protective role of exercise-derived factors

NP 17

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Background: Depression is a major cause of disability worldwide. Its pathogenesis is multifactorial, with chronic stress and glucocorticoid excess impairing hippocampal neurogenesis through maladaptive molecular changes. Exercise-derived myokines adiponectin and irisin may counteract these effects via neurotrophic and anti-inflammatory properties. This study investigates cortisol-induced alterations in human hippocampal progenitor cells and whether these myokines can rescue such impairments.

Methods: HIP-009 cells were treated with cortisol (100 µM) for 3 days. Long-term effects were assessed after 21 differentiation days without cortisol. RNAseq was performed at day 3 and day 3+21 (DESeq2, $p<0.05$, $|\log2FC|\geq0.26$). Protective effects were tested with adiponectin (25 ng/mL) or irisin (100 ng/mL). Neurogenesis was assessed by immunocytochemistry; candidate genes by RT-PCR.

Results: Cortisol induced widespread transcriptional changes, modulating pathways involved in inflammation and extracellular matrix remodelling with a subset persisting after 21 days, indicating lasting molecular reprogramming. Cortisol also reduced BDNF, BDNF VIb ($p < 0.05$), Ki67+, MAP2+ and GFAP+ cells ($p < 0.01$). Co-treatment with adiponectin or irisin partially restored neurotrophic factors (BDNF, NGF, VEGF) ($p<0.05$) and Ki67+ proliferation ($p<0.05$).

Conclusion: Cortisol induces stress-related transcriptional and neurogenic impairments in human hippocampal precursor cells, with persistent alterations lasting beyond the initial exposure. Exercise-associated myokines such as adiponectin and irisin partially counteract these effects by selectively restoring neurotrophic support and proliferation. Further studies should clarify the molecular mechanisms underlying their protective actions against stress-induced dysfunction.

From hyperexcitability to drug resistance: integrating behavioral and electrophysiological biomarkers in a longitudinal pilocarpine model of temporal lobe epilepsy

NP 18

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Altered brain networks are features of hyperexcitability-based diseases such as Temporal lobe epilepsy (TLE). However, how circuit dysfunction contributes to behavioural comorbidities and drug resistance remains unclear. Here, we aimed to characterize phase-specific behavioral and electroencephalographic (EEG) features of TLE progression in a pilocarpine (PILO) rat model, to identify distinct patterns associated with resistance to Anti-Seizure Medications (ASMs). After stereotaxic surgery and TLE induction, male Wistar rats were evaluated for behavioral and spectral EEG biomarkers at five timepoints: baseline, acute phase, latent phase, chronic phase, and after assessment of drug-resistant epilepsy (DRE). After the chronic phase, responsiveness to phenobarbital (PB), standardized approach for DRE characterization, was assessed via video monitoring-based scoring to identify drug-resistant (DRUG-R) and drug-sensitive (DRUG-S) subpopulations. Our data show that PILO rats exhibited progressive phase-dependent behavioral and EEG alterations. Behavioral profiling revealed a hypermotor activity, impaired burrowing, anxiety- and depressive-like behavioral paradigms, and progressive memory impairment. Following PB, DRUG-R and DRUG-S subpopulations have been identified, displacing different behavioral features, especially in anxiety-like and burrowing behaviors. Spectral EEG analysis revealed dynamic modulation of frequency bands across epileptogenesis, particularly in Theta and Delta power, and descriptive analyses attempted to stratify by DRE. Correlation analyses identified associations between EEG, seizures frequency and behavioral measures, especially in the anxiety and declarative memory domains. This study provides evidence that TLE induces phase-specific brain network reorganization that relapses on distinct electrophysiological and behavioral signatures, representing an innovative approach for the identification of translational biomarkers of epileptogenesis and DRE.

Selective upregulation of Nav1.6 underlies PRRT2-linked cerebellar dysfunction

NP 19

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Proline-rich transmembrane protein 2 (PRRT2) is a neuron-specific protein regulating neuronal function by interactions with presynaptic proteins and voltage-gated Na^+ (Nav) channels (Valente et al., 2016, 2018). PRRT2 loss-of-function mutations cause paroxysmal kinesigenic dyskinesia, and its high expression in cerebellar granule cells (GCs) suggests that cerebellar dysfunction contributes to the dyskinetic phenotype in patients and PRRT2 knockout (KO) mice (Michetti et al., 2017). Primary GCs from PRRT2 KO mice show increased Nav expression and increased cell excitability (Binda et al., 2021). Nav1.2 and 1.6, the main GC Nav subtypes, display distinct developmental expression patterns. 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 showed selective Nav1.6 upregulation in PRRT2 KO GCs, with AIS elongation and increased channel clustering, indicating AIS remodeling and enhanced Nav1.6 function. To assess subtype-specific contributions, we combined RNA interference with whole-cell patch-clamp recordings. Nav1.6 knockdown reduced Na^+ currents, with a stronger effect in KO than in WT cells, supporting its selective upregulation in PRRT2-deficient GCs. Consistently, current-clamp recordings showed reduced action potential amplitude and firing frequency, indicating that Nav1.6 upregulation drives the altered GCs excitability. In contrast, Nav1.2 silencing similarly reduced Na^+ currents in KO and WT GCs, indicating that PRRT2 preferentially regulates the less abundant developmental subtype Nav1.6, with minimal effects on Nav1.2, which is already highly expressed and stably enriched at the AIS early in differentiation. Our findings show that PRRT2 preserves GC activity by limiting Nav1.6-dependent excitability, whose upregulation causes hyperexcitability in KO cells, highlighting its key role during early GC functional differentiation.

Early-life stress shapes cerebellar circuits associated with opioid use disorder vulnerability

NP 20

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Early life stress (ELS) is considered a key contributor to increased vulnerability to substance use disorders. Maternal separation (MS), a widely used mouse model of ELS, has been shown to enhance morphine sensitivity and alter opioid signaling within brain regions involved in reward and emotional regulation. Although the cerebellum is the brain region with the highest density of μ-opioid receptors (MOR), little is known about the functional role of the cerebellar opioid signaling at the cellular level, particularly in the context of ELS. Here, we investigated the impact of MS on opioid responsiveness and MOR expression in the cerebellum, using electrophysiological and molecular approaches. To induce ELS, CD1 pups were separated from their mothers from postnatal day P2 to P18 for 4 h/day. Patch-clamp recordings, immunofluorescence, and western blot analyses were performed on cerebellar samples of MS and control mice at P20. In control (CTRL) mice, the MOR agonist DAMGO significantly reduced synaptic transmission at the parallel fiber–Purkinje cell (PC) synapse, whereas this effect was absent in MS mice. DAMGO application also reduced PC firing in CTRL but not in MS slices. To investigate the potential mechanisms underlying this effect, we focused on GIRK channels, a potential target of MOR. Western blot analyses revealed decreased levels of phosphorylated MOR in the cerebellar cortex of MS mice relative to controls. Together, these findings indicate that MS disrupts MOR-mediated neuromodulation of Purkinje cells. Such alterations may impair cerebellar processing and could contribute to motor disturbances, such as tremor and ataxia, observed in individuals with chronic opioid exposure or opioid abuse.

Cortico-striatal circuit mechanisms mediate D1 dopamine agonist effects on memory capacity through cAMP/PKA signalling

NP 21

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Working memory (WM), the ability to temporarily maintain and manipulate information over short-time intervals, is essential for everyday cognition and strongly correlates with fluid intelligence. However, WM is constrained by a limited capacity (WMC), estimated at approximately six elements in both humans and rodents, which is reduced in aging and neuropsychiatric disorders. Despite the fundamental role of this cognitive function, the neural and molecular mechanisms that determine and potentially modulate its capacity remain poorly understood. Dopamine is a key neuromodulator of cognitive control, yet how dopaminergic signalling influences this fundamental cognitive limit, rather than simply memory retention, remains unclear. In this study, we used the D1 receptor agonist SKF 38393 to investigate whether this capacity can be expanded through modulation of the medial fronto-striatal circuit, a network critically involved in working-memory functions. Using a behavioural paradigm designed to manipulate memory load in male mice, we found that low doses of SKF 38393 expanded WMC beyond its physiological limit, increasing performance from six to eight items through activation of the cAMP/PKA signalling pathway within the striatum. In contrast, higher doses of SKF 38393 disrupted this balance: excessive D1 stimulation recruited cAMP/PKA signalling in the medial prefrontal cortex, reduced striatal output via feed-forward engagement of parvalbumin-positive interneurons, and impaired WMC. Consistent with the canonical inverted U-shaped dose–response profile of dopaminergic agonists, these findings reveal both an enhancing (low-dose) and impairing (high-dose) effect on WMC, challenging the traditional view of PKA by showing that its functional impact critically depends on the site of activation. This refined understanding of dopamine's contribution to cognitive control opens new therapeutic perspectives for disorders characterised by working memory deficits, including Alzheimer's disease and schizophrenia.

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