



TORINO 2025

OCTOBER 29 • 30 • 31

BOOK OF ABSTRACTS

8th BRAINSTORMING RESEARCH ASSEMBLY
FOR YOUNG NEUROSCIENTISTS

AULA MAGNA "GIOVANNI AGNELLI"
POLITECNICO DI TORINO • C.SO DUCA DEGLI ABRUZZI 24

Dear Young Neuroscientists,

Welcome to the **8th BraYn Conference, the Brainstorming Research Assembly for Young Neuroscientists**, held this year in the vibrant city of Turin.

Conceived and organized by young researchers, BraYn has evolved into the **leading European conference entirely dedicated to young neuroscientists**. Year after year, it brings together a diverse community united by one mission: to connect, collaborate, and shape the neuroscience of the future.

BraYn was born from the idea that innovation thrives when disciplines meet, ideas circulate freely, and generations work side by side. Today, it stands as a unique platform where basic, translational, and clinical neuroscience converge — covering virtually **all areas of modern neuroscience, from neurodegeneration and neuroinflammation to neuro-oncology, neuroimaging, neural plasticity, epilepsy, brain development, and neurogenetics**.

Following the success of last year's edition, we are proud to confirm the dedicated **Clinical Neuroscience session**, which bridges laboratory discoveries with patient-oriented research, emphasizing biomarkers, translational models, and therapeutic innovation.

BraYn is not just a conference, it is a growing ecosystem of ideas, opportunities, and shared vision. It represents a new way of doing science: open, interdisciplinary, and deeply human.

We thank you for being part of this journey. Your curiosity, creativity, and passion are the driving forces behind the future of neuroscience.

Welcome to BraYn Conference 2025. Welcome to the next generation of neuroscience.

BraYn Team

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Michela Matteoli	Humanitas University, Rozzano (Italy)
Thomas C. Südhof	Nobel Laureate • Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute, Stanford University School of Medicine (USA)
Antonio Uccelli	IRCCS San Martino Hospital, Genoa (Italy)

INVITED SPEAKERS

Burkhard Becher	Institute of Experimental Immunology, Universität Zürich (Switzerland)
Benjamin Deneen	Baylor College of Medicine, Houston, Texas (USA)
Michael Heneka	LCSB – Luxembourg Centre for Systems Biomedicine, The University of Luxembourg (Luxembourg)
Simona Lodato	Humanitas University, Rozzano (Italy)
Edvard Ingjald Moser	Kavli Institute for Systems Neuroscience, Norwegian University of Science and Technology (Norway)
Marzia Munafò	European Molecular Biology Laboratory (EMBL), Rome (Italy)
Gaia Olivo	Psykologiska Institutionen, Göteborgs Universitet (Sweden)
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Alessandro Bombaci	IRCSS Policlinico San Donato, San Donato Milanese (Italy); Vita-Salute San Raffaele University, Milan (Italy)
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Samuele Negro	University of Padova (Italy)
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Aleksandra Rutkowska	Medical University of Gdańsk (Poland)
Maria Velasco	CNIO, Madrid (Spain)

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**NEUROIMAGING &
CLINICAL NEUROLOGY**

Neuroimaging & Clinical Neurology 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.

NEUROINFLAMMATION

Neuroinflammation is a scientific session focused on exploring the mechanisms 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) 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.

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.

NEURO-ONCOLOGY

Neuro-Oncology session will cover the field of investigation that studies nervous system tumors. As many of brain tumors can cause severe nervous system damage, neuro-oncology represents a trending research area in neuroscience, which may identify the molecular mechanisms involved in tumor pathogenesis. This session will also discuss the development of novel therapeutic approaches for the treatment of life-threatening diseases such as glioma, and medulloblastoma.

**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

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.

9:30 Registration

10:30 Opening Ceremony • **G. Ferrara, E. Boda, M. Romeo**

BRAYN STARTING GRANT SESSION

Chairpersons: C. Calì, V. Chiurchiù, P. Infante, N. Marino

10:45 **Veronica Ceci** (Starting Grant 2024 Winner)
Specialized pro-resolving lipid mediators modulate choroid plexus inflammatory activity.

11:00 **Alessandra Martello** (Starting Grant 2024 Winner)
Neural and Cardiac Dysfunctions in a Parkinson's Mouse Model.

11:15 Lectio Magistralis | **Edvard Ingjald Moser** (Chairmen: G. Ferrara, M. Cambiaghi)
Neural network computation in the entorhinal cortex: From space to time.

12:15 Poster Session 1 with Lunch Box

PARALLEL SESSION (13:00-14:30)

• For scheduled groups only •

BraYn meets **Edvard Ingjald Moser**

SESSION 1 • NEURODEVELOPMENT ORAL COMMUNICATIONS

Chairpersons: E. Tagliatti, G. Borgonovo, M.M. Chellali

14:15 Lecture | **Simona Lodato** (Chairwoman: E. Tagliatti)
Development on the Edge: From Cortical Circuits to Brain Barriers.

14:45 **Elena Ricci** • *Exposure to EDs during pregnancy and lactation alters maternal behavior.*

15:00 **Chiara Tremolanti** • *Heparan sulfate SULFatases ensure correct patterning of stem cell-derived midbrain dopaminergic neurons.*

15:15 **Francesca Postogna** • *Cholesterol dysregulation in Rett syndrome: implications for synaptic function.*

15:30 SpeedTalk | **Antonia Gurgone** • *Extracellular Vesicle-derived miRNAs as non-invasive biomarkers and therapeutic targets for CDKL5 Deficiency Disorder.*

15:35 SpeedTalk | **Tiziana Romanazzi** • *Functional characterization of GAT1 variants involved in SLC6A1-related neurodevelopmental disorder: not all mutations act alike.*

15:40 SpeedTalk | **Livia Metani** • *FLVCR1a Regulates Cortical Interneuron Development and Migration in the Embryonic Cortex.*

15:45 SpeedTalk | **Giovanni Francesco Porcheddu** • *Investigating the pacemaking role of Hcn1 during cortical development.*

SESSION 2 • NEUROINFLAMMATION ORAL COMMUNICATIONS

Chairpersons: M. Bottero, A. Baldassarro, M. Rasile, T. Giannattasio

- 15:50** Lecture | **Burkhard Becher** (Chairwoman: B. Bettegazzi)
Neuroinflammation: Cytokine miscommunication of autoimmune attack?
- 16:20** **Enrica Marzani** • *CDK9 inhibition prevents neuroinflammation and preserves blood-brain barrier integrity in sepsis-associated encephalopathy.*
- 16:35** **Erica Staurenghi** • *Deepening the impact of ApoE4 astrocytes on Alzheimer's disease: a focus on lipid homeostasis alterations and astrocyte reactivity.*
- 16:50** BraYn Educational Symposium ► **Novartis**
Alessia Di Sapia • *The role of Astrocytes in Neuroinflammation and Degeneration in CNS autoimmune Disease.*
Simona Malucchi • *Implementation of GFAP in addition to sNFL in MS patients: a potential tool for objective measuring clinical progression.*
Cecilia Irene Bava • *Longitudinal sNFL and GFAP dynamics in AQP4 positive and negative NMOSD patients: a real-world experience.*
- 17:20** **Antonella Potenza** • *Circulating Biomarkers in Moyamoya Angiopathy: Translating Molecular Signatures into Clinical Needs.*
- 17:35** **Edoardo Mazzone** • *Role of pro-inflammatory T-cells in brain injury evolution after experimental subarachnoid hemorrhage.*
- 17:50** BraYn Educational Symposium ► **Beckman Coulter**
Valerio Chiurchiù • *How to decipher the Resolution Code of Inflammation: Suspicious clues from Parkinson's disease.*
- 18:05** SpeedTalk | **Marica Pagliarini** • *C3 and CD14 modulate diffuse but not focal neuroinflammation in TBI associated with polytrauma.*
- 18:10** SpeedTalk | **Martino Bonato** • *Short-term exposure to particulate matter triggers selective molecular and behavioral alterations in a mouse model of Multiple Sclerosis.*
- 18:15** SpeedTalk | **Fernando Josa-Prado** • *Sex differences in CSF levels of FGF-2 and Anosmi-1 as novel bio-signatures in Multiple Sclerosis progression.*
- 18:20** SpeedTalk | **Sneha Anand** • *Characterization of astrocyte phenotype during epileptogenesis in a mouse model of acquired epilepsy.*
- 18:25** SpeedTalk | **Evridiki Asimakidou** • *Impairment of spatial memory in chronic CNS inflammation is associated with hippocampal microgliosis and inefficient neuronal recruitment.*

18:30 Closing remarks

SESSION 3 • NEURODEGENERATION ORAL COMMUNICATIONS

Chairpersons: S. Bonomi, E. Battocchio, P.A. Raposo Afonso

- 9:30** [Anna Grasso](#) • AAV-mediated gene therapy rescues motor function in a mouse model of Marinesco-Sjögren Syndrome.
- 9:45** [Su-Chun Huang](#) • Disease-Specific Inner Retinal Signatures in Alzheimer's Disease and Cerebral Amyloid Angiopathy Revealed by VBM-OCT.
- 10:00** [Federico Carlini](#) • A new AAV-Based Mouse Model of Parkinson's Disease targeting dopamine synthesis to study levodopa-induced dyskinesia.
- 10:15** [Silvia Castiglioni](#) • Modeling the corticospinal tract in ALS by TARDBP-human assemblies.
- 10:30** [Cecilia Delprete](#) • 10-year retrospective study: cervical and leg dual-site skin biopsy accurately detects pS129- α -Syn in LBD and MSA as a less invasive alternative to three-site protocols.
- 10:45** [Giovanna Menduti](#) • A novel therapeutic candidate for Spinal Muscular Atrophy identified through drug repositioning: evidence from a mouse model and patient-derived cell.
- 11:00** [Athina Grigoriou](#) • Investigating the reciprocal relationship between DNA damage and Dementia with Lewy bodies.
- 11:15** BraYn Educational Symposium ► **Euroclone**
[Laura Koering](#) • The perfect match for RT-QuIC: FLUOstar Omega microplate reader from BMG LABTECH.
- 11:30** SpeedTalk | [Davide Marangon](#) • A human oligodendrocyte-based platform for dissecting myelin dysfunction and uncovering therapeutic targets in Niemann-Pick type C disease.
- 11:35** SpeedTalk | [Zuzanna Kula](#) • GBA2 as a potential therapeutic target in GBA1-PD.
- 11:40** SpeedTalk | [Marcella Palumbo](#) • Cerebrospinal fluid and plasma HDL (dys)function in Multiple Sclerosis.
- 11:45** SpeedTalk | [Luther Loose](#) • Tau protein regulates age-related cognitive decline and structural white matter abnormalities.
- 11:50** BraYn Educational Symposium ► **Vector Builder**
[Connie Rich](#) • Bigger and Better: Optimizing AAV for research and therapies.
- 12:00** Poster Session 2 with Lunch Box

SESSION 4 • NEURO-ONCOLOGY ORAL COMMUNICATIONS

Chairpersons: E. Stanzani, E. Vannini, M. Ibáñez Navarro

- 14:30** Lecture | **Benjamin Deneen** (Chairwoman: E. Vannini)
Decoding the Language of Brain Tumor and Neuron Communication.
- 15:00** **Valentino Ribecco** • Postsurgical ischemia in glioblastoma induces proneural-to-mesenchymal transition, therapy resistance and immunosuppression.
- 15:15** **Alice Reccagni** • Animal-protein diet shapes glioblastoma growth via microbial and neurogenic hydrogen sulfide signaling along the gut-brain axis.
- 15:30** BraYn Educational Symposium ► **Miltenyi**
Stefano Abbiati • Shining Light on Neural Complexity: Imaging Workflows for neuroscience discovery.
- 15:45** **Marta Nowakowska-Desplantes** • Modulation of Calcium Oscillations in Glioblastoma: An Electrophysiological Approach.
- 16:00** **Beatrice Tremonti** • Targeting glioblastoma stem cells: triamterene inhibits CLIC1-mediated proliferation and invasiveness.
- 16:15** BraYn Educational Symposium ► **Revvity**
Guillaume Reveillon • Optical Imaging solutions for Neuroscience.
- 16:30** SpeedTalk | **Massimiliano Del Bene** • Liquid Biopsy in Glioblastoma: Leveraging Plasma Extracellular Vesicles for Tumor Diagnosis, Real-Time Monitoring, and Patient Stratification.
- 16:35** SpeedTalk | **Paolo Cabras** • Multiparametric approach to the identification of novel prognostic biomarker for high grade gliomas.
- 16:40** SpeedTalk | **Lucía Córdoba Esteban** • BRAINTOX: Uremic toxins in chemotherapy response in the brain.
- 16:45** SpeedTalk | **Anastasija Neimane** • Magnetite Nanodiscs in a Sinusoidal Magnetic Field Induce Non-Thermal Mechanical Ablation of Glioblastoma Cells In Vitro.
- 16:50** SpeedTalk | **Sofia Remedía** • A systematic evaluation of Ferroptosis induction as a therapeutic approach in Temozolomide-resistant glioblastoma.
- 17:00** Lecture | **Michael Heneka** (Chairwoman: R.C. Paolicelli)
Innate Immunity in Alzheimer disease.

17:30 Closing remarks

SESSION 5 • NEUROPHYSIOLOGY & NEURAL PLASTICITY ORAL COMMUNICATIONS

Chairpersons: G. Calabrese, P. Lippiello, S. Zucca

- 9:30** [Lara Pizzamiglio](#) • *Excitatory glycine receptors (eGlyRs) in the ventral hippocampus control synaptic plasticity and anxiety-related behaviors.*
- 9:45** [Mario De Gregorio](#) • *From spine shape to synaptic function: exploring the role of SKT/KIAA1217, a novel component of the postsynaptic density complex.*
- 10:00** [Mattia Lorenzo Di Francesco](#) • *Ziapi2 and derivatives for long-term modulation of neuronal activity tuned by light.*
- 10:15** [Eleonora Pali](#) • *Age-dependent dysregulation of the E/I balance in the prefrontal cortex of the IB2 KO mouse model of autism.*
- 10:30** Lecture | [Tommaso Pizzorusso](#) (Chairman: G. Sansevero)
Unraveling the Net: Perineuronal Nets, Critical Periods, and Lifelong Plasticity.
- 11:00** SpeedTalk | [Filippo Balducci](#) • *Expression and roles of Creatine biosynthetic enzymes and transporters in human and mouse oligodendroglia: an in silico and in vivo study.*
- 11:05** SpeedTalk | [Noemi Conte](#) • *Cystatin B as a mediator in cell-cell communication: its neuronal secretion through extracellular vesicle.*
- 11:10** SpeedTalk | [Erika Di Pietro](#) • *Microglia-neuron crosstalk in the remodelling of peritumoral circuits.*
- 11:15** SpeedTalk | [Cecilia Traldi](#) • *Modelling nervous tissue complexity through biofabrication technologies.*
- 11:20** SpeedTalk | [Sonia Sonda](#) • *Psilocybin (un)locked: intracellular drug retention as a novel strategy to modulate neuroplasticity in depression.*

SESSION 6 • NEUROIMAGING & CLINICAL NEUROLOGY ORAL COMMUNICATIONS

Chairpersons: U. Manera, A. Bombaci, R. Rabbito

- 11:25** Lecture | [Gaia Olivo](#) (Chairman: S. Cocozza)
Rapid brain plasticity: Insights from high-resolution imaging and task-based structural MRI.
- 11:55** SpeedTalk | [Maria Martinez-Serrat](#) • *Temporal Dynamics of Serum Neurofilament Light Chain in Multiple Sclerosis: A Retrospective Study in a Clinical Routine Setting.*
- 12:00** SpeedTalk | [Valentina Berni](#) • *Metformin Boosts Temozolomide Response in Glioblastoma: Insights from Patient-Derived Models and Multimodal Imaging.*
- 12:05** SpeedTalk | [Sara Cabras](#) • *Exploring the phenotypic heterogeneity of Amyotrophic Lateral Sclerosis through blood biomarkers: a prospective study.*
- 12:10** SpeedTalk | [Antonio Canosa](#) • *Use of brain 2-[18F]FDG-PET to discriminate ALS and ALS-mimics.*

12:15 Technical Talk (30 min) | **Marzia Munafo** (Chairwoman: I. Battistella)
Creative science illustrations.

12:45 Poster Session 3 with Lunch Box

PARALLEL SESSION (13:00-14:30)

• For scheduled groups only •

BraYn openLab ► Beckman Coulter
(Maria Velasco, Mario Picozza)

Unlocking the brain's secrets with precision neuroscience and advanced cytometry tools.

14:30 BraYn Educational Symposium ► **Evident**
Matteo Lorenzi • *Transforming Precision Imaging: empower your microscopy imaging experiment with quantitative image data.*

14:45 **Gemma Gorla** • *Elucidating RNF213-Mediated Vascular Dysfunction in Moyamoya Angiopathy Pathogenesis: Insights from a Human Vascular Cell Model.*

15:00 **Carlo Ferritto** • *Two Modalities, One Network: A Multilayer Graph Framework for Alzheimer's Disease Biomarkers).*

15:15 **Syed Bilal Nizami** • *Focused Ultrasound Protocol for Safe Delivery of Large Magnetic Nanodiscs for Multimodal Neurostimulation.*

15:30 Closing remarks & BraYn Awards
(Chairpersons: G. Ferrara, M. Romeo, E. Boda, C. Calì, V. Chiurchiù).

SESSION 1 • NEURODEVELOPMENT**Elena Ricci**

Exposure to EDs during pregnancy and lactation alters maternal behavior

Chiara Tremolanti

Heparan sulfate SULFatases ensure correct patterning of stem cell-derived midbrain dopaminergic neurons

Francesca Maddalena Postogna

Cholesterol dysregulation in Rett syndrome: implications for synaptic function

SESSION 2 • NEUROINFLAMMATION**Enrica Marzani**

CDK9 inhibition prevents neuroinflammation and preserves blood–brain barrier integrity in sepsis-associated encephalopathy

Erica Staurenghi

Deepening the impact of ApoE4 astrocytes on Alzheimer's disease: a focus on lipid homeostasis alterations and astrocyte reactivity

Antonella Potenza

Circulating Biomarkers in Moyamoya Angiopathy: Translating Molecular Signatures into Clinical Needs

Edoardo Mazzone

Role of pro-inflammatory T-cells in brain injury evolution after experimental subarachnoid hemorrhage

SESSION 3 • NEURODEGENERATION**Anna Grasso**

AAV-mediated gene therapy rescues motor function in a mouse model of Marinesco-Sjögren Syndrome

Su-Chun Huang

Disease-Specific Inner Retinal Signatures in Alzheimer's Disease and Cerebral Amyloid Angiopathy Revealed by VBM-OCT

Federico Carlini

A new AAV-Based Mouse Model of Parkinson's Disease targeting dopamine synthesis to study levodopa-induced dyskinesia

Silvia Castiglioni

Modeling the corticospinal tract in ALS by TARDBP-human assembloids

Cecilia Delprete

10-year retrospective study: cervical and leg dual-site skin biopsy accurately detects pS129- α -Syn in LBD and MSA as a less invasive alternative to three-site protocols

Giovanna Menduti

A novel therapeutic candidate for Spinal Muscular Atrophy identified through drug repositioning: evidence from a mouse model and patient-derived cells

Athina Grigoriou

Investigating the reciprocal relationship between DNA damage and Dementia with Lewy bodies

SESSION 4 • NEURO-ONCOLOGY**Valentino Ribecco**

Postsurgical ischemia in glioblastoma induces proneural-to-mesenchymal transition, therapy resistance and immunosuppression

Alice Reccagni

Animal-protein diet shapes glioblastoma growth via microbial and neurogenic hydrogen sulfide signaling along the gut-brain axis

Marta Nowakowska-Desplantes

Modulation of Calcium Oscillations in Glioblastoma: An Electrophysiological Approach

Beatrice Federica Tremonti

Targeting glioblastoma stem cells: triamterene inhibits CLIC1-mediated proliferation and invasiveness

SESSION 5 • NEUROPHYSIOLOGY & NEURAL PLASTICITY**Lara Pizzamiglio**

Excitatory glycine receptors (eGlyRs) in the ventral hippocampus control synaptic plasticity and anxiety-related behaviors

Mario De Gregorio

From spine shape to synaptic function: exploring the role of SKT/KIAA1217, a novel component of the postsynaptic density complex

Mattia Lorenzo Di Francesco

Ziapin2 and derivatives for long-term modulation of neuronal activity tuned by light.

Eleonora Pali

Age-dependent dysregulation of the E/I balance in the prefrontal cortex of the IB2 KO mouse model of autism

SESSION 6 • NEUROIMAGING & CLINICAL NEUROLOGY**Gemma Gorla**

Elucidating RNF213-Mediated Vascular Dysfunction in Moyamoya Angiopathy Pathogenesis: Insights from a Human Vascular Cell Model

Carlo Ferritto

Two Modalities, One Network: A Multilayer Graph Framework for Alzheimer's Disease Biomarkers

Syed Bilal Nizami

Focused Ultrasound Protocol for Safe Delivery of Large Magnetic Nanodiscs for Multimodal Neurostimulation

POSTER SESSION 1 • October 29, 12:15-14:15

NEURODEVELOPMENT

- NDV 01** | Testing a novel ERK signalling modulator to counteract neuronal deficits associated with 16p11.2 copy number variants • **Cecilia Zuglian**
- NDV 02** | Investigating Oxytocin system organization in the hypothalamus of CDKL5-deficiency disorder mouse model • **Inas Daher**
- NDV 03** | Whole-Brain Encoding of Opposite-Sex Odors in Mice • **Flavia M.D. Di Fiore**
- NDV 04** | Molecular and synaptic alterations in iPSC-derived glutamatergic neurons from Noonan Syndrome patients • **Dounia Fadel**
- NDV 05** | Sexual dimorphisms in early neuronal network spontaneous activity • **Edoardo Fraviga**
- NDV 06** | Maternal Care in Fragile X Syndrome: Shaping Neuroplasticity and Behavior in Mice • **Alessandra Lodi**
- NDV 07** | Subtype-specific ion channels drive early spontaneous activity during cortical development • **Sara Mancinelli**
- NDV 08** | Maternal Low-Protein Diet and Genistein Exposure Disrupt HPA Axis Development and Induce Sexually Dimorphic Behavioral Outcomes in Offspring • **Marilena Marraudino**
- NDV 09** | Loss of the X-linked Intellectual Disability gene ArhGEF6 affects the migration and electrical functions of forebrain inhibitory neurons • **Giorgio Merlo**
- NDV 10** | Stream of consciousness: dissecting cognitive circuits in schizophrenia MAM model – a proof of concept. • **Barbara Parisi**
- NDV 11** | Unravelling WASHC4 brain-specific function and its implication in intellectual disability • **Aurora Pistella**
- NDV 12** | ARHGAP10 variants impact neurogenesis and cause neurodevelopmental disorders in human patients • **Beatrice Risso**
- NDV 13** | Repurposing of Histone Deacetylase Inhibitors as a potential therapeutic strategy for Myoclonic-Astatic Epilepsy • **Chiara Tesoriero**

NEUROINFLAMMATION

- NI 01** | Nrf2/NOX2 Pathway Signaling and Oxidative Stress Biomarkers Evaluation in the Pathophysiological Link Between Gaucher Disease and Parkinsonism • **Alessio Ardizzone**
- NI 02** | Regulation of Myeloid Immune Cells in Alzheimer's and Parkinson's Disease Patients by the TREM2 Ligand Sulfavant A • **Dalila Carbone**
- NI 03** | Maresin-1 promotes neuroprotection and modulates inflammatory responses in preclinical models of multiple sclerosis • **Giulia Carrera**
- NI 04** | Generation of an integrated cellular model to study demyelination and remyelination • **Arianna Contato**
- NI 05** | Targeting inflammation in depression: a stratified approach with minocycline • **Floriana De Cillis**
- NI 06** | Functional Analysis of EBI2 Signaling in Glial Cell Biology and Remyelination Using Organotypic Cerebellar Slices • **Fatimah Fatimah**

- NI 07** | Exploring the impact of neuronal inflammatory response in an in vitro model of Huntington's disease • **Giulia Rossi**
- NI 08** | Alpha-synuclein in an experimental animal model of Parkinson's disease: morphological, neuroinflammatory, and molecular dynamics • **Federica Servillo**
- NI 09** | Receptor-Specific Neuroimmunomodulatory Actions of Psychedelics: The Roles of 5-HT_{2A} and Sigma-1 receptors • **Yu Ying**

NEURODEGENERATION

- NDG 01** | CRISPR/Cas9 Targeting of AAVS1 Locus for Doxycycline-Inducible NGN2 Expression Improves Neuronal Differentiation from iPSCs • **Andrea Alloni**
- NDG 02** | Boosting NAD in senescent microglia, a useful strategy to counteract neurodegeneration? • **Cecilia Astigiano**
- NDG 03** | Investigating autophagy modulation at the interface between Herpes Simplex Virus 1 infection and APOE4 allele in Alzheimer's disease • **Elisabetta Battocchio**
- NDG 04** | NAD-related dysregulation in asymptomatic phases of ALS: early biomarkers of the disease? • **Andrea Benzi**
- NDG 05** | SARS-CoV2 Nucleocapsid Protein induces Tau pathological changes that can be counteracted by SUMO2 • **Marco Bosica**
- NDG 06** | Engineering the spinal cord in vitro: a multi-scaffold 3D system for modeling cytoarchitecture and neural activity • **Silvia Chasseur**
- NDG 07** | Functional Integration and Circuit Reconstruction by hESC-Derived Striatal Progenitors in the QA-Lesion Model • **Simone Diana**
- NDG 08** | Role of mesocortical dopaminergic pathway in cognitive dysfunctions of Multiple Sclerosis • **Laura Doro**
- NDG 09** | Neural activities and brain function network alteration in a mouse model of Alzheimer's disease. • **Simona Francia**
- NDG 10** | A new perspective on SMA disease beyond spinal motor neurons: what role do cortical projection neurons play? • **Federica La Rocca**
- NDG 11** | Dietary phytoestrogens preserve brain health by targeting mitochondria • **Manuela Leri**
- NDG 12** | Understanding mechanisms of neurodegeneration in Riboflavin Transporter Deficiency (RTD): development of innovative approaches to rescue the diseased RTD neural phenotypes using astrocyte-derived exos • **Valentina Magliocca**
- NDG 13** | Analysis of deregulated microRNAs in plasma-derived small vesicles and their target genes in Frontotemporal Dementia • **Evelyne Minucchi**
- NDG 14** | RNA-seq and miRNA-seq analysis across the Alzheimer's disease continuum • **Evelyne Minucchi**
- NDG 15** | TREM2 structural variants insight in Alzheimer's disease and Frontotemporal Dementia • **Andrea Ninni**
- NDG 16** | Lysosomal Impairment Reshapes Membrane Composition and Metabolism in GCase-Deficient Dopaminergic Neurons • **Carola Pedroli**
- NDG 17** | Transcranial direct current stimulation (tDCS) of the prefrontal cortex modulates dorsal raphe serotonergic activity in an EAE mouse model of multiple sclerosis • **Alessandro Pigozzi**

- NDG 18** | The Effects of Amyloid Beta Oligomers and Fibrils on Oligodendrocyte Progenitor Differentiation and Myelination • **Piotr Pobiaryzyn**
- NDG 19** | Unveiling TREM2 mediated microglia to neuron crosstalk in neurodegeneration • **Paola Poliseno**
- NDG 20** | Targeting Endocannabinoid Signaling to Enhance Adult Neurogenesis and Stress Resilience • **Lucia Scipioni**
- NDG 21** | Changes in AQP4 expression and localization during ALS and SMA progression • **Alessandra Tuninetti**
- NDG 22** | Modeling sensory neuropathy with patient derived-iPSCs: the role of the choline importer FLVCR1 • **Diletta Isabella Zanin Venturini**

NEURO-ONCOLOGY

- NO 01** | PIEZO1 in glioma: the force that fuels a tumour • **Pablo Blanco Carlón**
- NO 02** | Unveiling Key Drivers of Glioma Progression: A Comparative Transcriptomic and Functional Analysis • **Elena Causa**
- NO 03** | Targeting CITK in high-grade brain tumors: a comparison of polypharmacological and specific Inhibitors • **Alessia Ferraro**
- NO 04** | Beyond Brain Tumor Control: Addressing Radiotherapy's Impact on Cognitive Function in GBM • **Noemi Marino**
- NO 05** | Monoclonal antibodies as a therapeutic strategy against Glioblastoma • **Francesca Michela Narcisi**
- NO 06** | Modeling neuron–glioma interactions in vitro using co-culture systems • **Ersilia Nicorvo**
- NO 07** | Cancer-neuronal crosstalk: how neuronal cells contribute to glioblastoma malignancy • **Chiara Saulle**
- NO 08** | Development of an immunocompetent murine model of EGFRvIII-expressing glioblastoma • **Agnese Vincenzi**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

- NP 01** | Oxytocin System Dysregulation and Social Reward Impairment in a Rat Model of Gaming Disorder • **Antonino Casile**
- NP 02** | A Cre-lox technique to address olfactory dopaminergic cell functional heterogeneity • **Ilaria Ghia**
- NP 03** | GluN2A(N615S) Mutation Drives Sex-Specific Seizure Susceptibility and Cardiac Ischemia: Modulation by Ketogenic Diet • **Alessandra Lodi**
- NP 04** | Effects of transcranial electrical stimulation on hippocampal synaptic activity in awake mice • **Ángela Martínez Zafra**
- NP 05** | Impact of diabetic neuropathy in small fiber alterations across sexes • **Emma Merlin**
- NP 06** | Sex differences in the recruitment of spinal dorsal horn inhibitory interneurons in inflammatory pain • **Emma Merlin**
- NP 07** | Chronic microdosing with psilocybin as a novel strategy to promote visual recovery after occipital stroke • **Sofia Nasini**

- NP 08** | Interactions Between Phytoestrogens and Chronic Anorexia-Like Phenotype: Behavioral and Neuroendocrine Changes in Female Rats • **Nebai Ontiveros**
- NP 09** | Combined environmental and P3HT nanoparticle-based approaches to improve visual function in Retinitis pigmentosa • **Edoardo Porzano**
- NP 10** | Decreased neuronal excitability and enhanced frequency adaptation in medial prefrontal cortex pyramidal neurons of depressed mice • **Anita Maria Rominto**
- NP 11** | Spatiotemporal Regulation of CXCR4 in Nerve Regeneration: H₂O₂ as Key Factor • **Marika Tonellato**

NEUROIMAGING & CLINICAL NEUROLOGY

- NIM 01** | Development of Allele-Specific Gene-Silencing siRNAs for MPZ-D61N in CMT1B • **Mattia Camera**
- NIM 02** | Longitudinal assessment of neurodegeneration in a mouse model of tauopathy using multiparametric magnetic resonance imaging • **Annacarla Martucci**
- NIM 03** | Cerebral mapping during dynamic autoregulation using functional near-infrared spectroscopy (fNIRS) • **Marco Romanelli**
- NIM 04** | Enhancing Safety in BBB Opening: A Statistical Approach to Cavitation Monitoring • **Chiara Maria Salzano**
- NIM 05** | The MYSTICALS study protocol: Microorganisms, Toxins and Immunity InterSeCTion In Amyotrophic Lateral Sclerosis Frontotemporal Dementia - targeting disease heterogeneity • **Salvatore Tafaro**

POSTER SESSION 2 • October 30, 11:50-14:30

NEURODEVELOPMENT

- NDV 14** | Investigating therapeutic approaches in Congenital Hydrocephalus • **Diletta Isabella Zanin Venturini**
- NDV 15** | Disrupted CystatinB-TRiC/CCT complex interaction in EPM1: from molecular dysregulation to neural dysfunction • **Natalia Abate**
- NDV 16** | Dissecting the role of the synaptic adaptor protein p140Cap in human cortical development through brain organoids • **Beatrice Bersia**
- NDV 17** | Enhancing human cerebellar organoids with enrichment of region-specific glia • **Olga Teresa Bianciotto**
- NDV 18** | A single cell atlas to unveil the diversity of mouse cerebellar astrocytes: insights into their molecular identities, development, and functions • **Valentina Cerrato**
- NDV 19** | Loss of NR2F1 Impairs Mitochondrial Function in Mouse and Human Models of the Neurodevelopmental Disorder BBSOAS • **Eleonora Dallorto**
- NDV 20** | Modeling infantile neurological disorders linked to Gi/o-cAMP signaling pathway in C. elegans: insights into pathogenic mechanisms and therapeutic responses • **Martina Di Rocco**
- NDV 21** | Impact of Interleukin-17 on neuronal metabolism and synaptic functioning • **Riccardo Grassi**

NDV 22 | Mechanisms and functions of RNF12/RLIM in human neurodevelopment and their disruption in intellectual disability • **Namanpreet Kaur**

NDV 23 | NR2F1 Haploinsufficiency Impairs Hippocampal Oligodendrocyte lineage in BBSOAS mouse models • **Annapaola Lippolis**

NDV 24 | Investigating the role of genetically regulated molecular traits in the phenotypic heterogeneity of autism spectrum disorder • **Camilla Marinelli**

NDV 25 | Genetic determinants of Social Cognition in Amyotrophic Lateral Sclerosis: a cross-sectional study. • **Francesca Palumbo**

NDV 26 | Modelling Tbc1d24 loss of expression in-vivo to study epileptogenesis and test novel therapeutic strategies • **Sara Tessitore**

NEUROINFLAMMATION

NI 10 | Piezoelectric Barium Titanate Nanoparticles Combined with Low-Intensity Pulsed Ultrasound Modulate Microglial Activation • **Massimo Adesse**

NI 11 | Therapeutic effects of short-chain fatty acids on seizures and comorbidities in a mouse model of acquired epilepsy • **Akash Ashok Bera**

NI 12 | Role of astrocytes in the initiation and progression of epilepsy: the focus on TRPV4 channel • **Francesca Buffelli**

NI 13 | Olive Oil Polyphenols as Modulators of Fibrinogen-Driven Neuroinflammation: Implications for Neurodegenerative Disease Prevention • **Federica Carnemolla**

NI 14 | Study of Peripheral Nervous System involvement in early ALS using SOD1G93A mouse model • **Irene Di Patrizi**

NI 15 | TREM2 in Microglia: A Central Player in the Hypothalamic Control of Metabolic Stress? • **Iris Giupponi**

NI 16 | Enhancing anandamide signaling via FAAH inhibition resolves aberrant neuroinflammation in a presymptomatic Alzheimer's disease mouse Model • **Alessandro Leuti**

NI 17 | Phenotypical analysis of innate immune system in neuropathy by antibodies anti-myelin associated glycoprotein • **Emanuela Maria Mobilia**

NI 18 | Sex-specific responses to inflammation in the dorsal and ventral hippocampus • **Giovanni Signorini**

NI 19 | Hydroxytyrosol of olive oil prevents oxysterol-induced neuroinflammation in Alzheimer's disease through sirtuin-1 induction. • **Gabriella Testa**

NI 20 | Morphological Transformation and Metabolic Shift in Friedreich's Ataxia Cerebellar Microglia • **Fabio Zaccaria**

NEURODEGENERATION

NDG 23 | Generation and Characterization of a Knock-in Mouse Model for FLVCR1-Related Sensory Neuropathy • **Raluca Elena Abalai**

NDG 24 | CXCR4: an early injury response marker at the NMJ and a target for functional recovery in ALS • **Chiara Baggio**

NDG 25 | Generation of isogenic lines using CRISPR/Cas9 technology from TBCE mutated iPSCs • **Federica Benigni**

NDG 26 | Altered hematopoiesis in the skull bone marrow in a mouse model of Parkinson's disease. • **Marta Bottero**

NDG 27 | Ultrastructural and Mitochondrial Alterations in a Mouse Model of Lafora Disease • **Vanessa Chiappini**

NDG 28 | Transglutaminase 2 as a novel therapeutic target to study ER-mitochondria contact sites in Alzheimer disease using iPSC model • **Fiorella Colasuonno**

NDG 29 | LRRK2 G2019S impairs GABAergic signaling and perturbs striatal E/I balance in Parkinson's Disease. • **Angela Di Iacovo**

NDG 30 | Exploring the effect of microgravity on beta-amyloid aggregates in transgenic C. elegans lines: insights into gene-environment interactions in neurodegeneration • **Francesca Carmen Follo**

 **NDG 31** | GBA2 as a potential therapeutic target in GBA1-PD. • **Zuzanna Kula**

NDG 32 | Exploring miRNA-mediated regulation of oligodendrocyte progenitor differentiation during brain aging • **Davide Lecca**

 **NDG 33** | Tau protein regulates age-related cognitive decline and structural white matter abnormalities • **Luther Loose**

 **NDG 34** | A human oligodendrocyte-based platform for dissecting myelin dysfunction and uncovering therapeutic targets in Niemann-Pick type C disease. • **Davide Marangon**

NDG 35 | Impaired mitophagy and mitochondrial dynamics in RTD iPSC-Derived Motor Neurons • **Chiara Marioli**

NDG 36 | Analysis of Neurofilament-light chain concentration in cerebrospinal fluid of an ALS patient carrying a novel SOD1 p.A90del mutation, treated with Tofersen and that developed myelitis • **Ilaria Merulla**

NDG 37 | NfL levels during therapy with the antisense oligonucleotide Tofersen in an ALS patient carrying a SOD1 variant and the C9orf72 pathogenic expansion • **Ilaria Merulla**

 **NDG 38** | Cerebrospinal fluid and plasma HDL (dys)function in Multiple Sclerosis • **Marcella Palumbo**

NDG 39 | Correlation between oxidative stress and mitochondrial dysfunction in Alzheimer's disease • **Elisa Pellegrini**

NDG 40 | Evaluating the neuroprotective effects of the novel oestrogen receptor GPER1 in tau related dementia. • **Patricia Alexandra Raposo Afonso**

NDG 41 | A map of transcriptomic profile of Different Brain areas and a longitudinal based study of mitochondrial DNA (mtDNA) methylation in whole blood and brain areas in Alzheimer's disease. • **Bartolo Rizzo**

NDG 42 | Towards ultrasonic neuromodulation mediated by piezoelectric nanoparticles (NPs): a biophysical study on a neuronal cell model • **Martina Valenzuela**

NDG 59 | Small molecules inhibitors of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) as potential pharmacological strategy in Alzheimer's Disease (AD) • **Bianca Papotti**

NEURO-ONCOLOGY

- NO 09** | Longitudinal Tracking of Glioblastoma Clones Acquiring Immune-Evasive Properties • **Davide Ceresa**
- NO 10** | Investigating non-genetic drivers of intra-clonal competition in glioma using a multiomic barcoding strategy • **Sebastian Garcia Mena**
- NO 11** | The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and is involved in medulloblastoma tumorigenesis • **Magdalena Z. Gładysz**
- NO 12** | Modeling Glioblastoma-Microglia Crosstalk in a Patient-Derived 3D Assembloid System • **Arianna Ioni**
- NO 13** | AMBRA1 controls the Sonic Hedgehog signaling pathway and SHH-medulloblastoma • **Shirin Navacci**
- NO 14** | PX-478-mediated HIF-1 α inhibition as a possible mechanism to revert GBM chemoresistance: molecular and biochemical studies • **Martina Nespoli**
- NO 15** | In vitro modeling of early glioma progression using neural organoids • **Chiara Riviera**
- NO 16** | Genome-Wide ATAC-seq Profiling Reveals the Impact of MEOX2 on Chromatin Accessibility in Glioblastoma Stem Cells • **Chiara Maria Salzano**
- NO 17** | Succisa Pratensis: A Natural Enhancer of Chemotherapy in Glioblastoma • **Francesca Servidio**

NEUROPHYSIOLOGY AND NEURAL PLASTICITY

- NP 12** | Unraveling the role of emotional memory in anxiety-related behaviors in rats • **Felice Ciccirelli**
- NP 13** | Oligodendroglia-specific ablation of Citron Kinase is associated with cortical hypomyelination and cognitive impairment which are mitigated by PTEN co-deletion • **Niccolò Di Cintio**
- NP 14** | Importin alpha3 controls age-dependent axonal branching via a ribosomal protein • **Sofia Dotta**
- NP 15** | Decoding the Neural Mechanisms Linking Emotion and Social Behavior in Autism Spectrum Disorder • **Camilla Giglio**
- NP 16** | Cortical dysregulations of GABA signaling and metabolism in Spinal Muscular Atrophy: results from SMA Δ 7 mice • **Giovanna Menduti**
- NP 17** | Evaluating potential Immune System dysregulation in Type 1 Narcolepsy patients • **Emanuela Maria Mobilia**
- NP 18** | Sex-age dimorphism in Importin alpha3 KO mice • **Lucrezia Muraglia**
- NP 19** | Evidence for Food-related Pathological Decision-making in a Mouse Model of Binge Eating Disorder • **Enrico Patrono**
- NP 20** | Cortical immature neuron density in macaques (Rhesus monkey) and its place in the phylogenetic variation. • **Alessia Pattaro**
- NP 21** | Paw posture is a robust indicator of injury, pain, and age. • **Christian Pritz**
- NP 22** | Molecular diversity of adult mouse cerebellar astrocytes • **Giacomo Turrini**

NEURO-IMAGING AND CLINICAL NEUROLOGY

- NIM 06** | Brain metabolic connectivity in ALS due to C9ORF72 hexanucleotide expansion: a 2-[18F]FDG-PET study • **Alessandra Maccabeo**
- NIM 07** | Rubik: a fluorescent reporter to label and manipulate specific neuronal populations • **Giada Pessina**
- NIM 08** | Functional transcranial Doppler (fTCD) neurofeedback for voluntary modulation of cerebral blood flow • **Rosita Rabbito**
- NIM 09** | Sulcal morphometry and cortical morphology in children and adolescents with different APOE genotypes • **Mahan Shafie**

POSTER SESSION 3 • October 31, 12:45-14:30

NEURODEVELOPMENT

- NDV 27** | CDKL5 deficiency disorder and gut disfunctions: exploring the role of Cdkl5 in the organization of the intestinal epithelial barrier • **Giorgia Valetti**
- NDV 28** | Functional characterization of the InSyn1-CDKL5 interaction for dystrophin/dystroglycan complex dependent inhibitory synapse formation • **Serena Baldin**
- NDV 29** | Negr1: a New Possible Convergent Hub for Autistic Spectrum Disorders • **Mariam Marie Chellali**
- NDV 30** | Targeting myelination deficits in CDKL5-KO mice with positive allosteric modulation of mGlu5 receptors • **Sunaina Devi**
- NDV 31** | Anticodon-edited tRNAs: a novel strategy to suppress premature stop codons in Rett syndrome • **Elena Fara**
- NDV 32** | TBC1D24 regulates presynaptic vesicle acidification and recycling in hippocampal neurons via V-ATPase modulation • **Simone Giubolini**
-  **NDV 33** | Extracellular Vesicle-derived miRNAs as non-invasive biomarkers and therapeutic targets for CDKL5 Deficiency Disorder • **Antonia Gurgone**
-  **NDV 34** | FLVCR1a Regulates Cortical Interneuron Development and Migration in the Embryonic Cortex • **Livia Metani**
-  **NDV 35** | Investigating the pacemaking role of Hcn1 during cortical development • **Giovanni Francesco Porcheddu**
-  **NDV 36** | Functional characterization of GAT1 variants involved in SLC6A1-related neurodevelopmental disorder: not all mutations act alike • **Tiziana Romanazzi**
- NDV 37** | CDKL2 as a potential compensatory kinase for CDKL5: common targets and neuronal functions? • **Serena Valastro**
- NDV 38** | Functional Maturation and Network Integration in Human Cortical Organoids and Assembloids • **Tomas Fanutza**

NEUROINFLAMMATION

-  **NI 21** | Characterization of astrocyte phenotype during epileptogenesis in a mouse model of acquired epilepsy • **Sneha Anand**
-  **NI 22** | Impairment of spatial memory in chronic CNS inflammation is associated with hippocampal microgliosis and inefficient neuronal recruitment • **Evridiki Asimakidou**
-  **NI 23** | Short-term exposure to particulate matter triggers selective molecular and behavioral alterations in a mouse model of Multiple Sclerosis • **Martino Bonato**
- NI 24** | “”Activation of Somatostatin-Expressing AgRP Neurons contribute to the pathogenesis of experimental autoimmune encephalomyelitis • **Eleonora Cornacchia**
- NI 25** | Minimally Invasive Neuromodulation: Biocompatibility Assessment of Wireless Sub-100- μ m μ Bots in the Mouse Hippocampus • **Teresa Giannattasio**
-  **NI 26** | Sex differences in CSF levels of FGF-2 and Anosmi-1 as novel bio-signatures in Multiple Sclerosis progression. • **Fernando Josa-Prado**
-  **NI 27** | C3 and CD14 modulate diffuse but not focal neuroinflammation in TBI associated with polytrauma • **Marica Pagliarini**
- NI 28** | EGFR activation correlates with intracranial pressure and survival in a mixed intracranial bleeding porcine model • **Marica Pagliarini**
- NI 29** | Interleukin-9 Modulates Microglial Activation and Inflammatory Signaling in an Alzheimer’s Disease Mouse Model • **Daniel Tortolani**
- NI 30** | Investigating the Impact of Chronic Palmitoylethanolamide on Microglial Immunophenotype and Functional Responses in Neuroinflammatory Contexts • **Daniel Tortolani**

NEURODEGENERATION

- NDG 43** | Integrating different approaches for establishing a multi scale functional validation platform for RNA-based drugs in the CNS (MULTIVAL) • **Chiara Aliprandi**
- NDG 44** | Mutant TAU (P301L) enhances global protein translation in differentiated SH-SY5Y cells • **Osvaldo Artimagnella**
- NDG 45** | Neuronal Loss of the Alzheimer’s disease Risk Factor CALM Perturbs Synaptic Plasticity and Exacerbates Amyloid Pathology • **Domenico Azarnia Tehran**
- NDG 46** | Exploring Cognitive and Behavioral Reactions to Monoclonal Anti- β -Amyloid Antibody Treatment in Wild-Type Mice • **Negin Biglari**
- NDG 47** | α -SYNUCLEIN/ α -TUBULIN INTERPLAY IN PARKINSON’S DISEASE • **Brigitta Bonaldo**
- NDG 48** | Development of a chemically aged cerebral organoid model to investigate the pathogenesis of fatal familial insomnia • **Eleonora Busani**
- NDG 49** | Endocannabinoid-driven modulation of progranulin expression in a model of senescent Tg2576-derived microglia • **Francesca Ciaramellano**
- NDG 50** | Evaluation of 6 monoclonal antibodies against Ser129-phosphorylated α -synuclein: Critical role of proteinase K antigen retrieval and superior sensitivity of the D1R1R clone in human skin biopsies • **Cecilia Delprete**
- NDG 51** | Comparison between D-loop methylation level and mtDNA copy number in Duchenne and Becker muscular Dystrophies patients • **Francesca Dragoni**

- NDG 52** | Discovering MAP1B: a new phenotypic determinant in sporadic ALS patients • **Francesca Dragoni**
- NDG 53** | Cerebral organoids as a model of FrontoTemporal Dementia (FTD) • **Clelia Ferrero**
- NDG 54** | Increased levels of 24-hydroxycholesterol reduce okadaic acid-induced tau hyperphosphorylation and neuronal morphological degeneration • **Serena Giannelli**
- NDG 55** | Cortical Dendritic Remodeling in a Murine Model of Oxaliplatin-Induced Neurotoxicity • **Giulia Iezzi**
- NDG 56** | Restoring Cognitive Function in 3xTg-AD Mice by Targeting the Prefrontal Cortex–Raphe Circuit via transcranial Alternating Current Stimulation (tACS) • **Federica Marchiotto**
- NDG 57** | Multimodal study of brain reconfigurations in an experimental model of multiple sclerosis • **Giulia Vasirani**
- NDG 58** | The role of Extracellular vesicles in the spreading of tauopathies • **Noemi Orsini**
- NDG 60** | Investigating the interaction between α -synuclein and Connexin 32 channels in the context of Parkinson's disease • **Barbara Rijtano**
- NDG 61** | The potential role of PCSK9 in the pathogenesis of Alzheimer's disease • **Sara Rosano**
- NDG 62** | CAP2 as a novel regulator of mitochondrial dynamics in neurons • **Ramona Stringhi**

NEURO-ONCOLOGY

-  **NO 18** | "Multiparametric approach to the identification of novel prognostic biomarker for high grade gliomas" • **Paolo Cabras**
-  **NO 19** | BRAINTOX: Uremic toxins in chemotherapy response in the brain • **Lucía Córdoba Esteban**
-  **NO 20** | Liquid Biopsy in Glioblastoma: Leveraging Plasma Extracellular Vesicles for Tumor Diagnosis, Real-Time Monitoring, and Patient Stratification • **Massimiliano Del Bene**
- NO 21** | Targeting pediatric CNS tumors with NKG2D-CAR T cells: A preclinical success • **Marta Ibáñez Navarro**
- NO 22** | Studying STAG2-driven medulloblastoma by in vitro and in vivo models • **Antonella Lettieri**
- NO 23** | The Impact of Healthy Fecal Material Transplantation on Glioma • **Xingzi Lin**
-  **NO 24** | Magnetite Nanodiscs in a Sinusoidal Magnetic Field Induce Non-Thermal Mechanical Ablation of Glioblastoma Cells In Vitro • **Anastasija Neimane**
- NO 25** | Deciphering and Targeting miR675-5p derived MOONLIT sequences: Unraveling Hypoxia-Driven Resistance in Glioma • **Chiara Pellizzer**
-  **NO 26** | A systematic evaluation of Ferroptosis induction as a therapeutic approach in Temozolomide-resistant glioblastoma. • **Sofia Remedia**

NEUROPHYSIOLOGY AND NEURAL PLASTICITY

-  **NP 23** | Expression and roles of Creatine biosynthetic enzymes and transporters in human and mouse oligodendroglia: an in silico and in vivo study • **Filippo Balducci**
- NP 24** | Brain-wide analysis of perineuronal nets and parvalbumin-positive interneurons in fragile X syndrome mice across postnatal development highlights region-specific alterations influenced by age and sex • **Lorenzo Cifarelli**

NP 25 | Sex- and region-specific modulation of perineuronal nets, parvalbumin interneurons, and microglia by ketogenic diet in a Grin2a mouse model of developmental and epileptic encephalopathy • **Lorenzo Cifarelli**



NP 26 | Cystatin B as a mediator in cell-cell communication: its neuronal secretion through extracellular vesicle • **Noemi Conte**



NP 27 | Microglia-neuron crosstalk in the remodelling of peritumoral circuits • **Erika Di Pietro**

NP 28 | Overexpression of Anosmin-1 alters the myelination pattern and the architecture of axonal components in cerebellar Purkinje cells • **Alessia Fieromonte**

NP 29 | The interplay between importin alpha3 and CBX2 in axonal regeneration and pain • **Giada Musso**

NP 30 | Alcohol mixed with energy drinks increases compulsive operant behavior by altering the mesocorticolimbic dopaminergic system • **Roberta Puliga**



NP 31 | Psilocybin (un)locked: intracellular drug retention as a novel strategy to modulate neuroplasticity in depression • **Sonia Sonda**



NP 32 | Modelling nervous tissue complexity through biofabrication technologies • **Cecilia Traldi**

NP 33 | Multisensory Integration of Social Cues in the ventromedial Prefrontal Cortex • **Stefano Zucca**

NEURO-IMAGING AND CLINICAL NEUROLOGY



NIM 10 | Metformin Boosts Temozolomide Response in Glioblastoma: Insights from Patient-Derived Models and Multimodal Imaging • **Valentina Berni**



NIM 11 | Exploring the phenotypic heterogeneity of Amyotrophic Lateral Sclerosis through blood biomarkers: a prospective study. • **Sara Cabras**



NIM 12 | Use of brain 2-[18F]FDG-PET to discriminate ALS and ALS-mimics • **Antonio Canosa**



NIM 13 | Temporal Dynamics of Serum Neurofilament Light Chain in Multiple Sclerosis: A Retrospective Study in a Clinical Routine Setting • **Maria Martinez-Serrat**

NIM 14 | Sex Differences in Cognitive Reserve Mechanisms in Amyotrophic Lateral Sclerosis: A 2-[18F] FDG-PET Study • **Enrico Matteoni**

COMMUNI
ORAL
CATIONS

Exposure to EDs during pregnancy and lactation alters maternal behavior

[Elena Ricci](#) ⁽¹⁾ - Emily Guarini ⁽²⁾ - Marilena Marraudino ⁽¹⁾ - Stefano Gotti ⁽¹⁾

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Endocrine disruptors (EDs) are compounds that affect the endocrine system by interfering with hormone synthesis and action, altering organ development, reproductive and metabolic processes. Bisphenols and perfluoroalkyl compounds are key EDs studied, used in industrial processes to produce everyday products, i.e. plastics for food packaging. This creates a direct pathway for human exposure, with potential risks for pregnant women and their offspring, about which little is known.

This study examined how maternal exposure to bisphenol A (BPA), bisphenol S (BPS), and perfluorooctanesulfonic acid (PFOS) affects mothers' spontaneous behavior and anxiety levels. CD1 mouse mothers were orally treated during pregnancy and lactation and divided into four groups: 1) control, 2) BPA (0.2 ng/kg), 3) BPS (0.2 ng/kg), and 4) PFOS (0.7 ng/kg). The mothers were subsequently tested for maternal and anxiety-related behaviors. Feeding behavior indicated an increase in food intake among PFOS-treated mothers, while their body weight remained unchanged. The pups from these mothers were significantly heavier than the controls, whereas the pups exposed to BPA were smaller. These characteristics persisted throughout development. Behavioral tests (elevated plus maze and open field) revealed increased anxiety in PFOS mothers, while BPA mothers showed hyperactivity. Maternal care analysis from PND2 to PND8 revealed that PFOS mothers exhibited fewer maternal behaviors, spending less time nursing and more time outside the nest. Additionally, immunohistochemical analysis of vasopressin immunoreactivity revealed altered vasopressin content in the paraventricular nucleus of PFOS-treated mothers, suggesting a neuroendocrine basis for behavioral changes.

In conclusion, BPA, BPS, and PFOS exposure during pregnancy and lactation resulted in diverse behavioral and metabolic effects. Future analyses will focus on the sexual and anxiety behavior of adult puppies and neural markers linked to these topics.

Heparan sulfate SULFases ensure correct patterning of stem cell-derived midbrain dopaminergic neurons

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Parkinson's disease (PD) is characterized by the degeneration of midbrain dopaminergic (mDA) neurons, driving interest in cell replacement therapies that rely on understanding mDA developmental programs. While intrinsic transcriptional networks (e.g., *OTX2*, *FOXA2*, *LMX1A*) and patterning cues (*SHH*, *WNT1*, *FGF8*) are well-studied, the role of extrinsic niche factors—particularly heparan sulfate (HS)-modifying enzymes, such as the extracellular SULFases (*SULF1/2*)—remains unclear. Recent single-cell RNA sequencing datasets identified *SULF1/2*-enriched radial glia in the human developing ventral midbrain, suggesting these enzymes shape the signaling microenvironment critical for mDA neurogenesis and regional identity. We hypothesized that *SULF1/2* regulate mDA differentiation by modulating HS interactions with key morphogens like *WNT* and *FGF8*, influencing their activity and downstream signaling. Using CRISPR/Cas9, we generated *SULF1/2* knockout (KO) human iPSCs and differentiated them into mDA neurons *via* an accelerated 16-day protocol. *SULF1/2* double KO (DKO) cells exhibited reduced expression of midbrain floor plate markers (*OTX2*, *FOXA2*, *LMX1A*) and reduced TH+ mDA neuron generation. Moreover, DKO cells exhibited elevated expression of multiple hindbrain/spinal cord *HOX* genes, alongside the axial elongation factor *CDX2*, indicative of a shift toward posterior identities. Remarkably, blocking FGF signaling partially rescued the DKO phenotype, leading to restored expression of the midbrain marker *OTX2*, a reduction in *CDX2*+ posterior progenitors, and increased yields of TH+ mDA neurons. These findings indicate that SULFs regulate the balance between midbrain and hindbrain/spinal cord cell fates, primarily through modulation of FGF signaling. Furthermore, our single-nucleus RNA sequencing of WT, DKO, and rescued-DKO cells demonstrated a pronounced disturbance of regional patterning and cell fate acquisition in *SULF1/2* DKO cells, reflected by a more complex cellular heterogeneity. Our findings position *SULF1/2* as crucial niche regulators of mDA development by fine-tuning anterior-posterior patterning, thus offering potential targets for improving mDA neuron differentiation protocols and ultimately PD cell therapies.

Cholesterol dysregulation in Rett syndrome: implications for synaptic function

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder mainly caused by mutations in the X-linked *MECP2* gene, which represents the main genetic cause of intellectual disability in girls worldwide. Since *MeCP2* is highly expressed in mature neurons, the majority of studies are focused on this cell type. However, besides neurons, astrocytes have been identified as active contributors to RTT pathogenesis, as *Mecp2* knock-out (KO) astrocytes fail to correctly support neuronal maturation and synaptogenesis. Indeed, culturing wild-type (WT) neurons with KO astrocytes or treating them with KO astrocyte-conditioned medium (ACM) affects their synaptic phenotype. Of note, one of the key synaptogenic factors released by astrocytes is cholesterol, which plays a crucial role in synapse formation and functioning. Several data highlight a defective cholesterol metabolism in RTT, supporting the hypothesis that abnormalities in astrocyte-produced cholesterol might contribute to synaptic dysfunctions. In this study, we report a downregulation of genes involved in cholesterol synthesis and secretion in *Mecp2* KO astrocytes and an alteration in the activation of *Srebp2*, that represents the master regulator of cholesterol metabolism. In a therapeutic perspective, we found that Trofinetide, the only FDA-approved drug for RTT, attenuates in KO astrocytes the transcriptional defects of cholesterol-related genes, whereas cholesterol treatment completely rescues the synaptic alterations both in WT neurons treated with KO-ACM and *Mecp2* heterozygous (HET) neurons. To corroborate *in vitro* evidence, we moved to investigations in adult mouse models, proving a deregulation of key factors involved in cholesterol metabolism in the brain of *Mecp2* deficient animals. Collectively, these data demonstrate an alteration of cerebral cholesterol metabolism in RTT and suggest cerebral cholesterol supply as a possible therapeutic strategy for repairing synaptic dysfunctions in RTT.

CDK9 inhibition prevents neuroinflammation and preserves blood–brain barrier integrity in sepsis-associated encephalopathy

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Sepsis is a complex health condition displaying a hyperinflammatory state leading to sepsis-associated encephalopathy (SAE), causing blood brain barrier (BBB) disruption and glial activation. Recent findings proved that cyclin-dependent kinase 9 (CDK9) hyperactivation elicits endothelial derangements, but its effect on cerebral microvessels and glial cells is still unknown. We tested the effects of CDK9 selective inhibition on sepsis-related BBB impairment and neuroinflammation *in vitro* and *in vivo*.

In vivo sepsis was induced by cecal ligation and puncture (CLP) in C57BL mice. 1h *post* CLP/Sham procedure, animals received the selective CDK9 inhibitor LDC (50mg/kg) or vehicle. Organs were harvested at 24h. *In vitro* LPS 500ng/ml+TNF α 5U/ml+IFN γ 10U/ml (LTI) reproduced sepsis on human brain microvascular endothelial cells (TY10) in presence/absence of LDC. Statistical significance ($p < 0.05$) was assessed by one-way ANOVA.

Septic mice developed encephalopathy, as led by lethargy and tremors' incidence (nil in SHAM group, while 70% of CLP mice witnessed them) and the increase in plasma concentrations of neurofilament light chain – indicative BBB disruption – as well as inflammatory markers (CCL-2, IL-6 and ICAM-1) in brain. Interestingly, LDC reduced their expression and improved neurological symptoms. Consistently, analysis on microglial cells volume showed that LDC prevented the microglial remodeling induced by sepsis and restored the microglia in its quiescent state. To deepen whether these outcomes are mediated by BBB, we tested LDC *in vitro* on TY10. Endothelial leakage increased with LTI – a 40%-raised permeability to dextran and claudin-5 degradation were recorded– while its barrier function ameliorated inhibiting CDK9, decreasing permeability and restoring membrane levels of claudin-5.

Our data suggests that pharmacologically targeting CDK9 may represent an innovative approach to prevent neuroinflammation and BBB dysfunction during sepsis-associated encephalopathy.

Deepening the impact of ApoE4 astrocytes on Alzheimer's disease: a focus on lipid homeostasis alterations and astrocyte reactivity

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Sporadic Alzheimer's disease (AD) accounts for the vast majority of AD cases and arises from a complex interplay of genetic, environmental, and lifestyle factors, making it a central focus of research. The $\epsilon 4$ allele of the apolipoprotein E gene (ApoE4) is the strongest genetic risk factor for sporadic AD. ApoE, mainly produced by astrocytes, plays a crucial role in brain cholesterol transport and homeostasis. We investigated the impact of the ApoE genotype on astrocyte cholesterol metabolism and reactivity, with a focus on the differences between ApoE3 and ApoE4 isoforms. Using immortalized mouse astrocytes derived from human ApoE3 or ApoE4 knock-in mice, we demonstrated that ApoE4 astrocytes show significant alterations in cholesterol metabolism, including abnormal levels of total cholesterol, cholesterol precursors, oxysterols, fatty acids, and disrupted expression of key lipid-related genes. In particular, the transcription factor peroxisome proliferator activated receptor γ (PPAR γ), which supports lipid homeostasis and has anti-inflammatory and neuroprotective roles, was markedly downregulated in ApoE4 astrocytes. This was confirmed at the protein level and aligns with findings from human AD brain tissues carrying the $\epsilon 4$ allele. Additionally, the expression of lipid transporters and receptors such as ABCA1, LDLR, LRP1, and ApoER2 was altered, further supporting a dysregulation of lipid handling in ApoE4 astrocytes. In a complementary human-induced pluripotent stem cell (hiP-SC)-based model, ApoE4 astrocytes exhibited increased reactivity to pro-inflammatory stimuli compared to ApoE3 counterparts, potentially linked to PPAR γ suppression. These findings suggest that ApoE4 promotes both lipid dyshomeostasis and a reactive astrocyte phenotype, contributing to AD pathology. Targeting pathways that restore PPAR γ function and lipid balance may represent promising therapeutic strategies for mitigating AD progression, particularly in high-risk ApoE4 carriers.

Circulating Biomarkers in Moyamoya Angiopathy: Translating Molecular Signatures into Clinical Needs

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Moyamoya Angiopathy (MA) is a rare cerebrovascular disorder depicted by steno-occlusive lesions in cerebral arteries and formation of fragile collateral vessels. It affects children and young adults, leading to ischemic/haemorrhagic strokes. Surgical revascularization is the only treatment. Since MA pathogenesis is still unknown, the identification of circulating biomarkers represents a key plan for unravelling disease mechanisms. This study aims at performing cellular/molecular profiling of selected circulating markers in MA peripheral blood, plasma, and cerebrospinal fluid (CSF) samples. By integrating molecular findings with clinical data, this study seeks to uncover candidate markers for a more accurate disease course monitoring. A cohort of 121 adult MA patients was recruited at the Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan. Demographic, clinical, neuroradiological/neurosurgical features were collected. Fluorescence-activated cell sorting analyses were performed on fresh whole blood, by identifying circulating endothelial progenitor cells (EPCs). Different immunoassays approaches were used to assess specific factor levels (e.g., epiregulin, EREG; endoglin) in CSF and plasma samples. Cellular/molecular markers were then correlated with MA clinical features. Suitable statistical analyses were performed. A reduced EPC% was detected in MA patients exhibiting haemorrhagic stroke as first event and characterized by bilateral cerebral hemisphere involvement vs those experiencing an ischemic stroke and monolateral disease presentation. Increasing trends of plasma EREG and CSF endoglin levels were identified in patients who experienced novel cerebrovascular events upon diagnosis vs those who did not. Overall, our findings indicate that specific cellular/protein factors - primarily involved with neovascularization/ neuroinflammation - may serve as promising circulating markers for an improved MA patient stratification and disease progression monitoring.

Role of pro-inflammatory T-cells in brain injury evolution after experimental subarachnoid hemorrhage

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Background. Subarachnoid hemorrhage (SAH) triggers sustained neuroinflammation, including immune cell recruitment and activation. T-cells, in particular, are known to promote secondary brain injury after ischemic stroke; however, their role in SAH remains unclear. This study characterized T-cell recruitment and pathological contribution in a clinically relevant mouse SAH model. **Methods.** Adult wild-type (WT) and CD3 knockout (KO, T-cell-depleted) mice underwent pre-chiasmatic cistern blood injection to induce SAH or sham procedure. At 7 days post-injury (dpi), brains were collected for neuroinflammatory gene expression (Nanostring® transcriptomic analysis and RT-PCR) and histology. Additionally, three SAH patient cohorts were evaluated for T-cell levels in blood and CSF at 2, 5, and 10 dpi. **Results.** Transcriptomic profiling revealed a major involvement of T-cells and microglia after SAH. Histology showed CD3+ T-cell infiltration, predominantly CD4+ (80%), in brain parenchyma, meninges and choroid plexus in SAH WT mice ($p < 0.01$ vs sham). Similarly, in human patients, a progressive CD4+ T-cell increase was reported in CSF ($p < 0.05$ 10d vs 2d), supporting clinical relevance of experimental observations. In SAH WT mice, neuronal death ($p < 0.01$) and microglial activation ($p < 0.001$) were observed in the same area of T-cell infiltration and hippocampus. In contrast, CD3-KO mice displayed widely reduced microglial activation, evaluated by Iba1 stained area (ns vs sham), with key pro-inflammatory microglial genes that were upregulated in SAH WT mice ($p < 0.01$ vs sham) remained unaltered in CD3-KO mice after SAH (ns vs sham). Importantly, CD3-KO mice also preserved hippocampal neuronal viability and showed reduced microglial reactivity (ns vs sham) after SAH. **Conclusions.** These findings indicate T-cells as key modulators of neuroinflammation and secondary injury evolution, by driving microglial pro-inflammatory activation and promoting neuronal death after SAH.

AAV-mediated gene therapy rescues motor function in a mouse model of Marinesco-Sjögren Syndrome

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Marinesco-Sjögren syndrome (MSS) is a rare genetic disorder with onset in early infancy, primarily characterised by cerebellar ataxia and myopathy. It is caused by loss-of-function mutations in the SIL1 gene, encoding a co-chaperone of BiP, leading to endoplasmic reticulum (ER) stress and maladaptive unfolded protein response. The woozy mouse, which carries a spontaneous Sil1 mutation, closely recapitulates the human disease, exhibiting progressive cerebellar ataxia with Purkinje cell (PC) degeneration and skeletal muscle myopathy. To explore the feasibility of gene therapy for MSS, we developed adeno-associated viral (AAV) vectors encoding either mouse or human SIL1wt, under the control of a ubiquitous (CAG) or PC-specific (L7-6) promoter. These constructs were packaged into PHP.eB capsids, and correct ER localization of transgenic SIL1 was confirmed in vitro in mouse embryonic fibroblasts. Newborn woozy mice were injected intracerebroventricularly (ICV) into the lateral ventricles. Motor performance was evaluated using the beam-walking and accelerated rotarod tests and calbindin immunostaining was used to quantify PC survival. ICV delivery of AAV encoding either mouse or human SIL1 under the CAG promoter resulted in robust SIL1 expression and significant motor improvement. At 9 weeks of age, treated woozy mice performed comparably to wild-type controls and showed a significant preservation of cerebellar PCs. Results were similar using AAVs encoding SIL1 under the L7-6 promoter, indicating that SIL1wt expression in PCs is sufficient to rescue cerebellar ataxia. In a separate long-term study, motor function was sustained up to 26 weeks post-injection, at which point animals were sacrificed for histopathological analyses. In conclusion, ICV administration of AAVs encoding SIL1 results in robust transgene expression in the brain and provides long-lasting protection against motor dysfunction in woozy mice. Ongoing studies are currently evaluating the extent of cerebellar neuroprotection and the potential impact of SIL1 gene therapy on skeletal muscle pathology.

Disease-Specific Inner Retinal Signatures in Alzheimer's Disease and Cerebral Amyloid Angiopathy Revealed by VBM-OCT

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Background Alzheimer's Disease (AD) and cerebral amyloid angiopathy (CAA) are characterized by amyloid- β (A β) deposition and brain atrophy. We lack non-invasive, low-cost biomarkers to diagnose and monitor AD and CAA progression. Voxel-based morphometry with Optical Coherence Tomography (VBM-OCT) is a novel, non-invasive imaging method capable of detecting focal retinal changes. We employed VBM-OCT to investigate inner retinal changes in AD and CAA and their associations with A β pathology and brain atrophy.

Methods We included patients with biomarker-confirmed AD (N=19, age=71 $\pm$ 8y, MMSE = 22 $\pm$ 5) and CAA (N=16, age=76 $\pm$ 6y, MMSE = 26 $\pm$ 2). All patients underwent CSF analysis (A β 40, A β 42, Tau, pTau), brain MRI (global [GCA], medial temporal [MTA], posterior cortical atrophy [PCA]), and macula OCT scans. VBM-OCT quantified focal changes of retinal layers in each subject relative to a healthy database (N=28, age=49 $\pm$ 11y). Correlations between the retinal, CSF, and MRI markers were assessed.

Results VBM-OCT revealed coexisting focal thickening and atrophy across all inner retinal layers, with distinct spatial distribution. In pooled analyses, VBM-ganglion cell layer (GCL) atrophy correlated with CSF A β 40 level ($r=-0.56^{**}$), and VBM-inner nuclear layer (INL) thickening with MTA score ($r=0.45^*$), predominantly driven by AD patients. In AD, VBM-INL thickening correlated with lower CSF A β 42 ($r=-0.62^*$). In CAA, VBM-GCL and inner plexiform layer (IPL) atrophy correlated with higher CSF Tau ($r=-0.69^*$ & -0.77^{**}), while VBM-INL thickening was correlated with lower MMSE score ($r=-0.62^*$). No other correlations were found.

Discussion AD and CAA demonstrated distinct retinal signatures on VBM-OCT. In CAA, inner retina abnormalities were linked to Tau-related neurodegeneration and cognitive decline. In contrast, VBM-INL thickening in AD reflected AD-specific pathology, including CSF A β 42 and MTA. These findings support the potential of VBM-OCT to detect disease-specific retinal alterations.

A new AAV-Based Mouse Model of Parkinson's Disease targeting dopamine synthesis to study levodopa-induced dyskinesia

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Levodopa Induced Dyskinesia (LID) is a pathological condition resulting from prolonged treatment of Parkinson's Disease (PD) with Levodopa (L-DOPA). Despite its efficacy, L-DOPA remains the most widely used drug for PD. However, after a few years, it leads to the development of LID, characterized by involuntary, stereotypical movements and an irreversible decline in therapeutic benefit.

The 6-hydroxydopamine (6-OHDA) murine model of PD involves unilateral damage to dopaminergic neurons in the substantia nigra pars compacta (SNc) via injection of the neurotoxin into the medial forebrain bundle (MFB), which contains projections from these neurons. Although adeno-associated viruses (AAVs) now enable direct in vivo genetic manipulation, and AAV α -Synuclein PD models successfully replicate synucleinopathy, the 6-OHDA model remains the gold standard for studying LID.

Our aim is to develop a novel AAV-based PD model using a Cre-dependent Reversible Silencing System (RSS) to target dopamine (DA) synthesis, thereby enabling the study of LID mechanisms. The system uses siRNAs against DOPA decarboxylase (DC) and Tyrosine Hydroxylase (TH), delivered by retrograde AAV. Upon Cre recombinase expression, the silencing cassette is removed, switching fluorescent labeling from EGFP to mCherry. The system was validated in murine primary hippocampal neurons and will next be tested in the dopaminergic MN9D cell line.

We are currently testing direct SNc injection. Mice will be monitored daily, and LID will be induced four weeks post-AAV injection and scored in PD-positive subjects.

This AAV-based reversible tool offers a new approach to modeling PD and LID, allowing both the induction and reversal of DA synthesis impairment. It provides a platform to study DA-dependent plasticity in LID and could support the development of models mimicking the progressive DA decline observed in PD.

Modeling the corticospinal tract in ALS by TARDBP-human assembloids

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder affecting both upper and lower motor neurons, leading to muscle paralysis and early death. While most cases are sporadic, approximately 10% are familial and involve pathogenic germline variants in several genes, including *TARDBP*, which encodes TDP-43, a DNA/RNA-binding protein essential for multiple steps of RNA metabolism. TDP-43 is ubiquitously expressed, with higher levels in neurons, where it regulates alternative splicing, stability, and transport of coding and noncoding RNAs, including those involved in neuronal survival and neurodegenerative pathways. In ALS, both genetic mutations and post-translational modifications lead to cytoplasmic mislocalization and aggregation of TDP-43, contributing to motor neuron degeneration. We aim to model and investigate the molecular and cellular mechanisms driving degeneration of the corticospinal tract in ALS using human cortical–spinal assembloids carrying pathogenic *TARDBP* variants. To this end, we generated cortical and spinal organoids from human iPSC lines harboring *TARDBP* mutations and their isogenic controls. Organoid identity and maturation at time of assembly were confirmed through immunostaining and molecular markers, such as EMX1, NESTIN, PAX6 and MAP2 for cortical identity, and SOX2, HOXB4, ISLET1, and SMI32 for spinal lineage. Subsequently, cortical and spinal organoids were fused to create corticospinal assembloids. Neuropathological and electrophysiological analyses were performed on mature assembloids to assess ALS hallmarks, focusing on TDP-43 pathology. Our human cortical–spinal assembloid recapitulates key pathological features of ALS associated with *TARDBP* mutations and represents a promising tool to study the molecular and cellular mechanisms underlying corticospinal tract degeneration. This model enables analysis of neurodegenerative processes and complex neuronal interactions in a human 3D context.

10-year retrospective study: cervical and leg dual-site skin biopsy accurately detects pS129- α -Syn in LBD and MSA as a less invasive alternative to three-site protocols

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The in vivo detection of phosphorylated α -synuclein (pS129- α -syn) in cutaneous nerve fibers via immunofluorescence has emerged as a promising biomarker for the diagnosis of synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA). Despite its potential, heterogeneity in skin biopsy protocols—particularly in the selection and number of anatomical sampling sites—represents a major limitation to clinical standardization and broad applicability. This study aimed to evaluate the diagnostic accuracy of different anatomical site combinations for detecting pS129- α -syn in a large retrospective cohort, with the goal of identifying a sampling strategy that maximizes sensitivity while minimizing patient burden and promoting methodological consistency. A total of 227 patients with confirmed synucleinopathies—194 with Lewy body diseases (LBD) and 33 with MSA—were included. All patients had undergone skin biopsies from three anatomical sites: cervical region (CE), thigh (TH), and distal leg (LEG). Diagnostic sensitivity was calculated for each site and all possible combinations, with stratification by sex and disease duration. The results demonstrated that the combination of CE and LEG provided the highest diagnostic sensitivity, achieving 97.68% in the LBD group and 100% in the MSA group. These outcomes were consistent across sex and disease duration subgroups. The addition of the TH site contributed minimal diagnostic value when combined with CE and LEG. In conclusion, the CE+LEG dual-site biopsy protocol offers high diagnostic accuracy for both LBD and MSA, representing a less invasive and more clinically practical alternative to the tri-site approach. The observed variation in pS129- α -syn deposition patterns between disease subtypes highlights the need for further research to refine the biomarker's diagnostic and pathophysiological significance.

A novel therapeutic candidate for Spinal Muscular Atrophy identified through drug repositioning: evidence from a mouse model and patient-derived cells

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Spinal Muscular Atrophy (SMA) is a severe neuromuscular disorder caused by *Survival Motor Neuron 1* (*SMN1*) gene mutations, leading to reduced SMN protein levels and progressive motor neuron (MN) degeneration. The homologous gene, *SMN2*, due to incorrect splicing, fails in rescue SMA phenotypes. Some limitations of current therapies still claim the research for new approaches aimed at increasing SMN levels. As identified through a screening of FDA-approved drugs, the antipsychotic GT5 (code name) emerged as a potent enhancer of *SMN2* transcript expression and splicing across multiple disease models. Using the delta 7 SMA mouse model, here we assessed the therapeutic effects of GT5 administration on survival, motor function, neuroprotection, and neuroinflammation, by histological, molecular and RNA-seq analyses of spinal cord and muscle samples. Compared to untreated condition, GT5 treatment increased lifespan (+15%) and improved motor performance in SMA mice, upregulated SMN expression in spinal cord ($\geq 50\%$) and muscles (≥ 1 -fold), reduced MN loss (cleaved caspase-3 apoptotic marker levels: $\leq 63\%$; MN density $\geq 90\%$), and attenuated neuroinflammation by decreasing GFAP signaling ($\leq 37\%$) and modulating microglial activation. Moreover, GT5 enhanced neuromuscular junction integrity and muscle trophism, suggesting additional peripheral benefits. Notably, RNA-seq analysis of GT5-treated spinal cords revealed extensive splicing changes, including direct SMN target transcripts, supporting enhanced SMN activity. We also evaluated GT5 efficacy in human SMA models using co-cultures of patient iPSC-derived MNs and myotubes, and observed enhanced MN survival and increased SMN expression, consistent with both SMN-dependent and neuroprotective mechanisms. Given its central nervous system penetrance and existing clinical approval, GT5 emerges as a promising candidate for SMA therapy.

Investigating the reciprocal relationship between DNA damage and Dementia with Lewy bodies

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Dementia with Lewy bodies (DLB) is characterised by the accumulation of alpha-synuclein (aSyn) in Lewy bodies and neurites, with early and prominent pathology in the neocortex and limbic system, corresponding to cognitive fluctuations and visual hallucinations. Although the nuclear presence of aSyn remains debated, growing evidence, including our own, supports its role in DNA damage repair. However, in DLB, nuclear aSyn appears to be pathologically altered, contributing to DNA damage and disease progression. Accordingly, we have previously reported the alteration of DNA damage repair (DDR) proteins in the nuclear proteome of DLB cortical tissue. To further elucidate DDR changes across neurodegenerative diseases (NDs) and to evaluate alterations in both nuclear and mitochondria DDR, we have quantified cytoplasmic and nuclear DDR changes in postmortem pre-frontal cortex tissues from control, prodromal, Alzheimer's disease (AD) and DLB cases. Specifically, markers of nuclear double strand break repair (DSBR) and mitochondrial and nuclear base excision repair (BER) were investigated. Preliminary results suggest an early downregulation of cytoplasmic DDR protein with corresponding increases in nuclear DDR proteins in prodromal cases. Consistent with the broad involvement of DNA damage across NDs we have observed nuclear proteome alterations in both DSBR and BER pathways in AD, DLB and mixed cohorts. On-going work will utilise tissue microarray slides immunostained for DSBR and BER markers, alongside aSyn pathology to investigate the temporal relationship between hallmark pathological changes and DNA damage. Moreover, we will employ dopaminergic-differentiated SH-SY5Y cells and cortical organotypic cultures exposed to pre-formed α Syn fibrils and DNA-damaging genotoxins, individually and in combination, to dissect the role of aSyn in DNA damage and repair pathways. These studies aim to delineate pathogenic mechanisms linking genomic instability to neurodegenerative progression, thereby informing the development of targeted therapeutic interventions.

Postsurgical ischemia in glioblastoma induces proneural-to-mesenchymal transition, therapy resistance and immunosuppression

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Glioblastoma (GBM) has a dismal prognosis, with a median survival of 8 months and a 5-year survival rate of just 6.9%. Despite extensive research, GBM resistance mechanisms remain unclear. While GBM's resistance to chemoradiation is well established, the impact of surgical resection on residual tumor cells and their response to therapy remains unexplored. To study how resection affects residual GBM plasticity and the tumor microenvironment (TME), we developed a stereotaxic fluorescence-guided resection of GBM on two syngeneic and one patient-derived mouse models. We employed time-resolved bulk- and single-cell transcriptomics, proteomics, epigenomics, digital pathology, and intravital microscopy. To assess the specific impact of surgery on tumor progression, we assembled a rare international cohort of GBM patients who underwent two surgeries in a short timeframe. For these uncommon cases, we performed a longitudinal analysis including neuropathology, single-nucleus, and spatial transcriptomics. In both preclinical and clinical samples, we found that surgery causes acute vascular disruption and leakage, causing hypoxia which promotes proneural-to-mesenchymal transition (PMT), enhances DNA repair, radiotherapy resistance, self-renewal, and tumorigenicity. Surgery also reshaped the immune TME, reducing T cells, NK cells, B cells, dendritic cells, and increasing immunosuppressive populations such as hypoxic macrophages, regulatory T cells, and neutrophils, along with diminished antigen presentation. Integrated multi-omics analyses identified hypoxia-inducible factor 1-alpha (HIF-1 α) as a key node in postsurgical signaling. Importantly, these molecular changes increase resistance to conventional therapies. Although surgery is a key step in GBM treatment, it may induce an ischemic, immunosuppressive state that undermines adjuvant therapy. Nevertheless, these findings reveal a post-surgical window of vulnerability where early interventions could improve GBM outcomes.

Animal-protein diet shapes glioblastoma growth via microbial and neurogenic hydrogen sulfide signaling along the gut-brain axis

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Glioblastoma (GB) is the most aggressive primary brain tumor, with limited therapeutic options and poor prognosis. Recent research highlighted the gut-brain axis (GBA) role in influencing central nervous system pathophysiology, including tumor progression. One possible mechanism embroils the production of hydrogen sulfide (H₂S), a gaseous signaling molecule mainly produced by gut microbiota through the metabolism of dietary proteins by gut microbiota. In this study, we explored how gut-derived H₂S affects glioblastoma growth via both direct (blood circulation) and indirect (vagal afferents-mediated) mechanisms. Using a glioma-bearing model of GB, we administered a standard animal-protein (AP) diet or a control diet containing animal-derived protein for two weeks prior to tumor inoculation and for the next three weeks. To block bacterial H₂S production in the gut, mice received aminooxyacetic acid (AOAA), an inhibitor of cystathionine β-synthase (CBS), ad libitum in drinking water. Contextually, we investigated the vagal pathway by pharmacologically blocking TRPV1 channels, highly expressed on vagal afferents, with capsazepine (CPZ). Further, at the end of the experiment, fecal samples were collected to assess gut-microbiota modification. Our findings reveal that an AP diet reduced tumor growth, likely through shifts in H₂S production and AOAA treatment reversed its antitumor effect and modify tumor microenvironment, suggesting a protective role of gut-derived H₂S. Accordingly, CPZ treatment promoted tumor growth, implicating the involvement of the vagal afferent signaling via TRPV1 in H₂S effects on the brain. These results suggest that H₂S has both direct and indirect effects on GB progression. Increasing dietary H₂S bioavailability could be a novel therapeutic approach that could be used alongside GB's standard of care to improve patient outcomes.

Modulation of Calcium Oscillations in Glioblastoma: An Electrophysiological Approach

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Glioblastoma (GBM) is a highly aggressive malignant brain tumour with a median survival of only 8 to 14 months despite standard surgery and chemotherapy. One defining feature of GBM cells is their ability to form an interconnected network through calcium (Ca^{2+})-mediated communication. A subset of these cells (5-10%) shows spontaneous Ca^{2+} oscillations, which promote tumour progression even after surgical resection. These oscillations are regulated by a complex interplay of internal Ca^{2+} stores, store-operated Ca^{2+} channels, and Ca^{2+} -activated potassium channels. The latter modulate the membrane potential, which regulates Ca^{2+} transport into the cells. In our novel approach, we aimed to modulate GBM Ca^{2+} oscillations using electrophysiological interventions. In our preliminary study on U87 cells transfected with the NEMOc Ca^{2+} reporter, we demonstrated that external positive electrical field stimulation (200 ms monophasic pulses at 1 Hz, +2 V, 15 min) significantly reduced the frequency of Ca^{2+} oscillations (frequency before stimulation: 0.75-1.5/min; during and after stimulation: 0-0.75/min). Negative electrical fields and alternating positive and negative fields had no significant effect on the Ca^{2+} oscillation frequency. In another experiment, NEMOc-transfected U87 cells grown as a 3D tumour on the chorioallantoic membrane of a chick embryo were subjected to an external electrical field stimulation. Alternating positive and negative fields (biphasic pulses at 100 Hz, ± 2 V, 4 min) resulted in a large Ca^{2+} influx at the beginning of the stimulation, followed by focused oscillations throughout the session. After the stimulation ended, the oscillations ceased. These findings highlight the potential to manipulate GBM Ca^{2+} dynamics through targeted electrical interventions. Further research is needed to determine whether these approaches can influence tumour progression *in vitro* and *in vivo*, opening new possibilities for therapeutic strategies aimed at treating GBM.

Targeting glioblastoma stem cells: triamterene inhibits CLIC1-mediated proliferation and invasiveness

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Glioblastoma (GBM) is the most frequent malignant primary brain tumor in adults, characterized by rapid recurrence and limited survival. Glioblastoma stem cells (GSCs), resistant to chemo- and radiotherapy, are key drivers of recurrence and treatment failure. Therefore, GSC pharmacological targeting is critical for eradicating GBM.

The chloride channel CLIC1, constitutively active in GSCs, plays a pivotal role in sustaining pro-oncogenic GBM features, thus representing a promising pharmacological target. Through a drug repurposing strategy, and based on structural similarity with biguanides like metformin, we identified the potassium-sparing diuretic triamterene (TMT) as a potential CLIC1 inhibitor. In whole-cell patch-clamp experiments, TMT inhibited CLIC1 activity in GSCs, with an EC_{50} ~200-fold lower than metformin. Molecular dynamics simulations revealed four potential binding sites likely to increase protein rigidity and prevent its activation via membrane insertion. CLIC1 blockade by TMT reduced GSC proliferation in 2D and 3D cultures, delayed G1-S transition, and decreased tumor growth in zebrafish xenografted with GSCs. TMT depleted GSCs *in vitro*, as confirmed by limiting-dilution assays. TMT activity was selective for GBM cells, showing significantly lower effects on human astrocytes, oligodendrocytes, and brain endothelial cells. Notably, TMT impaired GSC invasiveness in 3D culture independently of antiproliferative effects. Co-treatment with TMT enhanced temozolomide's anti-tumor potency ~80-fold (dose-reduction index >300). These findings support TMT as a promising adjuvant in GBM therapy and a valuable lead compound for developing new CLIC1-targeting agents.

Work supported by #NEXTGENERATIONEU (NGEU) and funded by the Ministry of University and Research (MUR), National Recovery and Resilience Plan (NRRP), project MNESYS (PE0000006) – A Multiscale integrated approach to the study of the nervous system in health and disease (DN. 1553 11.10.2022)

Excitatory glycine receptors (eGlyRs) in the ventral hippocampus control synaptic plasticity and anxiety-related behaviors

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Excitatory glycine receptors (eGlyRs) have recently emerged as a novel neuronal signaling modality that challenges the traditional view of glycine as an inhibitory neurotransmitter. Composed of the glycine-binding NMDA receptor subunits GluN1 and GluN3A, eGlyRs are insensitive to glutamate and activated by glycine only. Unlike conventional GluN1/GluN2 NMDARs, the distribution and role of eGlyRs remain poorly understood. Recent data from our laboratory and others have evidenced that they are functionally expressed in specific areas of the adult brain including the medial habenula (MHb), the basolateral amygdala (BLA) and the neocortex. Here, combining anatomical, electrophysiological and behavioral studies, we investigate eGlyR expression and operation in the adult mouse hippocampus. We show that eGlyRs are highly enriched in the ventral hippocampus (VH), conferring distinct property to this brain region. eGlyRs display a striking dorsoventral gradient within CA1 pyramidal cells, being expressed in the ventral but not dorsal hippocampus (VH vs DH). In contrast, SST-positive and PV-positive interneurons express eGlyRs in both subregions. eGlyRs mediate tonic inward currents and regulate neuronal excitability. They are also responsible for the attenuated long-term potentiation (LTP) in the VH compared with the DH, and are necessary for the stress hormone corticosterone to control the extent of VH LTP. In line with their important role in VH circuitry and function, eGlyRs contribute to the modulation of anxiety-related behaviors. Overall, our data establish eGlyR signaling as an important modality of intercellular communication in the adult brain, with strong implications in neuropsychiatric-relevant regions that control internal states and emotional processing.

From spine shape to synaptic function: exploring the role of SKT/KIAA1217, a novel component of the postsynaptic density complex

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The postsynaptic density (PSD) is a highly organized protein network essential for excitatory synaptic transmission and plasticity. Disruption of PSD architecture is increasingly implicated as a mechanism underlying neurodevelopmental disorders such as autism and intellectual disability. SKT/KIAA1217 emerges as a novel and uncharacterized brain-enriched protein with specific enrichment at the PSD. Biochemical and imaging approaches indicate that SKT functions as a scaffold protein, forming specific interactions with core PSD constituents such as PSD-95 and SHANK family proteins. In SKT knockout (KO) mice, loss of SKT results in reduced dendritic spine density and a shift toward immature spine morphologies characterized by increased filopodia and decreased mushroom spines. These structural alterations are accompanied by a significant reduction in PSD-95 cluster size and intensity, consistent with impaired synaptic organization. Remarkably, re-expression of full-length SKT or its N-terminal region is sufficient to rescue spine defects, demonstrating a direct role for SKT in PSD organization. Electrophysiological recordings from SKT KO hippocampal slices and primary neurons reveal attenuated excitatory synaptic transmission, characterized by reduced AMPAR-mediated responses and a diminished readily releasable vesicle pool, without alterations in receptor levels or presynaptic release probability. MEA analysis indicates delayed neuronal development and reduced network synchronization. Finally, SKT KO mice exhibit motor coordination deficits and excessive self-grooming, recapitulating hallmark phenotypes of PSD-95 and SHANK3 knockout models. These findings establish SKT as a novel PSD scaffold critical for synaptic structure and function, with potential implications for the pathophysiology of neurodevelopmental synaptopathies.

Ziapi2 and derivatives for long-term modulation of neuronal activity tuned by light

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Ziapi2 is a novel photochromic compound engineered for membrane penetration, enabling the modulation of the passive membrane properties that is tunable by light. Ziapi2 is particularly efficient in evoking neuronal action potentials (APs) triggered by light, via a direct modification of membrane capacitance, leaving unaltered the activity of ion channels, receptors, and other membrane proteins unaltered, and without requiring gene manipulation. The increased membrane capacitance occurring in the dark is responsible for a reduction in AP dynamics, with an overall decrease in the network firing activity. On the contrary, the molecular isomerization from *trans* to *cis*, elicited by cyan illumination, generates a phasic drop in capacitance, triggering neuronal firing. Ziapi2 has the potential to tune AP frequency in a bimodal fashion, by inhibiting/activating neurons in the dark vs light configuration. To exploit these properties, we investigated the ability of Ziapi2 to decrease the firing rate of neurons pharmacologically subjected to hyperexcitability by administration of the K-channel blocker 4-aminopyridine (4AP). Interestingly, Ziapi2 normalized the paroxysmal activity of hyperactivated neurons and epileptic-like networks, as assessed by patch-clamp and multielectrode array (MEA) electrophysiology. We are currently confirming the ability of Ziapi 2 to normalize the epileptic phenotype in human iPS-derived excitatory neurons and, *in vivo*, in epileptic Zebrafish and murine models. Our study aims to develop novel strategies to treat neurological disorders characterized by hyperexcitability, offering the possibility to tune neuronal firing, in the dark or under light stimulation, by interfering exclusively with membrane capacitance.

Funded by “Ricerca Finalizzata 2021” (GR-2021-12374630), and “5xmille” (5M-2022-23685330).

Age-dependent dysregulation of the E/I balance in the prefrontal cortex of the IB2 KO mouse model of autism

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Autism spectrum disorders (ASDs) are neurodevelopmental conditions characterized by social deficits and repetitive behaviors. Disruptions during critical periods of brain development can significantly impact cortical function and are recognized as hallmarks of ASD. To this end, we investigated electrophysiological alterations in the medial prefrontal cortex (mPFC) of the IB2 knockout (KO) mouse model of ASD across key developmental stages. In juvenile IB2 KO mice (P18–23), high-density multielectrode array recordings revealed increased mPFC network activity in acute brain slices. Voltage-sensitive dye imaging further confirmed an elevated excitation/inhibition (E/I) ratio within cortical columns, peaking at layer 5 (L5). Consistently, whole-cell patch-clamp recordings from L5 pyramidal neurons showed increased excitability that was abolished by inhibition blockade, enhanced NMDA receptor-mediated excitatory postsynaptic currents (EPSCs), and reduced inhibitory postsynaptic currents (IPSCs). We also found a more depolarized GABA_A reversal potential and a decreased frequency of spontaneous IPSCs (sIPSCs). This suggests inhibitory dysfunction and cortical hyperexcitability in the mPFC of juvenile mutant mice. Surprisingly, in early adult IB2 KO mice (P28–35), the GABA_A reversal potential normalized, indicating a delayed maturation of chloride homeostasis. However, sIPSC frequency was markedly increased, hinting at maladaptive inhibitory compensation for the increased E/I ratio at early stages. *In vivo* recordings from anesthetized early adult mice (P28–35) further confirmed this shift toward hyperinhibition, revealing a decreased basal firing of IB2 KO pyramidal neurons in the mPFC. Overall, these findings point to a shift in the E/I ratio from early hyperexcitability to late hyperinhibition in the mPFC circuitry, likely driven by delayed GABAergic development and aberrant circuit rearrangements, revealing a critical age-dependent cortical dysfunction in this ASD model.

Elucidating RNF213-Mediated Vascular Dysfunction in Moyamoya Angiopathy Pathogenesis: Insights from a Human Vascular Cell Model

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Moyamoya angiopathy (MA) is a rare steno-occlusive disease characterized by an unstable network of compensatory collateral vessels, that leads to ischemic and hemorrhagic strokes. The disease etiology is still unknown; however, the E3-ubiquitin ligase RNF213 has been identified as a susceptibility gene in the East Asian population, where the p.R4810K variant exhibits a founder effect, leading to a higher incidence compared to Caucasians. The age of disease onset shows a bimodal distribution (0–10 and 40–49 years), with the adult peak characterized by a stronger female prevalence (ratio 3:1). Angiography remains the current diagnostic gold standard. No specific pharmacological therapy is available for MA; therefore, patients are treated with antiplatelet and anticoagulant agents. The most effective treatment approach is neurosurgical revascularization. Microfluidic chips or miniature tissues, including human 3D primary cell models, are increasingly considered more representative than animal models, especially for neurological diseases or species-specific cellular phenotypes. To investigate the molecular and cellular mechanisms underlying MA pathogenesis, we developed an in vitro model using human endothelial and vascular smooth muscle cells. A hypoxic stimulus was induced to mimic ischemic injury, following transient RNA interference targeting two potentially involved factors (RNF213 and its putative phosphatase PTP1B). The mRNA and protein expression of angiogenic factors were evaluated through RT² Profiler arrays, qRT-PCR assays, and Western blot analyses; angiogenic function was assessed via tube-formation assays. Our findings contributed to unveiling the elusive function of RNF213, providing the first evidence of an interaction between PTP1B and RNF213 in MA. These factors may be involved in vascular modulation and regulation of angiogenesis during hypoxic stress. Moreover, our results indicate RNF213 itself as a critical factor for cerebral vessel formation.

Two Modalities, One Network: A Multilayer Graph Framework for Alzheimer's Disease Biomarkers

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Alzheimer's disease (AD) is a progressive neurodegenerative condition associated with widespread disruptions in both functional and structural brain connectivity. Recent advances in network neuroscience have highlighted the value of combining multiple imaging modalities to better understand these alterations. In this work, we adopt a multilayer graph (MG) framework to integrate structural connectivity derived from diffusion MRI (dMRI) and functional connectivity extracted from resting-state functional MRI (rs-fMRI). To address acquisition-related variability of the Alzheimer's Disease Neuroimaging Initiative (ADNI) multi-center dataset, we applied the CovBat harmonization method to connectivity matrices, ensuring consistency across acquisition protocols while preserving biological variability. Each MG was constructed by linking the functional and structural layers via replica nodes. This representation preserves the topological information of each modality while capturing their interactions. From the harmonized MGs, we extracted a rich set of node-level metrics that characterize networks in terms of centrality, modularity, segregation/integration and efficiency. These features were used to train machine learning classifiers to distinguish between individuals with AD, mild cognitive impairment (MCI), and healthy controls. Our results show that the harmonized MG framework enhances classification performance and provides greater interpretability in identifying network-based biomarkers of disease. The integration of multimodal imaging through multilayer models, combined with advanced harmonization and graph-theoretical analysis, offers a robust and scalable approach for studying complex brain disorders such as Alzheimer's disease. This method supports the discovery of meaningful, reproducible biomarkers and opens new avenues for multimodal network-based diagnostics.

Focused Ultrasound Protocol for Safe Delivery of Large Magnetic Nanodiscs for Multimodal Neurostimulation

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Magnetic iron-oxide nanodiscs (MNDs, $\leq 130\text{nm}$) permit multimodal neurostimulation—thermal and mechanical—by engaging distinct neuronal pathways. Their intrinsic magnetism also makes them MRI contrast agents, linking local concentration to modulatory efficacy. Yet their relatively large size complicates non-invasive delivery to the brain. We therefore refined a focused-ultrasound (FUS) strategy that drives MNDs across the blood–brain barrier (BBB) while preserving tissue integrity. FUS transiently enlarges tight junctions and alters efflux transporters, including P-glycoprotein (P-gp). Whereas most BBB-permeable nanoparticles are $<100\text{nm}$, our approach targets significantly larger MNDs. We systematically varied acoustic settings that dictate cumulative tissue pressure (CTP) to maximize nanodisc deposition and safely influence efflux activity. Wild-type mice underwent bilateral FUS with a motorized 1.5 MHz transducer operating at a continuous 100% duty cycle. The beam scanned a 6 mm path (four vertical tracks) at 10 mm s^{-1} for 2 min; each focal spot experienced $\sim 0.1\text{s}$ per pass, amounting to $\sim 5\text{s}$ total exposure. A bolus of $100\text{ }\mu\text{L}$ SonoVue microbubbles was injected 5s before sonication at peak negative pressures (PNP) of 0.6 or 0.75 MPa. MND biodistribution and P-gp levels were quantified by Perl's blue and immunofluorescence, whereas transmission electron microscopy evaluated particle dispersion and agglomeration. Delivering 0.75 MPa markedly increased MND accumulation versus 0.6 MPa and coincided with significant P-gp down-regulation (21 a.u. in treated vs 32 a.u. in contralateral cortex). Importantly, no hemorrhage or other overt damage was detected, underscoring the safety of the applied CTP for transporting oversized particles. Future experiments will employ this optimized 0.75MPa regimen to position MNDs for on-demand, precise, real-time, and robust, effective multimodal neuromodulation in vivo, paving the way for fully translatable, patient-oriented therapies tomorrow.

1

Testing a novel ERK signalling modulator to counteract neuronal deficits associated with 16p11.2 copy number variants

NDV 01

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Copy number variants (CNVs) at the 16p11.2 chromosomal region, encompassing both deletions and duplications, are associated with intellectual disability (ID), autism spectrum disorder (ASD), speech deficits, attention-deficit/hyperactivity disorder (ADHD), learning deficits and epilepsy. The 16p11.2 duplication is also associated with schizophrenia (SZ) and bipolar disorder. The 16p11.2 locus is a 600 kb genomic region, encompassing 27 protein-coding genes, including *MAPK3*. This gene encodes ERK1 that, together with ERK2, participates in the MAPK/ERK signalling cascade, a key player in cell survival, plasticity, behaviour, and brain development.

Our lab recently designed a novel ERK signalling modulator, RB5, a cell permeable peptide corresponding to amino acids 7–38 of the human ERK1 sequence. By interfering with ERK1's preferential binding to importin α 1, RB5 promotes ERK2 binding to importin α 1 and nuclear translocation, thereby enhancing overall ERK signalling.

In this work, we aimed to investigate the effect of RB5-mediated ERK enhancement on neuronal differentiation in neural progenitors derived from human induced pluripotent stem cells (iPSCs). Our preliminary data indicate that RB5 accelerates neurogenesis in neural progenitor cells. Ongoing work is aimed to further characterize RB5 activity across distinct neurodevelopmental stages and assess its potential as a therapeutic candidate for neurodevelopmental disorders associated with 16p11.2 CNVs.

Investigating Oxytocin system organization in the hypothalamus of CDKL5-deficiency disorder mouse model

NDV 02

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Cyclin-dependent kinase-like 5 (CDKL5) is a gene on the X chromosome encoding a serine/threonine kinase, predominantly expressed in the brain, that is crucial for neuronal and synaptic development, cortical circuits organization, sensorimotor and cognitive functions. Mutations in CDKL5 gene leads to a severe neurodevelopmental encephalopathy, CDKL5 deficiency disorder (CDD), characterized by neural and synaptic malfunctions and is manifested by early-onset of epileptic seizures, motor impairments, and autistic traits. Oxytocin (OT) is a neuropeptide that plays key roles in the regulation of important neural, cognitive functions and social behaviour. Additionally, there exists evidence associating the deficiency in OT signalling with autism pathophysiology. Accordingly, we here hypothesized that the cognitive and social deficits observed in CDKL5-Knock Out (KO) mice could be associated to an atypical morphofunctional organization of the OT system. To test this, we analysed OT-positive neurons distribution in the hypothalamus of adult CDKL5-KO male mice and heterozygous (HET) female mice compared to the respective wild-type (WT) littermates. The quantitative analysis of OT immunofluorescence on confocal microscopic images revealed that CDKL5 HET females show an increased mean number of OT-expressing neurons compared to WT controls, along with region-specific alterations in both KO males and HET females compared to WT littermates. These findings suggest that lack of CDKL5 interferes with the normal development of OT system, and thus offer new insights into the neurobiological basis of social dysfunction in CDD. Further exploration in CDKL5 mutant mice, including the analysis of the distribution of OT receptor expression, the morphology of OT-positive cells, and the organization of OT-positive projections into telencephalic areas is currently ongoing with the goal to clarify the impact of the CDKL5 kinase on the role of this crucial pleiotropic hypothalamic hormone.

Whole-Brain Encoding of Opposite-Sex Odors in Mice

NDV 03

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Sexual imprinting is a form of learned mate preference in which individuals develop attraction or avoidance to traits observed during their early social environment. These preferences, acquired during a critical developmental window, shape adult mate choice and promote pairing with partners likely to provide high-quality parental care. In rodents, olfaction is the major sensory modality and plays an important role in regulating social behaviors, particularly in mate selection. Here, we performed whole-brain analyses of females reared with and without their fathers and then exposed to familiar and unfamiliar olfactory stimuli. We also measured females' behavior during stimulus exposure, to correlate brain activation with behavioral performance. Additionally, for generalisation and ecological validation, we compared whole-brain activation and behavior in C57BL/6j laboratory females with wild house mice. Neuronal activity was measured using whole-brain immunolabeling for the immediate early gene c-Fos. Brains were cleared using the iDISCO protocol and imaged by light-sheet fluorescence microscopy. We used ClearMap for single-cell detection and brain atlas registration. Our results show clear differences between laboratory and wild mice in both recruited brain areas and behavior. Laboratory mice actively interact with stimuli, whereas wild mice have less interaction with stimuli, with increased contact to familiar compared to novel odor cues. Differences in behavior correlate with differences in brain activation. Although we see an overall overlap in macro-regions between experimental groups, wild mice show less activation compared to laboratory mice, and specific recruitment of different subregions. Brain activation correlates well with differences in behavioral performance between laboratory and wild mice highlighting different specific strategies for odor-driven social decision-making and providing insight into the neural basis of learned mate preferences.

Molecular and synaptic alterations in iPSC-derived glutamatergic neurons from Noonan Syndrome patients

NDV 04

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Noonan syndrome (NS) is a rare genetic disorder, most caused by mutations in the *PTPN11* gene. These mutations lead to hyperactivation of the encoded Shp-2 tyrosine phosphatase, which plays a key role in the Ras/mitogen-activated protein kinase/extracellular signal-regulated kinase (Ras/MAPK/ERK1/2) signaling cascade. Approximately 50% of individuals with NS experience cognitive impairments, ranging from moderate intellectual disability to deficits in higher-order cognitive functions, particularly learning and memory. To date, no pharmacological treatments are available for these symptoms, and the molecular mechanisms through which Shp-2 hyperactivation affects neuronal function remain poorly understood.

One of the major limitations in understanding the molecular aspects of diseases affecting the central nervous system (CNS) is the lack of suitable experimental models that accurately recapitulate key pathological features. Access to patient brain tissue is limited, and animal models often fail to fully recapitulate human phenotypes. These limitations can be addressed using induced pluripotent stem cells (iPSCs), which are generated by reprogramming somatic cells from patients and differentiating them into disease-relevant neuronal subtypes while preserving the patient's genetic background. This approach provides a powerful *in vitro* model for investigating disease mechanisms.

Since previous studies have shown that Shp-2 hyperactivity in neurons, driven by pathogenic *PTPN11* mutations, has been associated with dysregulation of glutamatergic signaling, we utilized the iPSC technology to generate human glutamatergic neurons for the study of NS-associated cognitive deficits. iPSCs were derived from peripheral blood mononuclear cells (PBMCs) of two NS patients carrying the *PTPN11* 188A>G mutation and exhibiting cognitive impairments. A specific differentiation protocol was developed to generate a mixed neuronal population, including approximately 70% glutamatergic neurons, from both patient-derived iPSCs and a commercially available, genetically normal control iPSC line. Cultures were characterized at three different time points using morphological, biochemical, and molecular techniques.

Immunofluorescence analysis and real-time PCR revealed a reduction in the expression of both glutamatergic and synaptic markers in patient-derived neuronal cultures, accompanied by increased gliogenesis. Moreover, Western Blot analysis showed elevated expression levels of both Shp-2 and ERK1/2 in these cultures, suggesting that Shp-2 hyperactivation may impair the development and maturation of the glutamatergic neuronal population in NS patients.

All together, these findings suggest that iPSC-derived neuronal models from NS patients with cognitive impairments represent a promising platform for elucidating the molecular basis of the disease's neurological manifestations and for identifying novel therapeutic targets.

Sexual dimorphisms in early neuronal network spontaneous activity**NDV 05**[Edoardo Fraviga](#) ⁽¹⁾⁽¹⁾ *Humanitas University, Humanitas University/Pharmacology and Brain Pathology, Rozzano, Italy*

Neuronal development represents a finely tuned process occurring during early postnatal life that paves the way for the proper brain functioning in adulthood. Among the factors found to be crucial for this process, the early electrical activity represents a key aspect whose alterations may have a deep impact for the proper establishment of neuronal circuits. By combining multiple functional approaches, we longitudinally assessed the early postnatal development stages in both male and female. We firstly employed high-density MEA (HD-MEA) technology to probe the dynamic changes of neural network activity along development in both male and female. Cortical-hippocampal acute brain slices were established at different postnatal (P), developmental stages (P5-P10-P15) and spontaneous network activity was recorded. Although network activity progressively increased during development in both sexes, female exhibited higher network activity during the first postnatal week (P5) compared to male. Moreover, at P5, female also showed higher intracellular chloride levels, measured via two-photon microscopy, suggesting a higher hyperpolarizing action of GABA signaling compared to males. Interestingly, this trend reversed, with female displaying lower intracellular chloride levels than male. These findings reveal a sexual dimorphism in the early stages of brain development. Understanding these differences may shed light on the sex-dependent susceptibility to neurodevelopmental disorders.

Maternal Care in Fragile X Syndrome: Shaping Neuroplasticity and Behavior in Mice

NDV 06

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Fragile X syndrome (FXS) is the most prevalent monogenic (Fmr1 gene) cause of intellectual disability and autism spectrum disorder. Behavioral symptoms are described by anxiety, hyperactivity, profound social deficits, as well as cognitive impairment; this latter, is strictly correlated to brain plasticity and closure of postnatal critical periods by perineuronal nets (PNNs). In addition to genetic influences, environmental factors play a critical role in shaping the behavioral phenotype in FXS: adverse environments, including maternal stress and insufficient caregiving, can exacerbate neurodevelopmental and behavioral symptoms.

We examined the maternal behavior of Fmr1 +/- and Fmr1 -/- KO mothers and their strain-matched control wild-types (C57BL/6J). Subsequently, analysis of behavior and PNNs expression (density and colocalization with Parvalbumin positive neurons), was performed on male offspring to evaluate the consequences of maternal neglect.

Our analysis revealed that both Fmr1 +/- and Fmr1 -/- KO mothers exhibited poorer maternal behavior compared to wild-type (WT) dams. Notably, heterozygous mothers showed hyperactivity and the poorest maternal care among the three genotypes. Significant consequences have been observed on the offspring's analysis, concerning anxiety-like behaviors, short-term memory, social interaction, and executive functions, as well as variation in neuroplasticity and in different brain areas.

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Subtype-specific ion channels drive early spontaneous activity during cortical development

NDV 07

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Cortical development is shaped by a complex interplay of genetic programs, environmental cues, and electrical activity, which allows developing neurons to form early networks and generate spontaneous spatiotemporal activity even before birth. Early neuronal activity - marked by a variety of oscillatory patterns - progresses through distinct stages and plays a critical role in the establishment of mature neuronal circuits. These activities influence several developmental processes, including neurogenesis, migration, synaptogenesis, and their dysregulation is often linked to the onset of neurological disorders, such as epilepsy and autism spectrum disorders. Despite the recognized importance of early spontaneous activity, the molecular underpinnings and cellular substrates of this phenomenon remain largely unexplored. We investigate whether the emergence of neuronal identity is linked to a specific ion channel repertoire in cortical neurons and how their early spontaneous activity is modulated. Using longitudinal single-cell transcriptomic analysis, we found that excitatory projection neuron (PN) subtypes possess unique ion channel configurations that are dynamically acquired along their maturation trajectories and contribute to their spontaneous activity. By integrating *in utero* genetic perturbations, pharmacological inhibition of selected ion channels, and single-cell transcriptomics, we examined how Hcn1, a channel known to play a fundamental role in stabilizing membrane potential, influences the connectivity and functional differentiation of the layer 5 Subcerebral Projection Neuron (ScPN) subtype. Our findings suggest that Hcn1 modulates spontaneous electrical activity in emerging ScPN circuits, implicating Hcn currents as pivotal signals for cortical wave propagation during development. These insights support the notion that specific ion channel repertoires are integral to the functional maturation of distinct cortical subtypes.

Maternal Low-Protein Diet and Genistein Exposure Disrupt HPA Axis Development and Induce Sexually Dimorphic Behavioral Outcomes in Offspring

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Offspring health can be affected by maternal nutrition during pregnancy. For instance, a low-protein diet may pose a greater risk to their physical and neurological development. Therefore, dietary supplements such as soybeans, which are rich in phytoestrogens, particularly Genistein (GEN), are recommended to address deficiencies in maternal diets during pregnancy. Phytoestrogens are classified as endocrine disruptors due to their ability to bind to estrogen receptors, which affects various estrogen-sensitive neural systems, including the stress axis. In this study, we examined the effects on the HPA axis from mothers and on offspring in Sprague Dawley rats due to a chronic maternal diet that was low in protein (8%), both with and without GEN. RT-PCR on the maternal brains revealed a significant alteration in the stress axis, primarily characterized by reduced expression of glucocorticoid receptors across all treated groups. The brain is susceptible to changes in energy production during chronic stress, as demonstrated by WB analysis, which indicated alterations in ATP production within the treated groups. Additionally, analysis of breast milk showed lower protein and fat content in experimental low-protein groups. Pups born to these mothers have been smaller since birth and have never fully recovered their normal development. At birth, females showed marked alteration of the stress axis compared with their male siblings, along with reduced energy production. In adulthood, these animals were subjected to behavioral tests, and females exhibited anxiety-like behaviors, particularly those born to mothers on GEN-low-protein diet. In conclusion, these results suggest that a maternal diet plays a crucial role in maintaining the stress axis during the sensitive phase of pregnancy. An inadequate diet could become a chronic maternal stressor, and when enriched with endocrine disruptors, it can lead to sexually dimorphic alterations in the neurodevelopment of the offspring.

Loss of the X-linked Intellectual Disability gene *ArhGEF6* affects the migration and electrical functions of forebrain inhibitory neurons

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Intellectual Disability (ID) has been consistently associated with altered neuronal migration, neuritogenesis, synaptogenesis, and plasticity that impact neuronal networks' function and lead to excitatory-inhibitory imbalance. Those processes involve cytoskeleton dynamics, whose key regulatory enzymes are small Rho GTPases. The *ARHGEF6* gene codes for a RAC1-specific positive regulator and has been found mutated in the X-linked form of non-syndromic ID MRX46. To gain knowledge on the endophenotype of this ID, we have examined the brain of *Arhgef6*^{-Y} mice. We observed that *Arhgef6* is highly expressed in the CA3 of the murine hippocampus and that its absence leads to defects in hippocampal interneurons (INs) morphology, quantity and apoptosis in each distinct hippocampal subfields (CA1, CA2, CA3 and Dentate Gyrus), along with an altered number of the specific INs subtypes PV+ and CCK+. Moreover, the same alteration was also observed in murine embryonic and adult cortical layers. In addition, electrophysiological recordings in brain slices shows that the loss of *Arhgef6* leads to an intrinsic hypoexcitability of hippocampal INs. Cumulatively, the data collected strongly indicate migratory defects in *Arhgef6*^{-Y} INs. In a parallel effort, we investigated the role of *ARHGEF6* in human: we mutagenized iPSCs to obtain a null mutation in *ARHGEF6* and used these to obtain neural progenitors and forebrain organoids. Immunohistochemistry analysis revealed a decreased expression of progenitor markers, an abnormal accumulation of neurons in the organoid core, and increased cell death. Dorso-Ventral assembloids revealed that *ARHGEF6*-KO basal neurons exhibit migratory defects consisting in decreased saltation frequency, length, and velocity. These deficits contribute to a reduction in the number of INs in the cortical compartment, as previously noted in the *Arhgef6*^{-Y} mice. In conclusion, these results highlight the critical role of the RAC1 GTPase pathway in regulating proliferation and migration of INs and provide a link between this endophenotype and the onset of Intellectual Disability.

Stream of consciousness: dissecting cognitive circuits in schizophrenia MAM model – a proof of concept.**NDV 10**Sebastian Sulis Sato ⁽¹⁾ - Marta Carè ⁽²⁾ - Andrea Escelsior ⁽³⁾ - [Barbara Parisi](#) ⁽⁴⁾

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Schizophrenia is a complex neurodevelopmental psychiatric disorder characterized by positive, negative and cognitive symptoms. It is marked by alterations in brain structure and neurotransmission, particularly within dopaminergic circuits. While positive psychotic symptoms have been extensively studied, cognitive impairments remain less understood and represent a critical therapeutic challenge. Understanding the neuronal bases of these cognitive deficits is crucial for developing new therapeutic strategies. In this context, preclinical models provide valuable platforms for exploring these deficits due to their translational relevance; an example is the methylazoxymethanol acetate (MAM) rat model, which replicates key neuroanatomical and behavioral features of schizophrenia.

This proof-of-concept study aims to investigate the neuronal mechanisms underlying cognitive dysfunctions in schizophrenia using the MAM model, focusing on the prefrontal cortex (PFC), a brain region critically involved in executive functions and structurally altered in schizophrenia patients and models.

We propose to employ miniature head-mounted microscopes (Miniscopes, UCLA) to perform real-time calcium imaging of neuronal activity in the PFC of freely moving MAM and control rats during cognitive task performance. Additionally, functional imaging will be combined with optogenetic techniques to selectively manipulate specific neuronal populations identified as key players in cognitive processing. This integrated strategy will help clarify the causal role of these neurons in cognitive deficits associated with schizophrenia.

By using this approach, we aim to elucidate the cellular and circuit-level alterations involved in cognitive impairments in schizophrenia, providing insights that may help develop more precise treatments to improve cognitive outcomes in affected patients.

Unravelling WASHC4 brain-specific function and its implication in intellectual disability

NDV 11

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Neuronal protein sorting and spatial organization of cellular components are highly regulated processes. They rely on intracellular vesicular trafficking pathways, and determine correct neuronal morphogenesis, information processing and synaptic plasticity. Deregulation of these processes has been observed in intellectual disability. The WASHC4 protein, or SWIP, is part of the WASH (Wiskott-Aldrich syndrome protein and SCAR homology) complex, which is involved in actin dynamics, endosomal trafficking and protein sorting in non-neuronal cells. Its role in neurons remains underexplored. Rare genetic variants in the human *WASHC4* gene cause autosomal recessive intellectual disability. A recent proteomics study revealed that a WASHC4 missense mutation identified in patients (p.Pro1019Arg) alters the abundance of several endo-lysosomal and excitatory post-synaptic proteins in SWIP^{P1019R} knock-in (KI) mouse brains, and this is associated with cognitive and movement impairments in adult mice. Our aim is to understand how WASHC4 mutations impact on synaptic structure, composition and function through a perturbation of intracellular vesicular trafficking and sorting of synaptic proteins. To do so, we are characterizing molecular, electrophysiological and behavioural phenotypes of the SWIP^{P1019R} KI mouse model. Preliminary results obtained with patch-clamp recording of hippocampal dentate gyrus granule cells showed significant alterations in neuronal excitability and spontaneous synaptic activity in KI mice vs controls. Moreover, we confirmed by Western blotting that endo-lysosomal markers are perturbed in KI mice as compared to controls. The expression and subcellular localization of AMPAR subunits and postsynaptic scaffolding proteins, as well as dendritic spine development and morphology are under investigation *in vitro* and *in vivo*. Our results are expected to provide new insights in the role of WASHC4 in neuronal cell biology, brain development and cognition.

ARHGAP10 variants impact neurogenesis and cause neurodevelopmental disorders in human patients

NDV 12

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The Rho GTPase family acts as a central signaling hub that controls cytoskeletal dynamics. Regulating actin polymerization, it plays a crucial role in cell morphology, migration, and neuronal development. Disruption of Rho GTPase signaling has been implicated in a variety of neurodevelopmental disorders (NDDs). ARHGAP10 is a GTPase-activating protein (GAP) that promotes the inactivation of RhoA and Cdc42 by accelerating GTP hydrolysis. Structural variants in the human *ARHGAP10* gene have been detected in individuals diagnosed with schizophrenia, but its role in neurodevelopment remains elusive.

Through exome sequencing, we identified *de novo* or biallelic *ARHGAP10* variants in a cohort of individuals with NDDs from five unrelated families. All variants were extremely rare, affected conserved residues in functionally relevant domains of the protein, and were predicted to be deleterious *in silico*. To investigate the potential pathogenic role of ARHGAP10 in morphogenesis and neuronal development, we employed human cell line and primary neuronal cultures. In HEK293 cells, wild-type ARHGAP10 overexpression induced marked morphological changes, including a significant reduction in cell area and the appearance of filopodia-like protrusions, indicative of its role in cytoskeletal remodeling. RT-PCR and western blot analysis in developing mouse cortical neurons and iPSC-derived neurons from human fibroblasts revealed a peak of expression at early stages of neuronal differentiation. In mouse cortical neurons, wild-type ARHGAP10 promoted robust neurite outgrowth and increased dendritic complexity. In contrast, patient-derived ARHGAP10 variants failed to elicit similar effects, supporting a loss-of-function mechanism. These findings validate ARHGAP10 as a novel regulator of human neuronal morphology and support its involvement in NDD pathogenesis, paving the way to the development of therapeutic strategies that target Rho-GTPase pathways in affected individuals.

Repurposing of Histone Deacetylase Inhibitors as a potential therapeutic strategy for Myoclonic-Astatic Epilepsy

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Epilepsy is one of the most prevalent neurological disorders, affecting approximately 70 million people worldwide. Often characterized by recurrent seizures, it imposes a substantial physiological and psychological burden on patients. Despite significant progress in understanding the pathogenetic mechanism of the disease and the availability of antiepileptic drugs (AEDs), around 30% of patients remain refractory to treatment. Moreover, current AEDs offer limited efficacy in addressing common comorbidities such as cognitive impairments and psychiatric disorders. Histone deacetylase (HDAC) inhibitors, including FDA-approved compounds like valproic acid and 4-phenylbutyrate, have demonstrated antiepileptic properties and hold potential for broader therapeutic applications beyond seizure control. To investigate their efficacy in a relevant genetic context, we performed a target-driven drug screening using our recently developed knockout zebrafish model of Myoclonic-Astatic Epilepsy (MAE)—a rare, childhood-onset epilepsy syndrome caused by pathogenic variants in the human SLC6A1 gene. With this purpose, a panel of 21 HDAC inhibitors was administered via acute exposure (24 hours) to 4-day post-fertilization (dpf) larvae. Notably, several compounds induced a significant transcriptional upregulation of *slc6a1a* and *slc6a1b* (the zebrafish orthologs of SLC6A1), indicating a drug-induced response that may contribute to functional rescue.

Interestingly, treatment with 4-phenylbutyrate led to a partial normalization of the altered behavioral alterations observed in the *slc6a1* mutant zebrafish, suggesting a rescue effect toward a wild-type-like phenotype. Although further validation is required, these initial findings support the idea that HDAC inhibitors represent promising and readily translatable candidates for epilepsy therapy, with the potential to accelerate the path toward clinical application.

Nrf2/NOX2 Pathway Signaling and Oxidative Stress Biomarkers Evaluation in the Pathophysiological Link Between Gaucher Disease and Parkinsonism

NI 01

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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 GBA1 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 signaling axis has emerged as a pivotal pathway in the modulation of neuroinflammation and neurodegeneration. This study aims to explore the pathogenic link between GBA1 mutations and PD, focusing on the redox imbalance and the role of Nrf2 signaling in an in vivo Gba D409V knock-in mouse model, compared to wild-type (WT) C57BL/6J controls. Animals 8-weeks old were evaluated over a five-month period, with tissue and behavioral assessments conducted at 7, 14, 30, 60, and 90 days. Early timepoints (7 and 14 days) did not reveal significant changes in behavioral performance, expression of PD-related markers (TH, DAT, α -synuclein), or oxidative stress indicators, including Nrf2, 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 GBA1 variants in promoting neurodegenerative processes. Unraveling these mechanisms is essential for the identification of early biomarkers and may offer new therapeutic insights for GBA1-associated PD.

Regulation of Myeloid Immune Cells in Alzheimer's and Parkinson's Disease Patients by the TREM2 Ligand Sulfavant A

NI 02

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There is a growing body of research about the role of the innate immune system in pathogenesis of neurodegenerative disorders, such as Alzheimer's Disease (AD) and Parkinson's disease (PD). Emerging evidence suggests that peripheral inflammation may affect central nervous system (CNS) pathology by activating immune cells in peripheral blood. This immune activation is hypothesized to contribute to the dysregulated and persistent inflammation that drives the chronic evolution of neurodegenerative diseases. In this context, this project investigates small molecules as potential modulators of immune cells through interaction with regulatory receptors as Triggering Receptor Expressed on Myeloid Cells 2 (TREM2). The study involves identifying bioactive compounds and developing an *in vitro* assay platform to assess their effects on myeloid cells, focusing on dendritic cells (DCs), that are critical effectors at the interface between neuroinflammation and peripheral immunity. DCs express TREM2, also found on microglia, where it regulates phagocytosis, inflammation, and immune functions involved in neurodegeneration. TREM2 binds various anionic lipids, including the synthetic ligand Sulfavant A (SULF A), which induces a novel "homeostatogenic" DC phenotype (home-DCs). Although preliminary studies indicating altered surface marker expression on DCs in AD and PD, a comprehensive phenotypic and functional characterization of these cells in neurodegeneration is lacking. Here we address this gap by phenotypically analysing DCs from peripheral blood of AD and PD patients and comparing them to healthy donors. Functional assessments include cocultures with healthy donor lymphocytes. The project also evaluates the effects of SULF A treatment on inflamed DCs to assess its capacity to restore immune homeostasis and mitigate neuroinflammation. Ultimately, this research seeks to uncover novel immunomodulatory strategies with potential therapeutic relevance for neurodegenerative diseases.

Maresin-1 promotes neuroprotection and modulates inflammatory responses in preclinical models of multiple sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system with an autoimmune component that affect young adults. During neuroinflammation, both resident and infiltrated immune cells are activated and contribute to disease onset and progression. Recent studies suggest that neuroinflammation could be due to a failure in resolving inflammation, the resolution of which is normally mediated by a newly characterized pathway that involves mostly omega-3 fatty acids-derived specialized pro-resolving lipid mediators (SPMs), that include resolvins and maresins. Thus, we investigated the impact of Maresin 1 (MaR1) on disease progression in a well-known, relapsing-remitting experimental autoimmune encephalomyelitis mouse model. Treatment with MaR1 ameliorated clinical signs of EAE, improving pathophysiology and promoting neuroprotection. In detail, MaR1 treatment significantly reduced disease severity at the peak stage and prevented further relapses in the treated group. Moreover, MaR1 treatment significantly reduced disease severity and symptom incidence.

Then, we investigated whether MaR1 could also impact the human disease. Peripheral blood cells were isolated from MS patients and pretreated with or without MaR1 upon stimulation with anti-CD3/CD28, analyzing cytokines expression by high-dimensional flow cytometry. MaR1 treatment significantly impacted all T-cells subsets resulting in decreased percentages of IFN- γ and IL-17 in CD4+ T cells and reducing also TNF- α and IFN- γ production in CD8+ T cells. Moreover, MaR1 potentiated Treg responses by significantly increasing the expression of FoxP3, a signature transcription factor, and the production of IL-10. In conclusion, all these observations together demonstrate the broad effect of MaR1 on the functional capabilities of immune cells associated with MS that leads to decreased severity and progression, suggesting MaR1 as a new hypothetical therapeutic agent.

Generation of an integrated cellular model to study demyelination and remyelination

NI 04

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Multiple sclerosis (MS) is a chronic, immune-mediated neurodegenerative disease characterized by demyelination within the central nervous system. While current immunomodulatory treatments slow disease progression, they fail to promote remyelination or prevent neurodegeneration. This therapeutic gap reflects both limited understanding of progressive MS mechanisms, where glial cells such as astrocytes and microglia play a central role, and the poor predictive value of current in vitro and in vivo models.

To address these challenges, we are developing an integrated and scalable 2D co-culture model composed of human iPSC-derived neurons and glia cells to study inflammatory de-/re-myelination, guided by insights gained from establishing a similar mouse system.

We first cultured both explant and dissociated mouse dorsal root ganglion (DRG) neurons, which displayed robust axonal outgrowth and long-term survival. These cultures were combined with MACS-sorted oligodendrocytes and astrocytes, leading to consistent glial integration and early axonal ensheathment by oligodendrocytes. By optimizing the timing and conditions of glia addition, we created a permissive environment to model pathology. Specifically, we established a lyssolecithin-based demyelination protocol compatible with neuronal survival and introduced pro-inflammatory cytokines (TNF α and IFN γ) to mimic inflammation. In parallel, we differentiated human iPSCs into sensory neurons that exhibit sensory-like morphology and express appropriate markers. We are now focusing on achieving efficient myelination of these neurons through co-cultures with hiPSC-derived glia.

With this system we aim to provide a controlled and flexible platform to study axo-glial interactions and model de-/re-myelination under inflammatory conditions, of potential interest for research in MS.

Targeting inflammation in depression: a stratified approach with minocycline

NI 05

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Inflammation is increasingly recognized as both a hallmark of depression and a key risk factor for its development. Evidence suggests that targeting inflammation may offer a novel therapeutic approach, particularly in treatment-resistant depression (TRD). Here, we investigated the effects of adjunctive minocycline, an antibiotic with anti-inflammatory properties, in patients with elevated peripheral inflammation (C-reactive protein [CRP] levels >2 mg/L) who had failed to respond to at least two selective serotonin reuptake inhibitors (SSRIs). Depressive symptoms were assessed using the Hamilton Depression Rating Scale (HDRS) and the Beck Depression Inventory (BDI), while state and trait anxiety were evaluated with the State-Trait Anxiety Inventory (STAI). Treatment response was measured using the Clinical Global Impression (CGI) scale. Peripheral inflammatory biomarkers were quantified in serum (BioPlex 200), and gene expression was analysed via absolute quantification using digital PCR (QuantStudio™). Statistical analyses included paired t-tests and repeated-measures ANOVA.

Clinical results revealed significant improvements following minocycline treatment, including reductions in HDRS ($p = 0.014$) and BDI ($p = 0.032$) scores, as well as decreased trait anxiety ($p = 0.009$). While CRP levels showed a trend toward reduction, serum IL-23 levels were significantly decreased ($p = 0.021$). Gene expression of IL-1 β and MIF was also reduced ($p < 0.047$). Significant associations were observed between peripheral biomarkers and clinical outcomes, including a positive correlation between MIF and CGI scores.

These findings suggest that minocycline may be an effective adjunctive treatment for TRD with elevated inflammation, improving both depressive symptoms and inflammatory profiles. Further studies are needed to replicate these results and clarify underlying mechanisms.

Functional Analysis of EBI2 Signaling in Glial Cell Biology and Remyelination Using Organotypic Cerebellar Slices

NI 06

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Epstein-Barr virus-induced gene 2 (EBI2, GPR183) and its oxysterol ligand 7 α ,25-dihydroxycholesterol (7 α ,25OHC) play a critical role in immune cell migration and have been implicated in neuroinflammatory and neurodegenerative diseases, including multiple sclerosis (MS). EBI2 is expressed in various immune cells such as B and T cells, dendritic cells, and natural killer cells as well as in astrocytes, oligodendrocytes, and hematopoietic stem cells. In the central nervous system (CNS), EBI2 is upregulated in inflamed glial cells and facilitates astrocyte-macrophage communication. Astrocytes and microglia produce oxysterols in response to inflammatory stimuli, further modulating EBI2 signaling. EBI2 is also transiently upregulated during oligodendrocyte maturation, and 7 α ,25OHC promotes oligodendrocyte progenitor cells (OPCs) migration, suggesting a role in remyelination. High expression of EBI2 in immune cells and their accumulation in MS lesions supports the idea that the EBI2/7 α ,25OHC pathway facilitates the migration of inflammatory cells to sites of CNS inflammation in MS. These findings suggest EBI2 signaling is a key regulator of neuroimmune interactions and myelin biology, highlighting its therapeutic potential in demyelinating diseases such as MS.

This study will further investigate the molecular and functional effects of EBI2 signaling in glial cells including astrocytes, microglia, and OPCs isolated from EBI2 knockout (KO) and WT mice. Cells will be treated with cytokines (e.g., TNF, IL-17), lysophosphatidylcholine (LPC) or lipopolysaccharide (LPS) and CF3-7 α ,25OHC to assess EBI2-dependent pathways. Functional outcomes in glial cells such as differentiation, maturation, and inflammatory responses will be evaluated using ELISA, qPCR, Western blot (WB), immunocytochemistry (ICC) along with (re)myelination in organotypic cerebellar slices. The central hypothesis suggests that EBI2 promotes glial cell function and enhances remyelination in the CNS.

Funding: This project received funding from the National Science Centre, Poland, grant registration number: 2022/47/D/NZ3/02613 (AR).

Exploring the impact of neuronal inflammatory response in an in vitro model of Huntington's disease

NI 07

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Huntington's disease (HD) is an inherited neurodegenerative disorder characterized by progressive motor, cognitive, and psychiatric decline, associated with the accumulation of mutant huntingtin protein (mHTT). While neuronal degeneration in HD has been extensively linked to toxic protein aggregation and mitochondrial dysfunction, increasing evidence points to chronic inflammation as a critical pathological component of disease progression. Although neuroinflammation has traditionally been attributed to glial cell activation, the involvement of neuron-intrinsic inflammatory mechanisms in HD pathogenesis remains underexplored. In this study, we focused on the role of the neuronal NLRP3 inflammasome using a striatal progenitor cell line (STHdh) derived from HD mice. We demonstrated that both wild-type and mHTT-expressing cells exhibit NLRP3 inflammasome activation, evidenced by IL-1 β and caspase-1 release and gasdermin D accumulation, under pro-inflammatory conditions. However, cells expressing mHTT display significantly higher vulnerability to inflammatory cell death, indicating that mutant huntingtin sensitizes neurons to inflammasome-mediated damage. This pathological susceptibility is further accompanied by mitochondrial dysfunction and increased oxidative stress, hallmarks of HD-related neuronal degeneration. Importantly, pharmacological inhibition of the NLRP3 inflammasome using MCC950 reduced basal levels of key inflammatory mediators, suggesting that targeting the neuronal inflammasome could potentially interfere with inflammatory stress and neuronal loss in HD. These findings highlight a novel and disease-relevant inflammatory mechanism in HD, supporting inflammasome modulation as a promising therapeutic strategy.

Alpha-synuclein in an experimental animal model of Parkinson's disease: morphological, neuroinflammatory, and molecular dynamics

NI 08

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A pivotal breakthrough in Parkinson's disease (PD) research was the identification of pathological aggregation of α -synuclein (α -syn), a protein whose abnormal forms are now recognized as a hallmark histopathological feature of Lewy bodies. These aggregates have been extensively studied in various experimental animal models, where they have demonstrated neurotoxic effects, including loss of dopaminergic neurons in the Substantia Nigra pars compacta (SNpc), disruption of the nigrostriatal pathway, and impairments in mitochondrial and lysosomal functions. Growing evidence also highlights a critical role for neuroinflammation in PD progression. To investigate these processes, we employed a rodent model characterized by bilateral striatal injections of α -syn preformed fibrils (α -syn-PFF). This experimental model aims to replicate many features of early PD stages, such as progressive neurodegeneration, impaired synaptic plasticity, and spreading of α -syn pathology across multiple brain regions. The severity of these alterations varied depending on the post-injection time points, which were assessed at 4, 8, and 12 weeks following injection. Inflammatory responses evolved in parallel, particularly in brain regions associated with PD, including the nucleus accumbens core (NAc), dorsolateral striatum (DL-STR), motor cortex, and SNpc. Glial cells, including astrocytes and microglia, responded dynamically to α -syn-PFF by displaying time-dependent changes in morphology and cell density. To explore the underlying molecular mechanisms, RNA sequencing performed 8 weeks post-injection revealed significant transcriptional changes in numerous genes, many of which are involved in innate and adaptive immune responses. Complementary preliminary analyses further examined markers of MHC-II and components of the complement system, suggesting a complex interaction between peripheral immune activity and central neuroinflammatory processes, potentially contributing to disease progression.

Receptor-Specific Neuroimmunomodulatory Actions of Psychedelics: The Roles of 5-HT_{2A} and Sigma-1 receptors

NI 09

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Psychedelics—such as lysergic acid diethylamide (LSD), N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (5-MeO), 3,4-methylenedioxymethamphetamine (MDMA), ketamine and psilocybin—are actively studied for their psychoactive properties, yet their direct cellular effects on neuroinflammation and neuroprotection remain unclear. These compounds activate serotonin 5-HT_{2A} and Sigma-1 receptors, which regulate neuronal survival, neuroplasticity and inflammatory signaling. However, their potential roles in promoting myelination or preventing inflammation-induced demyelination remain unexplored. Likewise, their impact on blood–brain barrier (BBB) integrity and permeability under normal and inflammatory conditions is unknown. This project evaluates whether psychedelics protect against demyelination and neuronal damage via anti-inflammatory signaling through 5-HT_{2A} and Sigma-1 receptors and assesses their impact on BBB function. Organotypic cerebellar slices were lysophosphatidylcholine (LPC)-demyelinated and treated with DMT alone or with antagonists: ketanserin (5-HT_{2A}) or BD-1063 (Sigma-1). Pro-inflammatory cytokine levels were measured using enzyme-linked immunosorbent assay (ELISA). Gene expression was assessed by reverse transcription quantitative polymerase chain reaction (RT-qPCR), including markers for oligodendrocyte precursor cells (OPCs), oligodendrocytes, neurons, transcription factors, BBB components and both receptors. A human tri-cell BBB model was treated with psychedelics with or without interleukin-17 (IL-17)/tumor necrosis factor alpha (TNFα) and receptor antagonists. Psychedelics reduced pro-inflammatory signaling in *ex vivo* and *in vitro* models. These effects were blocked by receptor antagonists indicating 5-HT_{2A} and Sigma-1 receptor mediation. This study provides a cellular basis for exploring psychedelics as potential therapies for neuroimmune, neurodegenerative, and neurovascular disorders such as multiple sclerosis, dementia, and stroke.

CRISPR/Cas9 Targeting of AAVS1 Locus for Doxycycline-Inducible NGN2 Expression Improves Neuronal Differentiation from iPSCs

NDG 01

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Induced pluripotent stem cell (iPSC)-derived neurons are valuable tools for modeling human neurological disorders. Two main protocols are used to generate neurons from iPSCs: a classical small molecule-based differentiation (SMAD protocol), which mimics neurodevelopmental signaling, and a faster transcription factor-mediated approach via NGN2 overexpression (iNGN2 protocol). While the SMAD protocol is time-consuming and yields mixed cell populations, iNGN2 offers a rapid, more direct method to produce excitatory neurons.

Early NGN2-based protocols used lentivirus to introduce the doxycycline (dox)-inducible NGN2 transgene, potentially causing genomic aberrations and batch variability. To address this, we used CRISPR/Cas9 genome editing to insert NGN2 into the AAVS1 safe harbor locus, generating stable iPSC lines for controlled expression. Next we compared neurons derived using both SMAD and iNGN2 protocols for molecular and functional properties.

iNGN2-derived neurons showed higher expression of structural markers, including Tau, β -II-tubulin, NF200, and MAP2, compared to SMAD neurons at day 21 in vitro. Synaptic proteins such as Synaptophysin and PSD95 were also expressed at higher levels, suggesting enhanced synapse development. Functional KCl-induced depolarization assays confirmed the ability of iNGN2-neurons to respond to stimulation via MAP kinase activation. Cell composition analysis showed high expression of the excitatory marker vGLUT1 and absence of the astrocytic marker GFAP in iNGN2 neurons, in contrast to SMAD-derived neurons. These results suggest that the iNGN2 protocol yields a more homogeneous, excitatory neuron-enriched population with minimal glial contamination. Overall, iNGN2-derived neurons display stronger neuronal features and offer a more efficient and reliable method for generating excitatory neurons from iPSCs.

Boosting NAD in senescent microglia, a useful strategy to counteract neurodegeneration?

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In neurodegenerative disorders and senescence, microglia, the brain immune cells, acquire a disease associated signature that may favour tissue repair in early disease state but at late stages lose its capability to restore brain homeostasis and protect neurons and oligodendrocytes from cell death. Senescent microglia exhibit a secretory associated senescence phenotype (SASP), and impaired metabolism, with depletion of the cofactor Nicotinamide Adenine Dinucleotide (NAD), which plays a central role in genome integrity and cell metabolism.

Emerging evidence highlighted lower levels of NAD in senescence and neurodegenerative diseases, with consequent impairment of sirtuins' activity.

In this study we investigated changes that occur during senescence in microglia developing an *in vitro* model of chronically exposure (up to 30 days) to high iron concentration. Initially, iron treatment induces microglia to proliferate more, enhances phagocytosis, and increase NAD levels suggesting microglia activation. After 30 days treated-microglia acquired a senescent-like phenotype with decreased proliferation and phagocytosis, upregulation of SASP markers with a significant increase in extracellular vesicles production. Biochemical and transcriptomic analyses showed decreased levels in NAD and NADPH content in iron-treated microglia, concomitantly to an increased expression of CD38 (the major NAD consuming enzyme). Moreover, the levels/activity of Sirtuin 6, downregulated in aged/senescent cells, were strongly reduced compared to control microglia. Senescent microglia co-cultured with healthy microglia induced senescent traits in healthy cells, as revealed by a significant increase in senescence-associated β -galactosidase and p21 positive cells and in reduced NAD levels. In conclusion, increasing NAD content, by boosting NAD synthesis or inhibiting its degradation, could represent a useful strategy to counteract senescence and/or senescence propagation to healthy microglia.

Investigating autophagy modulation at the interface between Herpes Simplex Virus 1 infection and APOE4 allele in Alzheimer's disease

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Alzheimer's disease (AD) affects 55 million people worldwide. Despite huge research efforts, a disease modifying treatment is still an unmet medical need. This might be related, at least in part, to the fact that the animal models available for testing treatment options mainly mimic genetic alterations found in familial cases which accounts only for 5% of AD patients. In contrast, 95% of AD cases are sporadic and are likely caused by an underexplored interaction between genetic and environmental factors. The $\epsilon 4$ variant of the APOE lipoprotein has been recognized as the most relevant genetic allele, and Herpes Simplex Virus-1 (HSV-1) reactivation has been recently found to play a role in AD onset. Interestingly, APOE4 and HSV-1 seem to converge at the molecular level on the autophagic pathway, whose dysfunction is well-documented in AD and other neurodegenerative diseases. Our goal is therefore to generate a new AD model by combining the APOE4 allele and HSV-1 infection, both *in vitro* and *in vivo*. To this aim, hippocampal neuronal cultures from APOE4 knock-in mice were infected with a replication-defective HSV-1 vector and compared to uninfected control cultures. Western blotting analyses of the autophagosome markers and autophagic regulators (LC3, p62, Beclin1, mTORC1, AMPK) were carried out. Quantification of β -amyloid and phospho-Tau content as well as the levels of synaptic markers (established AD hallmarks) are currently under investigation. The development of a sporadic AD model represents an impactful breakthrough in the field. Our results will advance the mechanistic understanding of the disease and help to guide successful development of effective treatments in AD patients.

NAD-related dysregulation in asymptomatic phases of ALS: early biomarkers of the disease?

NDG 04

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Nowadays, the lack of effective therapies for Amyotrophic Lateral Sclerosis (ALS) is largely attributed to late diagnosis and incomplete understanding of the mechanisms determining the disease.

Nicotinamide Adenine Dinucleotide (NAD) is an essential coenzyme, fundamental for energy metabolism and homeostasis. Deranged NAD levels and NAD-related pathways are emerging in ALS pathogenesis. Specifically, decreased NAD levels were observed both in mice and patients affected by ALS. In an ALS mouse model, supplementation with a NAD precursor delayed motor neuron degeneration and decreased neuroinflammation markers in spinal cords.

The aim of this study is to investigate whether dysregulations in NAD synthesis/degradation occur in asymptomatic phases of ALS in a swine model (*SOD1*^{G93A}).

Our data indicate that NAD(H) levels were increased in spinal cords of *SOD1*^{G93A} pigs at early (eALS) and late asymptomatic phases (laALS), whereas at symptomatic stage (sALS) a depletion in NAD(H) levels was observed. Thus, gene expression of the main NADases was evaluated: *CD38* (mostly expressed on inflammatory cells) was overexpressed in laALS and sALS, whereas *SARM1* (whose activation has a role in triggering neurodegeneration) expression was higher at all stages. Further, the amount of nicotinamide mononucleotide (NMN), a *SARM1* activator, was increased in laALS and NMNAT activity (converting NMN into NAD) was reduced in the different phases. Increased ROS levels were reported to be generated in ALS: in line with this, we found a decrease in NADPH content, primary defense against oxidative stress, in sALS. Accordingly, G6PD activity, the main cellular source of NADPH, was increased in eALS and subsequently decreased in sALS in comparison to age-matched WT animals.

In conclusion, our results unveil that NAD(P)(H) levels and NAD-degrading enzymes are dysregulated already in asymptomatic phases. Studies are in progress to link NAD metabolism and EVs in order to find novel biomarkers for ALS.

SARS-CoV2 Nucleocapsid Protein induces Tau pathological changes that can be counteracted by SUMO2

NDG 05

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Neurological manifestations are a direct and long-term consequence of SARS-CoV-2 infection, with COVID-19 patients exhibiting elevated levels of Alzheimer's-associated biomarkers, including phosphorylated Tau. In brain organoids, SARS-CoV-2 alters the phosphorylation and distribution of Tau. We hypothesize that these pathological changes are due to the recruitment of Tau into stress granules (SGs) operated by the nucleocapsid protein (NCAP) of SARS-CoV-2. Moreover, SUMOylation, a post-translational modification of NCAP and Tau, has emerged as an essential pathway in the modulation of SG dynamics. To test this hypothesis, we investigated whether NCAP interacts with Tau and localizes to SGs in hippocampal neurons in vitro and in vivo. We tested whether SUMOylation modulates their distribution in SGs and their pathological interaction. To investigate whether NCAP colocalizes and interacts with Tau, we transfected Hek293 cells with Tau and NCAP, performed an immunostaining for G3BP1 (SG marker), and evaluated the colocalization of Tau with NCAP in SG by confocal analyses. To test whether NCAP could induce pathological changes in Tau in vivo and whether SUMO2 might reduce them, we infected JNPL3 mice and age-matched C57 wild-type animals with adeno-associated viruses to express NCAP alone or in combination with SUMO2. We assessed motor and cognitive performances by the Rotarod test and Y-Maze test. We found that NCAP and Tau colocalize and physically interact. We also found that NCAP induces hyperphosphorylation of Tau and causes cognitive impairment in mice infected with NCAP in their hippocampus. Finally, we found that SUMOylation modulates NCAP SG formation in vitro and cognitive performance in infected mice. Our data demonstrate that NCAP induces Tau pathological changes both in vitro and in vivo. Moreover, we demonstrate the importance of the SUMOylation pathway as a target of intervention against neurotoxic insults, such as Tau oligomers and viral infection.

Engineering the spinal cord in vitro: a multi-scaffold 3D system for modeling cytoarchitecture and neural activity

NDG 06

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Recreating physiologically relevant spinal cord models in vitro requires not only appropriate cell types but also structural and topographical cues that mimic native tissue. While 2D cultures fall short in replicating the spatial complexity of the central nervous system, emerging 3D bio-printing strategies enable more faithful reconstructions of neural microenvironments. Here, we laid the basis to develop a 3D bioprinted spinal cord model by combining murine cell lines with a multi-material scaffold system. Neuronal/astrocytic (NE-4C) and motor neuron-like (NSC-34) cells were embedded in gelatin methacrylate (GelMA) hydrogels via extrusion-based bioprinting. To promote anisotropic fiber alignment, the hydrogels were layered on aligned polycaprolactone (PCL) microfibers produced by Melt Electrowriting. Our results showed that the matrix supported long-term cell viability, proliferation and differentiation: western blot at multiple time points (14, 21, 28 days) revealed progressive increase in neuronal (MAP2, β III-tubulin, with respective 1.5-fold and 13.5-fold increase at 14 DIV compared to 0 DIV) and glial (GFAP, with a 16-fold increase at 28 DIV compared to 0 DIV) markers over time, confirmed in immunofluorescence. Spontaneous network activity was detected through calcium imaging. The aligned PCL microfibers effectively guided cell orientation and neurite outgrowth, closely mimicking spinal cord architecture.

Pilot experiments using human neural progenitor cells derived from induced pluripotent stem cells (iPSCs) are underway to expand the model's translational relevance.

This versatile 3D system offers a biomimetic platform for modeling spinal cord structure and function, with potential applications in spinal cord injury and neurodegeneration research, and physiologically relevant drug screening.

The work was supported by Fondazione CRT and European Union – Next Generation EU within the PRIN 2022 program (D.D. 104 – 02/02/2022 Ministero dell'Università e della Ricerca).

Functional Integration and Circuit Reconstruction by hESC-Derived Striatal Progenitors in the QA-Lesion Model

NDG 07

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Human embryonic stem cell (hESC)-derived neuronal progenitors hold strong potential in the treatment of several neurodegenerative pathologies. To be effective, cell products need to properly restore lost neuronal populations as well as functionally integrate in the host tissue and reconstruct damaged circuits. Toward this end, we investigate the potential of second generation (sg) hESC-derived striatal progenitors (Conforti et al, 2022) to functionally restore damaged striatal circuitry when grafted in the quinolinic-acid lesion rat model.

Using rabies tracing technique, we demonstrated that grafted cells receive synaptic inputs from host neurons located in cortical and thalamic regions, and project towards appropriate striatal targets from early time points after transplantation. This connectivity pattern indicates a promising, although still partial, reconstruction of the lost striatal circuitry.

To assess the functional properties of the grafted cells, we performed ex vivo electrophysiological recordings using high-density microelectrode arrays at long term post-grafting. These analyses revealed spontaneous activity within the graft cells, indicative of functional maturation. Additionally, we used pharmacological treatments and chemogenetic tools to modulate graft activity ex-vivo or in-vivo. Notably, chemogenetic modulation of graft activity affected locomotion and fine motor skills including grooming, behaviors in which the striatal circuitries are directly involved.

Altogether these findings support the participation of sg-grafts in the reconstruction and functional integration of the host damaged circuits and offer new insights into the therapeutic potential of stem cell-derived cell replacement approaches.

Role of mesocortical dopaminergic pathway in cognitive dysfunctions of Multiple Sclerosis

NDG 08

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Multiple sclerosis (MS) is a chronic autoimmune disease in which the immune system, abnormally activated, attacks myelin, causing reduction or interruption of nerve transmission and leading to neurodegeneration, inflammation, and demyelination. Myelinic damage, present in different areas of the central nervous system, causes motor, cognitive, and sensory deficits.

In our study we administered cuprizone, a copper chelating agent, to rats, which induces apoptosis of oligodendrocytes and activation of microglia and astrocytes, leading to demyelination in different brain areas. Rats were administered cuprizone for six weeks (long-term treatment) or for three weeks followed by three weeks washout period (short-term treatment). The aim of the study was to assess whether long-term treatment could reduce cognitive performance, in relation to variation in the extracellular concentration of dopamine in the prefrontal cortex, and whether in short-term treatment these cognitive deficits could be repristinated. In this context, the T-maze test was carried out to analyze a) forced alternation and b) delayed not matched to position both as an index of working memory, c) reversal learning as index of behavioral flexibility, all dependent on the prefrontal cortex and d) spontaneous alternation as index of the spatial memory, dependent on the hippocampus.

The results show that long-term treatment impaired cognitive performance only in the tasks that are dependent on prefrontal cortex and significantly reduce extracellular concentration of dopamine in this area. On the contrary, animals exposed to the short-term treatment did not significantly affect performance or dopamine levels compared to controls.

These findings highlight the crucial contribution of mesocortical dopaminergic pathways to cognitive processes and provide key evidence for the identification of possible therapeutic targets in the treatment of cognitive symptoms of MS.

Neural activities and brain function network alteration in a mouse model of Alzheimer's disease.

NDG 09

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Alzheimer's disease (AD) is the most prevalent neurodegenerative cause of dementia worldwide, associated with progressive neurodegeneration in specific brain areas, which causes neural dysfunction from synapses to networks. Recent studies identified biomarkers for a preclinical stage of AD.

Different transgenic mouse models are studied to dissect the mechanisms involved in AD pathology. Here, we focused our project on the triple-transgenic mouse 3xTg-AD, a model that exhibits both A β and tau pathology, as well as synaptic dysfunction, that reflects the human form. 3xTg-AD model described extracellular plaques first appearing at 6 months of age until 12 months of age.

The triple transgenic mouse model 3xTg-AD will be subjected to different monoclonal antibodies (MAbs-A β) targeting A β protofibrils. At different stages of the AD progression, we are going to perform high-density electrophysiological recording and magnetic resonance imaging of 3xTg-AD treated with MAbs-A β or vehicle compared to controls, aimed to evaluate the neural activities and brain connectivity alterations during AD progression. These results will be compared to the cognitive and memory deficits observed in vivo and the identification of novel blood or cerebrospinal fluid biomarkers that will be modulated by the treatments in both experimental groups. This translational research project will dissect the mechanisms involved in the AD neurodegeneration for in vivo clinical applications.

A new perspective on SMA disease beyond spinal motor neurons: what role do cortical projection neurons play?

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease of the childhood, caused by survival motor neuron 1 (*SMN1*) gene mutations. The lack of functional SMN protein determines the degeneration of lower motor neurons (MNs); however, growing evidence indicates that SMN deficiency also impacts other structures, including brain. Thus, the involvement of cortical alterations in SMA remains unclear. Here, we examined the sensorimotor cortex of SMA Δ 7 mice, a severe SMA model, to determine the effect of SMN lack on the survival and morphology of cortical projection neurons. We analyzed early (postnatal day 5) and late (P11) symptomatic animals, comparing SMA mice with their WT littermates. Our findings indicate a selective reduction of 50% in corticospinal and of 36% callosal neurons within the SMA cortex at P11, suggesting that SMN reduction also impacts upper MNs. Interestingly, corticothalamic neurons appear resistant to SMN deficiency, as we observed no signs of degeneration in this population. In comparison to WT, SMA callosal and corticospinal neurons also exhibit alterations in morphological traits: we observed soma size decrease (- 17,8% and - 32%, respectively) and branching reduction (- 40% and - 41%, respectively), together with a less mature phenotype of dendritic spines. In contrast, we did not observe any morphological alterations in corticothalamic neurons. Interestingly, although the same analyses at P5 suggested cortical cell death concomitant with spinal MN death, the morphological changes described above already appear at the early stage of the disease, suggesting that specific populations of cortical projection neurons are more sensitive to the absence of SMN from the very beginning. Thus, the identification of any early markers of dysfunction or resistance in cortical cells, in SMA pathology, may be crucial in providing insights into disease progression and may lead to the identification of potential new therapeutic targets.

Dietary phytoestrogens preserve brain health by targeting mitochondria**NDG 11**

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Menopausal estrogen loss has been identified as a contributing factor to the progression of Alzheimer's disease (AD), as estrogen plays a critical role in supporting memory and cognitive functions through neuroprotective mechanisms. Despite the potential benefits of hormone replacement therapy (HRT), its neuroprotective effects have been inconsistent, leading to growing interest in safer alternatives, such as phytoestrogens—plant-derived compounds that exhibit estrogen-like activity. Among these, natural polyphenols have garnered significant attention due to their promising health benefits, particularly those found in extra virgin olive oil (EVOO). EVOO is rich in oleuropein aglycone (OleA) and its primary metabolite, hydroxytyrosol (HT), both of which have been shown to counteract amyloid aggregation and mitigate the toxic effects of various proteins involved in amyloidosis. Recent studies using SH-SY5Y human neuronal cells highlight the ability of these polyphenols to activate estrogen receptor beta (ER β) and insulin-like growth factor 1 (IGF1) receptors, both of which are crucial for neuronal survival. Moreover, these compounds regulate intracellular calcium signaling through ryanodine receptors (RyR), as well as AMPA and NMDA receptors, which play key roles in synaptic plasticity. In addition to their effects on synaptic function, EVOO polyphenols have been found to enhance mitochondrial biogenesis and metabolic efficiency, vital processes for maintaining healthy neuronal function. They also modulate the expression of amyloid-binding alcohol dehydrogenase (ABAD), an enzyme involved in the pathology of AD. Taken together, these findings suggest that polyphenols from EVOO may mimic estrogen's protective actions, potentially compensating for estrogen loss during menopause. This provides a promising strategy for reducing or reversing age-related cognitive decline and offering neuroprotection in the context of neurodegenerative diseases like Alzheimer's.

Understanding mechanisms of neurodegeneration in Riboflavin Transporter Deficiency (RTD): development of innovative approaches to rescue the diseased RTD neural phenotypes using astrocyte-derived exos

NDG 12

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Riboflavin transporter deficiency (RTD) is a rare and progressive inherited neuropathy of childhood onset, characterized by sensorineural hearing loss, bulbar dysfunction, diffuse muscle weakness that could lead to respiratory failure. RTD is caused by homozygous or compound heterozygous pathogenic variants in *SLC52A2* or *SLC52A3* genes, which encode for Riboflavin (Rf) transporter RFVT2 or RFVT3 respectively. The only known effective treatment is high-dose oral Rf, but unfortunately not all patients are responsive. To better understand the physio-pathology of RTD, we derived induced pluripotent stem cells (iPSCs) from patients with pathogenic variants in *SLC52A2* to obtain neuronal cells: i.e., motoneurons and astrocytes. In this study we decided to study the interaction among astrocytes and motoneurons (MNs), to understand if the altered neuronal phenotype can be found in both cell types or not. We demonstrated that RTD astrocytes are not affected by alterations of *SLC52A2* gene while RTD MNs are the most damaged in their morphology and function, measured by calcium influx. We also performed co-culture experiments with RTD motoneurons and RTD astrocytes to understand if in conditions closer to the neuronal physiological environment we also observe neuronal impairment or if the presence of astrocytes can rescue the altered RTD neuronal phenotype. In particular, we investigated the morphological changes and neuronal activity of RTD MNs in co-culture with Ctrl and RTD ASTROs in presence of cell-cell contact, and without contact. The present study shows that the astrocytes have positive effects on RTD motoneurons, improving both their morphology and function.

Analysis of deregulated microRNAs in plasma-derived small vesicles and their target genes in Frontotemporal Dementia

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Fluid biomarkers can be used to identify dementia such as FTD (Frontotemporal Dementia), among these Extracellular Vesicles (EVs) and their cargo are important tools. EVs are able to cross the blood-brain barrier and it means they can be used as an insight on the brain pathological state. EVs cargo include different kind of molecules such as miRNAs, which can be also studied in Peripheral Blood Mononuclear Cells (PBMCs). The aim of this work is identifying deregulated miRNAs in FTD and their relative target genes to study their involvement in FTD pathogenesis. Small EVs (SEVs) were isolated from 25 FTD patients and 25 healthy controls (HC), RNA was then isolated from SEVs. Four deregulated miRNAs were selected according to their fold change ($\log_2FC \geq 7$ or $\log_2FC \leq -7$) and validated. miRNAs-mRNAs interaction prediction was carried out on miRTarBase database for two of the validated miRNAs and the selected target genes were validated in PBMCs isolated from blood samples of 25 FTD and 25 HC. Hsa-miR-365a-3p and hsa-miR-132-3p appeared to be downregulated in FTD compared to HC, these two miRNAs are deregulated in FTD and have been already studied in Alzheimer's Disease (AD). Target genes were identified based on their function in the context of the pathology and validated in PBMCs. We found *CCND1*, *FOXO3a* and *IL-6* to be up-regulated and *SHC1* down-regulated. No studies have reported the role hsa-miR-132-3p plays in FTD, it might be involved in the development of the disease through dysregulated regulation of target genes, especially *SHC1* which is involved in apoptosis and oxidative stress and *CCND1* which has a role in the regulation of the cell cycle. Our data highlight the importance of miRNAs cargo analysis in EVs since their potential may be exploitable for biomarkers discovery and study of gene expression in dementia pathogenesis.

RNA-seq and miRNA-seq analysis across the Alzheimer's disease continuum

NDG 14

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Identifying reliable biomarkers obtainable through non-invasive methods is crucial for early diagnosis and monitoring of Alzheimer's disease (AD) progression. The study aimed to identify AD transcriptomic signatures using Peripheral Blood Mononuclear Cells (PBMCs) from healthy controls (HC), patients with mild cognitive impairment with no AD markers (MCI_non-AD), MCI patients with AD markers (MCI-AD), and AD patients. The goal was to uncover both stage-specific and over-time molecular signatures reflecting the continuum of disease progression. Total RNA was extracted from PBMCs of 25 HC, 25 MCI_non-AD, 25 MCI-AD and 25 AD patients. Total RNA libraries and miRNA libraries were prepared and sequenced on the Illumina NextSeq 550 platform and the NextSeq 2000 respectively. Data analysis was conducted in collaboration with the SING Research Group (Spain) using the Compi RNA-Seq pipeline for total RNA and the myBrain-Seq software for miRNA. Regarding total RNA analysis, 869, 944, and 258 differentially expressed (DE) genes were identified in the MCI_non-AD, MCI-AD, and AD groups, respectively. Examining expression patterns, a progressive trend in genes dysregulation was revealed along the disease continuum from preclinical stages to AD, involving processes like mitochondrial electron transport chain, oxidative phosphorylation and inflammatory response. In the miRNA-seq analysis, 38, 51, and 4 DE miRNAs were detected in the MCI_non-AD, MCI-AD, and AD groups, respectively and pathway enrichment analysis highlighted immune response dysregulation. These findings suggest that transcriptomic alterations in PBMCs might reflect key AD pathological processes and may represent possible biomarkers for disease staging and progression.

TREM2 structural variants insight in Alzheimer's disease and Frontotemporal Dementia**NDG 15**[Andrea Ninni](#)⁽¹⁾ - Davide Pietrafesa⁽¹⁾ - Daniele Lettieri Barbato⁽¹⁾⁽¹⁾ *Università degli studi di Roma Tor Vergata, Università degli studi di Roma Tor Vergata, Roma, Italy*

Single nucleotide variations in the gene encoding Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) have been identified as crucial factors in various neurodegenerative conditions. Specifically, some of these variations are associated with late-onset Alzheimer's disease (AD) and behavioral variant frontotemporal dementia (FTD). Models of the extracellular domain of TREM2 show that AD-associated variants localize to a putative ligand-interacting region on the extracellular surface, and that FTD-associated variants are found in the hydrophobic core. However, while these disease-associated residues are predicted to play a role in disrupting ligand binding to the extracellular domain of TREM2, how they ultimately lead to disease remains unknown. To address this gap, we employed *in silico* molecular modeling and molecular dynamics simulations to investigate all-atom models of TREM2. Our study characterized the effects of AD-associated R47H and R62H, and FTD-associated T96K, D86V, and T66M variants on TREM2 conformation and dynamical motion, comparing them to the benign N68K variant and the common variant. Analysis reveals how these variants interfere with the formation of the K48-D86 and R47-T66 hydrogen bonds, consequently disrupting the stability and complementarity determining regions (CDR) of TREM2. This instability ultimately compromises the tethering between these CDRs and the core of the protein domain, exposing a group of otherwise-buried, negatively charged residues. Furthermore, docking studies with beta-amyloid were carried out and they demonstrate impaired binding for AD- and FTD-associated mutations compared to healthy variants. These findings further suggest that neurodegenerative disease-associated variants may cause a loss of TREM2 function by altering the conformational stability of the ligand-interacting CDR, ultimately reducing ligand affinity or specificity.

Lysosomal Impairment Reshapes Membrane Composition and Metabolism in GCase-Deficient Dopaminergic Neurons

NDG 16

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Lysosomes maintain cellular homeostasis by recycling macromolecules, regulating metabolism, and ensuring mitochondrial quality control. In neurons, metabolically demanding long-lived cells, lysosomal integrity is especially crucial. Indeed, lysosomal dysfunction has emerged as a hallmark for both physiological aging and neurodegenerative conditions. One well-known example involves the deficiency of β -glucocerebrosidase (GCase), a lysosomal hydrolase responsible for degrading the glycosphingolipid glucosylceramide (GlcCer). Loss of GCase activity leads to the accumulation of GlcCer within lysosomes, resulting in lysosomal overload and dysfunction, mechanisms that underlie neurodegenerative disorders such as Gaucher disease and Parkinson's disease. However, the precise molecular mechanisms linking lysosomal impairment to neuronal damage remain incompletely understood. To explore how GCase deficiency and lysosomal dysfunction contribute to neurodegeneration, we used human iPSC-derived dopaminergic neurons chronically treated with conduritol-B-epoxide (CBE), a specific GCase inhibitor. CBE-treated neurons exhibit neurodegenerative features and GlcCer accumulation not only within lysosomes but also at the plasma membrane (PM). This redistribution resulted from aberrant activation of lysosomal exocytosis, which disrupted PM sphingolipid composition and altered membrane-associated protein content. Strikingly, we observed a marked reduction in the PM localization of the glucose transporter GLUT3, impairing glucose uptake and catabolism. This was confirmed by LC-MS/MS analysis following [U-¹³C₆]-glucose administration, which revealed profound metabolic rewiring. Our findings demonstrate that chronic lysosomal stress disrupts both lipid homeostasis and PM architecture, leading to compromised energetic metabolism and increased mitochondrial demands. This cascade of events likely contributes to the neuronal vulnerability observed in GCase-related neurodegenerative conditions.

Transcranial direct current stimulation (tDCS) of the prefrontal cortex modulates dorsal raphe serotonergic activity in an EAE mouse model of multiple sclerosis\

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Multiple sclerosis (MS) patients have a two- to five-fold higher risk of developing depression compared to the general population. The experimental autoimmune encephalomyelitis (EAE) mouse model is widely used to study MS, as it replicates key pathological features, including depression-like behavior. A promising intervention for depression is transcranial direct current stimulation (tDCS), a non-invasive neuromodulatory technique that delivers low-intensity electrical currents to increase (anodal, AtDCS) or decrease (cathodal, CtDCS) cortical excitability. The serotonergic (5-HT) system plays a significant role in depression, with a large body of literature suggesting that imbalances in 5-HT levels or circuit contribute to the disorder. Recently, we showed that in healthy mice, prefrontal AtDCS modulates 5-HT neuron activity in the dorsal raphe nucleus (DRN). Here, in EAE mice, we investigated the effects of prefrontal tDCS on 5-HT activity and depression-like behavior. Control (CFA) and EAE mice received anodal, cathodal or sham tDCS for five consecutive days post-immunization, before symptom onset. Behavioral assessments were conducted during both the acute and chronic phases of the disease, followed by extracellular single-unit in vivo recordings of DRN 5-HT neurons. Neurophysiological analysis revealed a reduced number of active DRN 5-HT neurons in EAE mice, partially reverted by AtDCS. Despite this, no clear effect of the stimulation was observed on depression-like behavior. Interestingly, CtDCS-EAE mice exhibited a tendency toward a reduced clinical score. Overall, prefrontal AtDCS partially restores 5-HT system impairments but shows no effects on disease symptoms; conversely, prefrontal CtDCS seems to positively modulate the clinical course of EAE, though not influencing DRN 5-HT neuronal activity.

The Effects of Amyloid Beta Oligomers and Fibrils on Oligodendrocyte Progenitor Differentiation and Myelination

NDG 18

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Introduction: Alzheimer's disease (AD) is characterized not only by amyloid beta (A β) deposition and neurofibrillary tangles, but also by widespread white matter (WM) abnormalities. These include myelin loss, parahippocampal WM fiber disruption, and oligodendrocyte dysfunction, and have been linked to cognitive decline. Emerging evidence suggests that both oligodendrocyte precursor cells (OPCs) and mature oligodendrocytes may be directly affected by A β species, impairing their capacity to generate and maintain myelin. However, the mechanisms by which A β oligomers and fibrils disrupt myelination remain poorly defined.

Aim: This project aims to investigate how A β aggregates influence OPC differentiation, oligodendrocyte function, and the myelination/remyelination process. Specifically, we seek to understand whether A β interferes with the ability of oligodendrocytes to form myelin and whether restoring myelination can counteract A β -associated neurodegeneration.

Methods: OPCs will be isolated from P4–P7 C57BL/6 mice using CD140a (PDGFR α)-based magnetic sorting and cultured under defined conditions. Cells will be treated with A β 1–42 oligomers and fibrils at various concentrations (0.01–10 μ M) for 72h. Ex vivo cerebellar slice cultures will be used to assess A β effects on normal myelination and remyelination following lysophosphatidylcholine (LPC)-induced demyelination. Expression of myelin-related markers (MBP, MOG, CNPase, PDGFR α , OLIG1) will be analyzed by qPCR and Western blot. Immunohistochemistry will assess the spatial organization of myelin and OPC populations.

Results: Preliminary data suggest A β exposure alters OPC differentiation and reduces myelin gene expression. Ongoing analyses will determine the extent to which fibrillar and oligomeric A β species impair myelin formation and whether these effects are reversible during remyelination phases in cerebellar slice models.

Conclusions: This study will provide insight into the direct effects of A β on the oligodendrocyte lineage and contribute to a deeper understanding of white matter involvement in AD. Targeting A β -induced myelin disruption may offer new avenues for neuroprotective strategies.

Unveiling TREM2 mediated microglia to neuron crosstalk in neurodegeneration

NDG 19

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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 brain, and neurons is critical for shaping neuronal circuits during development. Disruptions in this bidirectional communication contribute to various neurodevelopmental disorders, such as autism and schizophrenia. Among microglial receptors, the Triggering Receptor Expressed on Myeloid Cells 2 (TREM2) plays a pivotal role in modulating neurodevelopment by controlling synaptic refinement and influencing neuronal metabolism. Dysfunctional TREM2 signalling causes imbalances during early neuronal maturation, which may predispose the adult brain to increased vulnerability during aging and neurodegenerative conditions such as Parkinson's and Alzheimer's diseases. However, the precise mechanisms underlying TREM2's influence on early neuronal development remain unclear. In this project, I aim to elucidate how TREM2 modulates neurodevelopment and neurodegeneration by investigating both soluble factors and direct microglia-neuron interactions. Using trans-well systems, microglial conditioned media, and co-culture models, I assessed the effects of soluble factors or microglia-derived extracellular vesicles and identified potential novel TREM2 ligands on neurons. Moreover, by taking advantage of the availability of brain tissues from the alpha-synuclein (α -syn) injected mouse model, a widely used tool in Parkinson's disease (PD), we characterized the profile of microglial cells in the striatum. Together, these findings aim to deepen our understanding of microglial contributions to brain homeostasis during neurodevelopment and neurodegeneration, ultimately providing potential therapeutic targets for brain diseases involving microglia and TREM2.

This study is supported by the FRRB grant Linking Park.

Targeting Endocannabinoid Signaling to Enhance Adult Neurogenesis and Stress Resilience

NDG 20

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Adult neurogenesis plays a critical role in sustaining brain plasticity, cognitive function, and emotional regulation throughout life. Disruption of this process contributes to the pathophysiology of several neuropsychiatric and neurodegenerative disorders. The endocannabinoid system (ECS), an evolutionarily conserved and ubiquitously expressed signaling network, orchestrates brain homeostasis and is emerging as a key modulator of adult neurogenesis. Through its main endogenous ligands such as anandamide (AEA) and the activation of cannabinoid receptor type 1 (CB1), the ECS influences neuronal proliferation, differentiation, and survival. In this study, we investigated how modulation of endogenous AEA levels impacts neurogenic dynamics under physiological and stress-related conditions. Using flow cytometry, we quantified neural progenitor (Sox2, Nestin), proliferating (Ki-67), and immature neuronal (Doublecortin; DCX) populations, along with CB1 receptor expression. Pharmacological enhancement of AEA signaling via the fatty acid amide hydrolase (FAAH) inhibitor URB597 significantly increased Ki-67-positive proliferating cells and Sox2/Nestin-positive progenitors under baseline conditions. Conversely, chronic restraint stress (RRS) markedly reduced these neurogenic markers, reflecting impaired ECS function. Ongoing analyses aim to determine whether URB597 treatment can rescue neurogenesis under RRS, potentially counteracting stress-induced deficits. In parallel, we are exploring whether enhanced endocannabinoid signaling synergizes with environmental enrichment to further promote neurogenesis and stress resilience, highlighting the dynamic interplay between pharmacological and environmental modulatory strategies.

Changes in AQP4 expression and localization during ALS and SMA progression

NDG 21

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The glymphatic system is a perivascular network reliant on glial cells that plays a crucial role in removing waste products and neurotoxic substances from the brain. It includes aquaporin-4 (AQP4) water channels, expressed in astrocytes and located around blood vessels. Previous studies suggest that the glymphatic system dysfunction has been linked to various neurodegenerative conditions, such as Alzheimer's disease and Amyotrophic Lateral Sclerosis (ALS). In this context, we propose that changes in AQP4 expression may also play a role in the development of neurodegeneration in ALS and Spinal Muscular Atrophy (SMA). In this morphological study, we investigated AQP4 expression in the ventral horns of spinal cord in delta7SMN and SOD1^{G93A} mice, respectively SMA and ALS models. Three time points were selected to represent the pre-symptomatic, early symptomatic, and late disease stages. The lumbar spinal cord was collected, sectioned at 40 µm, and processed for immunofluorescence analysis using antibodies against AQP4, GFAP (astroglial marker) and ChAT (motor neuron marker). Images were acquired with a Zeiss Apotome microscope and analyzed with ImageJ. A significant increase in AQP4 expression was observed between the presymptomatic and early disease stages. Specifically, AQP4 levels rose by 116% in proximity to motor neurons and by 60% in total signal in the early stage compared to the presymptomatic stage, suggesting a peak phase of upregulation and polarization ($p < 0.01$). Expression patterns were similar in both diseases. Conversely, AQP4 levels decreased in the late stage, possibly due to neurodegeneration.

Our study highlights a possible role of the glymphatic system in the ALS and SMA onset and suggests AQP4 as a potential therapeutic target to delay neurodegeneration.

Modeling sensory neuropathy with patient derived-iPSCs: the role of the choline importer FLVCR1

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FLVCR1-related sensory neuropathies are rare genetic disorders of childhood onset, primarily characterized by progressive degeneration of sensory neurons and/or photoreceptors, resulting in sensory ataxia, pain insensitivity, and retinitis pigmentosa. These conditions are caused by mutations in the Feline Leukemia Virus Subgroup C Receptor 1 (FLVCR1) gene. FLVCR1 acts as a transporter of choline and ethanolamine and as interactor of the IP3R3–VDAC complex, regulating mitochondrial Ca^{2+} uptake at mitochondria-associated membranes (MAMs). Our findings showed that patient-derived fibroblasts carrying distinct pathogenic FLVCR1 variants exhibit reduced choline levels, altered membrane lipid composition, and impaired MAMs structure and function, leading to impaired mitochondrial bioenergetics. However, the mechanisms behind the disease remain unclear, and a therapy is lacking. To better dissect the pathogenic mechanism, we reprogrammed patient fibroblasts into induced pluripotent stem cells (iPSCs) and differentiated them into sensory neurons expressing the lineage-specific nuclear marker BRN3A. Interestingly, patient derived neurons exhibited a defective phenotype. Our current evidence suggests that FLVCR1 mutations lead to both mitochondrial dysfunction and lipid membrane disturbances, which may impair neurotrophic signaling and compromise axonal outgrowth and network formation as we observed in both developing and mature sensory neurons. Overall, our model highlights disrupted bioenergetics and membrane composition as key contributors to pathogenesis. Since sensory neurons critically rely on energy metabolism and membrane integrity to support axonal transport and receptor trafficking, we hypothesize that these defects may underlie their vulnerability in FLVCR1-related neuropathies. Further investigation is needed to better clarify disease mechanisms and identify therapeutic targets.

PIEZO1 in glioma: the force that fuels a tumour**NO 01**

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Glioblastoma multiforme (GBM) is one of the most aggressive types of brain tumour, with a median survival of less than 15 months. One of the main causes of this low survival rate is the absence of effective treatments, which have barely changed over the last 30 years. Currently, there is no known *bona fide* oncogene for this disease, which poses an additional challenge to developing effective therapeutic strategies. In this project, we explore the role of mechanosensitive ion channel Piezo1 in GBM. We have developed a novel transgenic mouse model of Piezo1 overexpression specifically in astrocytes (Piezo1^{Tg}/Gfap-Cre), observing that these mice developed gliomas with 45% of incidence and presented a reduced survival, which postulates *Piezo1* as the first discovered *bona fide* glioma driver oncogene. We also have performed a single cell RNAseq of pretumoral *Piezo1*^{Tg}/Gfap-Cre mouse brains to evaluate the molecular mechanisms underlying the astrocyte transformation driven by Piezo1. Additionally, to explore whether PIEZO1 contributes to GBM malignancy in humans, we correlated PIEZO1 expression in GBM biopsies with clinical outcomes of these patients, a cohort from Hospital 12 de Octubre (Madrid). We observed that PIEZO1 is a marker of worse prognosis exclusively in male patients. Finally, we generated an *in vitro* model of PIEZO1 overexpression in a human GBM cell line (U251 PIEZO1^{OE}). In this model, we observed that overexpression promoted an aggressive phenotype, with increased clonogenicity and migration. We also performed global transcriptomic and proteomic analysis of U251 PIEZO1^{OE} cells, observing alterations in pathways closely related with GBM progression, as inflammation response, epithelial-mesenchymal transition and WNT signaling. Overall, this project not only proposes Piezo1 as a new biomarker and oncogene in glioma, but also opens the door to its use as a therapeutic target for this disease.

Unveiling Key Drivers of Glioma Progression: A Comparative Transcriptomic and Functional Analysis

NO 02

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Gliomas, characterized by their aggressive behavior and resistance to treatment, pose a significant challenge in oncology. Understanding the molecular mechanisms that govern malignant progression is crucial for developing effective therapeutic strategies. In this study, we employed a glioma model based on retroviral transduction of PDGF-B oncogene in embryonic mice that simulate glioma progression.

The procedure generates both low-grade (LG) gliomas which exhibited an immunostimulatory phenotype, and high-grade (HG) gliomas capable of developing secondary tumors after orthotopic transplantation. These HG gliomas display an M2 pro-tumorigenic and anti-inflammatory profile promoting immune escape⁽¹⁾. Leveraging RNA sequencing, a comprehensive transcriptomic analysis of both LG and HG glioma samples had been conducted, to elucidate tumoral progression at the molecular level.

Through comparative analysis with transcriptomic data from human LG and HG gliomas obtained from publicly available databases, we identified key genes - Engrailed2, Hoxa5, Ulbp1, and Axl – overexpressed in both murine and human HG gliomas. To investigate their roles in glioma progression, we employed retroviral-mediated gene downregulation in our HG glioma cells, enabling us to assess the impact on cell proliferation and migration - hallmark features of high-grade gliomas. After conducting in vitro functional assays, our next step will be to evaluate the effects of silencing these genes in vivo to delineate their tumorigenic and immune evasive potential.

This integrative approach combining murine modeling, transcriptomics, and functional assays provides valuable insights into tumor progression and the dynamic interplay between gliomas and the immune system, offering potential avenues for therapeutic intervention aimed at disrupting tumoral progression and enhancing treatment efficacy.

1 Appolloni I et al., Cancer Lett. 2019 - PMID: 30312732

Targeting CITK in high-grade brain tumors: a comparison of polypharmacological and specific Inhibitors

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Medulloblastoma (MB) and glioblastoma (GBM) are the most frequent high-grade brain tumors (HGBT) in children and adulthood, respectively. Despite the improvements in patient survival, many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed. Citron Kinase (CITK), the primary product of the CIT gene, mutations of which are responsible for the MCPH17 primary microcephaly syndrome, is essential during neurodevelopment for proper cytokinesis and genomic stability in normal neural progenitor cells. CITK is validated as target for MB treatment as its depletion leads to cytokinesis failure and DNA double strand breaks (DSBs) accumulation in MB cells. Moreover, loss of CITK induces apoptosis and reduces tumor growth *in vivo*.

Methods After discovering that Lestaurtinib is an inhibitor of CITK, we tested it on different MB and GBM patient derived cell lines and *in vivo* injecting the drug in MBs arising in SmoA1 transgenic mice. In parallel, we conducted a Hit Discovery Program, which resulted in the identification of specific molecules targeting citron, which we subsequently tested on GBM patient derived cell lines.

Results Similar to CITK knockdown, treatment with Lestaurtinib leads to cytokinesis failure, accumulation of DNA double strand breaks, impairs cell proliferation and increases cell death in MB and GBM cells. Finally Lestaurtinib treatment reduces tumor growth and increases mice survival. The Hit Discovery Program resulted in the identification of molecules that inhibit CITK. When tested on GBM cells, these molecules produced phenotypes characteristic of CITK loss, although their effects were weaker compared to the polypharmacological compound Lestaurtinib.

Conclusions Specific inhibitors recapitulate the same effects, but to a lesser extent compared to a polypharmacological drug, and in a cell line-dependent manner. Given that the data on different cell lines show a varied response to CITK inhibition, we have begun to explore the genes and pathways that make a cell line sensitive to CITK, with the aim of predicting in the future which lines would be more responsive to treatment with CITK inhibitors.

Beyond Brain Tumor Control: Addressing Radiotherapy's Impact on Cognitive Function in GBM

NO 04

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Radiotherapy (RT) is a cornerstone treatment for glioblastoma multiforme (GBM), but its long-term impact on neurocognitive function, often called "brain fog," is a major concern. RT triggers neuroinflammation by activating microglia and astrocytes, contributing to cognitive decline. This, along with RT-induced endoplasmic reticulum (ER) stress and impaired proteasome activity, leads to the accumulation of misfolded proteins in neurons, intensifying neuroinflammation. To investigate these mechanisms, we used in vitro 3D co-cultures of patient-derived GBM cells and human microglia, and a patient-derived orthotopic xenograft (PDOX) GBM mouse model. Our Nanostring nCounter sequencing and RT-PCR analysis showed that RT induces both pro-inflammatory (M1) and anti-inflammatory (M2) microglial markers, with a predominance of M1 markers in co-cultured microglia and GBM cell lines. This suggests an immediate pro-inflammatory effect of RT, potentially damaging both tumor and healthy tissue.

Our data of the unfolded protein response (UPR) indicate that RT co-activates the ATF6 and IRE1 α pathways while decreasing PERK/eIF2 α expression, pointing that GBM prioritize adaptive mechanisms for survival under RT stress, rather than global protein synthesis suppression.

These findings demonstrate that RT-induced ER stress and UPR activation are key adaptive strategies in GBM cells under radiation. This nuanced UPR response, combined with the pro-inflammatory microglial phenotype, offers critical insights into RT resistance and the etiology of "brain fog", highlighting how cellular stress mechanisms might worsen clinical outcomes. Further studies are needed to explore these molecular pathways and identify neuroprotective strategies to improve cognitive function and quality of life for GBM patients undergoing RT.

Monoclonal antibodies as a therapeutic strategy against Glioblastoma**NO 05**

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults, characterized by rapid progression, high recurrence rates, and poor prognosis despite current standard-of-care therapies. Receptor tyrosine kinases of the ErbB family play key roles in tumor biology by regulating cellular proliferation, differentiation, survival, migration, and invasion. Among them, ErbB3 (HER3) is frequently overexpressed in GBM tissues. Upon binding to its ligand Neuregulin-1 (NRG1), ErbB3 activates the Phosphoinositide 3-kinase/Protein kinase B (PI3K/AKT) signaling pathway, promoting tumor cell proliferation, survival, and metabolic reprogramming. This study aims to assess the ability of an anti-HER3 monoclonal antibody (anti-HER3 mAb) to cross the blood-brain barrier (BBB) and evaluate its therapeutic potential in GBM mouse models. First, we use an *in vitro* BBB model composed of murine endothelial cell lines (bEnd.3) and murine primary astrocytes. This co-culture system creates a barrier, as revealed by the Trans-Endothelial Electrical Resistance (TEER) measurement, that is efficiently crossed by anti-HER3 mAb. We evaluated also the direct effects of anti-HER3 mAb treatment on glioma cell lines, GL-261 and CT-2A, observing impairment of survival, migration and proliferation. 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, our findings suggest that the anti-HER3 mAb is able to cross the *in vitro* BBB model and to exert an antitumor effect on glioma both *in vitro* and *in vivo*.

Modeling neuron–glioma interactions in vitro using co-culture systems**NO 06****Ersilia Nicorvo** ⁽¹⁾ - Alessia Ferraro ⁽¹⁾ - Paolo Pacca ⁽¹⁾ - Marina Boido ⁽¹⁾ - Ferdinando Di Cunto ⁽¹⁾ - Alessandro Vercelli ⁽¹⁾⁽¹⁾ *Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Department of Neuroscience, Torino, Italy*

The tumor microenvironment (TME) is a complex and highly dynamic niche composed of both cellular and acellular elements that play a crucial role in supporting cancer development and spread. In this context, the interactions between neurons and tumor cells have received increasing attention, especially in brain tumors, where they are believed to be key modulators of tumor aggressiveness. An expanding body of research supports the idea that neurons and glioma cells engage in reciprocal signaling, shaping one another's behavior. To explore the molecular basis of this neuron-glioma interplay, we established and refined multiple in vitro co-culture systems. We employed both primary cortical neurons from C57BL/6 mice and cortical neurons differentiated from human induced pluripotent stem cells (hiPSCs), co-cultured with U87 glioblastoma cells. Two experimental setups were used: transwell systems and treatment with glioma-conditioned media (CM), to dissect the effects of tumor-derived soluble factors on neuronal physiology. Neuronal viability and morphology were tracked in real-time using the Incucyte live-imaging platform with the NeuroTrack analysis module. Exposure to CM caused a 36% reduction in neurite outgrowth ($p = 0.0055$), a 44% drop in cell count ($p = 0.0024$), and elevated cytotoxicity after 48 hours ($p = 0.0116$), indicating the detrimental effects of glioma-secreted factors. Western blot analysis further revealed reduced levels of tubulin (–60%) and synapsin (–80%) in CM-treated neurons, pointing to disrupted cytoskeletal organization and impaired synaptic integrity. Ongoing studies aim to validate these findings in hiPSC-derived neurons and improve the biological relevance of our models using microfluidic-based systems. These advanced platforms may better reflect the in vivo scenario and help identify potential therapeutic strategies targeting neuron-glioma communication.

Cancer-neuronal crosstalk: how neuronal cells contribute to glioblastoma malignancy

NO 07

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The interaction between glioblastoma (GBM) and the brain microenvironment has gained increasing attention. Communication between neurons and glioma cells plays a key role in tumor growth and invasion. However, the underlying molecular mechanisms and how tumor heterogeneity influence this crosstalk remain to be fully elucidated. To address this, we developed an in vitro system where primary human GBM stem-like cell lines (hGSCs) were co-cultured with mature primary murine neurons. After 7 days of co-culture, neurons enhanced GBM cell proliferation in an activity-dependent manner. Notably, this supportive effect occurred even in the absence of direct cell-cell contact, suggesting the involvement of soluble factors. To account for GBM heterogeneity, we evaluated an heterogeneous panel of twelve hGSC lines. More than 80% of the lines exhibited increased proliferation when co-cultured with neurons ($p < 0.05$), and were classified as sensitive-hGSCs. In contrast, two cell lines did not respond to neuronal stimulation and were labeled as refractory-hGSCs. Interestingly, the proliferation blockade of refractory-hGSCs could be rescued when cells are co-cultured together with sensitive-hGSCs, suggesting a potential neuronal-dependent paracrine effect also among distinct GBM populations. These findings were paralleled by bulk RNA sequencing on hGSCs isolated through FACS-sorting following co-culture. Ingenuity Pathway Analysis revealed a specific enrichment of pathways associated to cell proliferation in sensitive-hGSCs, while refractory-hGSCs showed significant upregulation of apoptosis and cell death-related pathways (Z-score > 1.5). These findings highlight the pivotal role of the neuro-tumoral unit in GBM progression. Neuronal activity promotes glioblastoma cell proliferation, potentially via paracrine signaling. Ongoing analyses of neuron-induced pathways aim to further clarify the molecular mechanisms governing neuron-to-glioblastoma communication.

Development of an immunocompetent murine model of EGFRvIII-expressing glioblastoma

NO 08

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Glioblastoma, the most common and aggressive malignant brain tumor, has limited treatment options and a median survival of about 15 months after diagnosis. The epidermal growth factor receptor (EGFR) gene is frequently altered in glioblastoma, with the EGFR variant III (EGFRvIII) deletion mutation (exons 2-7) being highly prevalent. EGFRvIII is linked to tumor progression and poor prognosis, making it a crucial therapeutic target. To address this, we developed a pre-clinical model of EGFRvIII-expressing glioblastoma. We transduced two primary tumor cell cultures from our well-established glioblastoma model, based on platelet-derived growth factor subunit B (PDGF-B) overexpression in embryonic neural progenitor cells followed by inoculation into adult mice brains, with replication-defective retroviruses encoding EGFRvIII and the blue fluorescent protein (BFP) reporter gene. EGFRvIII+ cells were enriched by sorting for BFP expression, and stable EGFRvIII expression over time (75-90%) was confirmed by flow cytometry and immunocytochemistry. RNA sequencing of mRNA from EGFRvIII+ cells revealed that global transcriptomic profiles remained largely unchanged upon EGFRvIII overexpression. To test tumorigenicity, EGFRvIII+ cells were transplanted intracranially into immunocompetent BALB/c mice, forming secondary tumors. Flow cytometry and immunofluorescence analyses revealed that secondary tumors maintained EGFRvIII expression levels comparable to those of the parental cells. Preliminary data also indicate susceptibility of these cells to oncolytic viruses targeting EGFRvIII, highlighting the model's potential for evaluating targeted therapies. Our findings suggest that the developed model of EGFRvIII-expressing glioblastoma holds promise as a preclinical tool to evaluate EGFRvIII-targeted therapies and address other biological inquiries related to glioblastoma, thereby enhancing our understanding and potentially advancing treatment options for this aggressive brain tumor.

Oxytocin System Dysregulation and Social Reward Impairment in a Rat Model of Gaming Disorder

NP 01

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In 2018, the International Classification of Diseases (ICD-11) recognized Gaming Disorder (GD) as a mental health condition, with a peak prevalence during adolescence. Individuals with GD engage in compulsive gaming behavior, often associated with anxiety, social withdrawal, depression, and attention deficits. Although numerous clinical studies exist, they frequently present limitations related to exposure duration, observation windows, and gender variability. To address these issues and better elucidate the neurobiological mechanisms underlying GD, we developed a novel animal model.

Rats subjected to the GD protocol showed a significant increase, compared to control (CON) animals, in the frequency and duration of gaming behavior, time spent, and distance covered in front of the screen. This attachment to gaming altered their social interaction: GD rats consistently preferred the video game over novel social stimuli. Behavioral changes were further investigated in various contexts, including interactions with a familiar conspecific, an unfamiliar same-sex individual, and an unfamiliar opposite-sex individual, revealing a generalized reduction in social exploration. Moreover, the diminished effect of social reward in reducing gaming-related attachment was accompanied by a decrease in oxytocin-expressing neurons in the Paraventricular Nucleus and Supraoptic Nucleus of GD rats compared to controls.

Loss of control over gaming behavior, anxiety, and hyperactivity observed in GD rats further support the translational validity of this model. The reduction in social interaction and its influence on compulsive gaming behaviors suggest a critical role of the oxytocinergic system in GD. Oxytocin, a key neuroendocrine hormone, has been implicated in various psychiatric disorders and addictive behaviors; disruptions in this system may contribute to the compulsive and maladaptive patterns characteristic of addiction, potentially increasing adolescents' susceptibility to GD.

A Cre-lox technique to address olfactory dopaminergic cell functional heterogeneity

NP 02

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The main olfactory bulb (OB) is the first and only relay structure for odors before reaching the olfactory cortex and hosts the most numerous dopaminergic (DA) forebrain population. These inhibitory interneurons are typically identified by tyrosine hydroxylase (TH) expression and two main subpopulations can be recognized based on soma size, electrophysiological properties, and generation time. Although increasing evidence places the role of these cells in the early stages of sensory processing, their exact function remains unclear. Moreover, their high heterogeneity challenges the study of these cells and thus a specific method to target the two subpopulations differentially is required to study their properties and roles separately. To this aim, we validated a virally delivered, time-controlled Cre-lox technique that allows to express either morphological or functional reporters in the OB TH-positive cells selectively. The specificity of this labelling is comparable to that of the TH-GFP transgenic mouse line and can target TH-positive subpopulations enriched in either small or large cells whether it is performed in adult mice or in embryos and newborns, respectively. By expressing GCaMP indicators in TH-positive cells, we were able to perform in vivo two-photon functional calcium imaging experiments in anesthetized, head-fixed adult mice. This allowed the assessment of their spontaneous activity as well as their evoked responses to opposite-sex urine. Since we previously observed a specific TH-positive cell recruitment in the posterior OB of female mice exposed to male urine, our goal is to focus on this area while observing TH-positive cell function using our labelling strategy. Taking advantage of a multidisciplinary approach based on this OB DA cell targeting technique, our main aim consists in dissecting a possible differential role for the two DA subpopulations in processing ethologically relevant odors, specifically those associated to sexual behaviors.

GluN2A(N615S) Mutation Drives Sex-Specific Seizure Susceptibility and Cardiac Ischemia: Modulation by Ketogenic Diet

NP 03

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‘GRINpathies’ are rare pediatric neurodevelopmental disorders caused by mutations in the GRIN genes, encoding glutamate-gated Ca^{2+} -permeable NMDAR subunits. The Grin2a^{S/S} (GluN2A(N615S)) knock-in mouse model replicates key features of the human condition, including behavioral abnormalities and epilepsy¹. Sex differences have been observed, with Grin2a^{S/S} females demonstrating higher vulnerability to severe audiogenic seizures (AGS) leading to death and an improved response to antiseizure medications (ASMs)². Altered cardiac NMDAR function may lead to intracellular Ca^{2+} imbalance, contributing to arrhythmias and increased susceptibility to ischemia/reperfusion injury (IRI). We investigated cognitive deficits, seizure susceptibility, and IRI in male and female Grin2a^{S/S} mice by conducting behavioral testing, AGS-inducing protocols, and ex vivo ischemia-reperfusion assay³ followed by Western blot, respectively. We also assessed the therapeutic potential of a ketogenic diet (KD) by evaluating its effects on Grin2a^{S/S} phenotype, and on brain and cardiac plasticity, physiology and inflammation. Results suggest that Grin2a^{S/S} females, with heightened sensitivity to AGS leading to death, exhibited significantly larger cardiac infarcts compared to males. Interestingly, the ketogenic diet conferred a more pronounced protective effect in females, improving seizure outcomes and modifying both brain and heart parameters associated with the disease phenotype. In conclusion, our findings highlight marked sex differences in the Grin2a^{S/S} model, particularly in seizure susceptibility and cardiac vulnerability and, importantly, in the response to treatments, suggesting the potential need for sex-specific interventions targeting both neurological and cardiovascular comorbidities in GRINpathies.

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Effects of transcranial electrical stimulation on hippocampal synaptic activity in awake mice

NP 04

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Transcranial electrical stimulation (tES), including direct (tDCS) and alternating (tACS) currents, is increasingly explored as a neuromodulatory tool for cognitive and memory enhancement. However, its effects on hippocampal function remain unclear. We investigated the impact of single sessions of unilateral anodal and cathodal tDCS ($\pm 200 \mu\text{A}$, 20 min) and 40 Hz or 10 Hz tACS ($\pm 50 \mu\text{A}$, 30 min) on synaptic activity in the CA3–CA1 hippocampal pathway in awake mice. Electrophysiological recordings were performed using a paired-pulse protocol through Schaffer collateral stimulation in adult male and female mice. Anodal tDCS increased the amplitude of both evoked responses (P1 and P2), particularly in male mice, while cathodal tDCS selectively decreased P2 amplitude, resulting in a reduced P2/P1 ratio showing a presynaptic effect. Sex differences were observed: anodal tDCS had no significant effect on the ratio in males, whereas cathodal tDCS reduced it in females. In contrast, no significant changes were found in response amplitudes following 40 Hz or 10 Hz tACS, in either sex. These findings suggest that tDCS modulates hippocampal synaptic transmission in a polarity- and sex-dependent manner, while tACS at the tested frequencies does not induce detectable changes in evoked responses. Our results highlight the importance of stimulation parameters and biological variables such as sex when evaluating neuromodulatory interventions. This study provides insight into the differential effects of tES protocols on hippocampal excitability, which may be critical for optimizing stimulation-based strategies aimed at treating neurodegenerative and cognitive disorders.

Impact of diabetic neuropathy in small fiber alterations across sexes**NP 05****Emma Merlin**⁽¹⁾ - Patrizia Aimar⁽¹⁾ - Aruzhan Muratbek⁽¹⁾ - Francesco Ferrini⁽¹⁾ - Chiara Salio⁽¹⁾⁽¹⁾ *Università degli Studi di Torino, Dipartimento di Scienze Veterinarie, Grugliasco, Italy*

Diabetes is a chronic progressive disorder characterized by hyperglycemia, with a growing global prevalence affecting approximately 10% of adults. Diabetic neuropathy (DN), a common and severe complication that affects one-third of diabetic patients, lacks effective treatments to halt or slow its progression. The onset of DN is primarily linked to microvascular and metabolic alterations in the peripheral nervous system (PNS) and alteration of small C-fibers is clinically relevant for the development of pain symptoms. Yet, which type of small fibers is more affected by diabetes is still unclear. We adopted a streptozotocin (STZ)-induced T1D model in male and female mice expressing the green fluorescent protein *Venus* in a population of C-low threshold mechanoreceptors (C-LTMRs; *tafa4-Venus knock-in*), involved in touch hypersensitivity after injury. After a behavioral assessment, mice were sacrificed and three main types of C-fibers were investigated in glabrous and hairy skin, dorsal root ganglia (DRG), and spinal dorsal horn, namely: CGRP+ peptidergic fibers, Isolcectin B4 (IB4)+ non-peptidergic fibers and VGLUT3/TAFA4+ C-LTMR fibers. Interestingly, we found that females required higher STZ doses to develop diabetes, but also that they are more prone to display mechanical sensitivity at the von Frey test. Confocal microscopy analysis of small fiber alterations suggests that behavioral sex differences are accompanied by sexually dimorphic alterations of small fibers, particularly at the spinal level. Preliminary results indicate that females exhibit higher IB4 and VGLUT3 expression levels in controls, but after the STZ-treatment, a general reduction in these markers was observed. Conversely in diabetic males, CGRP modestly decreased, while IB4 remained unchanged, and VGLUT3 increased. These findings emphasize the necessity of including both sexes in diabetes research and suggest that specific types of sensory fibers are more likely to be affected in DN.

Sex differences in the recruitment of spinal dorsal horn inhibitory interneurons in inflammatory pain

NP 06

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Pain perception derives from the elaboration of sensory stimuli conveyed to different areas of the central nervous system (CNS) through nociceptive pathways. At the level of the spinal cord, the superficial dorsal horn (SDH), composed of laminae I (LI) and II (LII), is the site most involved in nociception, where modality-specific circuits for pain processing are activated. A complex network of inhibitory and excitatory interneurons exists, and their balance determines the way nociceptive signals are encoded and reach the brain. By immunofluorescence and confocal microscopy, we found that the density of inhibitory interneurons, identified by the phenotypic marker Pax2, is higher in females than in males in both the mouse and human spinal cord. To address the involvement of inhibitory neurons in nociception, we adopted an inflammatory pain model based on intraplanar injection of zymosan. Recruitment of inhibitory interneurons was investigated by measuring the expression of the immediate early gene c-fos, a common marker of neuronal activation, in Pax2+ neurons. Zymosan-induced inflammation produced comparable reductions in mechanical thresholds, as measured by the von Frey test, in both sexes, alongside similar increases in neuronal activation and recruitment of inhibitory neurons ipsilateral to the site of injection. Notably, baseline levels of neuronal activation and inhibitory interneuron recruitment were higher in females within both superficial and deep dorsal horn laminae. Collectively, our data suggest that a higher number of inhibitory interneurons is enrolled in the female dorsal horn to achieve the same level of control over inflammatory pain compared to males. These data highlight the importance of tailoring antinociceptive therapies to enhance spinal inhibition in a sex-specific manner.

Chronic microdosing with psilocybin as a novel strategy to promote visual recovery after occipital stroke

NP 07

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Stroke is a leading cause of disability, and occipital stroke often results in severe visual deficits. Currently, no treatments restore vision post-occipital stroke, necessitating novel therapeutic strategies. Psychedelics like psilocybin promote neuroplasticity and neurogenesis, potentially aiding recovery.

This study examines the effects of chronic low-dose psilocybin (0.05 mg/kg, 45 days) on visual recovery in adult mice subjected to photothrombotic unilateral visual cortex stroke. Visual abilities were assessed using the Visual Cliff, Looming, and Cued Morris Water Maze tests. Lesion size and dendritic spine density in perilesional and contralateral regions were also evaluated.

In the Visual Cliff test, psilocybin-treated stroke mice exhibited higher speed in the unsafe zone than vehicle-treated stroke mice, and speeds similar to controls, suggesting improved depth perception. In the Looming test, vehicle-treated stroke mice had delayed flight responses, while psilocybin-treated mice responded like controls, indicating preserved threat detection. In the Cued Morris Water Maze, psilocybin-treated stroke mice performed comparably to controls, unlike saline-treated mice.

These behavioural improvements were accompanied by reduced lesion volume after 45 days and increased spine density in both hemispheres.

Overall, chronic non-psychedelic psilocybin treatment, initiated 24h post-stroke, was well tolerated and improved visual task performance, likely via neuroplasticity enhancement. Psilocybin emerges as a promising candidate for post-stroke visual rehabilitation.

Interactions Between Phytoestrogens and Chronic Anorexia-Like Phenotype: Behavioral and Neuroendocrine Changes in Female Rats

NP 08

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Anorexia nervosa (AN) is a severe psychiatric disorder characterized by behavioral disturbances and profound physiological consequences. The activity-based anorexia (ABA) rat model mimics AN, consisting of a reduction of 25-30% weight using both a hypocaloric diet and daily exercise (2h running wheel). The phytoestrogen genistein (GEN) is known as an endocrine disruptor that may interfere with permanent morphological changes in the brain circuits sensitive to estrogen. Due to the frequency of a high-GEN soya diet in anorexic women, we aimed to study the effects of both conditions together. Female adult rats were used, creating two ABA-groups, control-ABA and altered-diet ABA (Gen-ABA). Weight and food intake were monitored daily during the three-month experiment, with reductions of 60% and 50% in the acute and chronic phases, respectively. Significant differences compared to control females have been observed in memory, locomotion (T-maze), and anhedonia (Sucrose test). Moreover, a global increase in hyperactivity, anxiety, and -progressively- depression in the open field and elevated plus maze tests was measured. Additionally, estrous cycle alterations have been noted, with an elongation of diestrus during the acute phase and total cessation of the estrous cycle in the chronic phase of ABA. Consistent with the increase in anxiety and cessation of estrus, aberrant sexual behavior has been observed. To better understand the underlying mechanisms, particularly those prone to being compromised due to these metabolic and behavioral alterations, further studies are recommended.

Combined environmental and P3HT nanoparticle-based approaches to improve visual function in Retinitis pigmentosa

NP 09

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Environmental enrichment (EE), a non-invasive strategy based on enhanced sensory and social stimulation, has shown neuroprotective and neurotrophic benefits in various neurodegeneration models. We recently demonstrated that EE delays retinal degeneration and enhances visual responses in Retinitis pigmentosa (RP), a progressive photoreceptor disorder. Retinal prostheses are currently the only means to restore light perception in late-stage RP; however, the visual system may become unable to properly process bionic visual signals. In this study, we investigated whether EE could enhance the efficacy of photoactive prosthetic stimulation by promoting neuroplasticity. We used blind retinal degeneration 10 (rd10) mice, a model of RP, and injected photoactive poly(3-hexylthiophene) nanoparticles into the subretinal space to deliver light-responsive input. After surgery, animals were either maintained in standard housing or exposed to EE for one month. We evaluated visual behaviour and electrophysiological responses in the retina and primary visual cortex (V1) in response to simple flashes and complex patterned light stimuli. This combined approach offers a promising avenue for improving visual outcomes and preserving retinal function in degenerative retinal diseases.

Decreased neuronal excitability and enhanced frequency adaptation in medial prefrontal cortex pyramidal neurons of depressed mice

NP 10

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Chronic social defeat stress (CSDS) is a widely used rodent model for studying the neurobiological mechanisms underlying depression, as it reliably induces depressive-like behaviors such as social avoidance, despair behavior and anhedonia. The medial prefrontal cortex (mPFC) is a central hub in the neural circuitry regulating mood and adaptive responses to stress, and its dysfunction has been implicated in the pathophysiology of depressive disorders. However, the cellular adaptations in mPFC neuronal activity caused by chronic stress remain not fully understood.

In this study, we subjected C57BL/6j male mice to a 10-day CSDS protocol and assessed behavioral outcomes using the social interaction test, allowing classification into stress-susceptible and resilient phenotypes. Whole-cell patch-clamp recordings were performed on pyramidal neurons in acute mPFC slices to investigate intrinsic excitability. Our results demonstrate that only stress-susceptible mice exhibited robust social avoidance, while resilient and control mice maintained normal social interaction. Electrophysiological analysis revealed that, although passive membrane properties were unchanged, mPFC pyramidal neurons from susceptible mice displayed a reduced intrinsic excitability. This hypo-excitability was driven by enhanced spike frequency adaptation, disrupting the neuron's ability to sustain repetitive firing. These findings provide direct evidence that chronic stress leads to a downregulation of intrinsic excitability in mPFC pyramidal neurons specifically in mice that show depressive-like behavior. Our data support the hypothesis that reduced mPFC activity is a key cellular correlate of susceptibility to chronic stress and may contribute to the emergence of depressive symptoms. Understanding these adaptations may help identify novel targets for therapeutic intervention in stress-related mood disorders.

Spatiotemporal Regulation of CXCR4 in Nerve Regeneration: H₂O₂ as Key Factor

NP 11

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Effective regeneration of an injured peripheral nerve requires a well-coordinated tissue response, relying on multiple mediators and signaling pathways within the surrounding milieu. In this context, the CXCL12 α -CXCR4 axis has emerged as a crucial driver of nerve repair. Well known for its role in development - where it guides motor axons exiting from the ventral root along the correct trajectory before being silenced upon target recognition - it is reactivated in adulthood in response to nerve damage. Its stimulation promotes rapid and effective motor axon regrowth upon acute nerve injuries. Here, we investigated the dynamics of CXCR4 re-expression at transcript and protein levels in the nerve, spinal cord and neuromuscular junction using various approaches, ranging from immunofluorescence, RNAscope, and electron microscopy. Our findings reveal central and peripheral mechanisms regulating receptor expression at different stages post-injury. Specifically, we show that hydrogen peroxide generated at the injury site acts as a trigger for CXCR4 signaling. Overall, these findings define the spatiotemporal regulation of CXCR4 expression in response to damage, highlighting the contribution of surrounding Schwann cells, muscle fibers, and of early local translation to CXCR4 expression and signaling.

Development of Allele-Specific Gene-Silencing siRNAs for MPZ-D61N in CMT1B

NIM 01

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We recently generated a mouse model carrying the D61N heterozygous mutation in the *MPZ* gene, which encodes a structural protein of peripheral myelin. This mutation causes, in humans, a severe early-onset form of CMT1B, characterized by extensive dys/demyelination. This model has also been extensively characterized in our laboratory using a sequential protocol that includes evaluation of motor performance, ultrasonography, magnetic resonance imaging, and whole-body micro-PET imaging to monitor in vivo the effects of any therapeutic intervention. In this study, we investigated the sensitivity and specificity of short-interfering RNA (siRNA) treatment for CMT1B caused by MPZ-D61N heterozygous mutation, using exogenous expression constructs in HeLa cells. A panel of 19 MPZ-D61N-specific siRNAs was assessed by a dual-fluorescent reporter assay and suppression of mutant MPZ expression was confirmed by Western blot. We also plan to perform a rescue experiment using dorsal root ganglia myelinating cultures from MPZ^{D61N/WT} heterozygous mice, which are characterized by severe myelination defects. Our screening identified several effective inhibitors for the mutant allele, with minor impact on wild-type MPZ. Our results will hopefully provide proof-of-principle that allele-specific RNAi has potential therapeutic efficacy for CMT subtypes caused by gain-of-function and dominant-negative mutations.

Longitudinal assessment of neurodegeneration in a mouse model of tauopathy using multiparametric magnetic resonance imaging

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Mouse models of Alzheimer's disease have been instrumental in investigating mechanisms of neurodegeneration, especially tau-mediated pathology and neurofibrillary tangle formation, key features of the disease. Despite advances, mechanisms driving tau-related neuronal loss and brain atrophy remain unclear. We used JNPL3 mice, expressing human Tau with the P301L mutation, to study neurodegeneration via multiparametric magnetic resonance imaging (MRI) in a longitudinal design. MRI was performed on wild-type (WT) and JNPL3 mice at 2, 4, 6, 11, and 16 months to assess volume changes in the cortex, striatum, and hippocampus. Diffusion tensor imaging (DTI) was applied to evaluate white matter integrity in the corpus callosum and fimbria. Motor and cognitive function were assessed with rotarod, Y-maze, and radial arm water maze (RAWM) tests. At 6 months, JNPL3 mice showed tau-related atrophy in cortex, striatum, and hippocampus. DTI parameters revealed white matter degeneration characterized by demyelination and axonal loss as early as 6 months, confirmed by Luxol fast blue histology. These changes coincided with early motor deficits detected by rotarod, while cognitive impairments became evident at 11 months in Y-maze and RAWM. Our study demonstrates that multiparametric MRI can elucidate progressive tau pathology in JNPL3 mice, revealing early white matter alterations preceding cognitive decline. These findings support the use of non-invasive MRI techniques to uncover early pathological events in Alzheimer's disease models and to evaluate the efficacy of therapeutic interventions targeting these processes.

Cerebral mapping during dynamic autoregulation using functional near-infrared spectroscopy (fNIRS)

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Cerebral autoregulation is a fundamental physiological mechanism that keeps cerebral perfusion stable despite fluctuations in systemic arterial blood pressure (ABP) and thus protects brain tissue from hypo- and hyperperfusion. Impairments in this mechanism have been associated with poor outcomes in various diseases, including stroke, traumatic brain injury and cerebrovascular stenosis. This study aims to spatially map dynamic cerebral autoregulation using functional near-infrared spectroscopy (fNIRS), a non-invasive technique that responds to haemodynamic changes in the cortex. Transcranial Doppler (TCD), photoplethysmography and standard near-infrared spectroscopy (NIRS) can be added to the setup. Two maneuvers were employed to produce controlled rapid fluctuations in mean arterial pressure (MAP): (1) a reduction in MAP achieved by the rapid deflation of thigh cuffs following a 90-second inflation at 200 mmHg, and (2) an increase in MAP induced by the sudden release of lower body negative pressure (LBNP) set at -40 mmHg for 90 seconds. Preliminary findings confirmed that these methods effectively triggered MAP changes of approximately -10 to +15 mmHg within 5 to 10 seconds of stimulus onset. Additionally, early recovery patterns observed in NIRS and TCD signals indicated the activation of dynamic cerebral autoregulation. Upcoming experiments using fNIRS will aim to examine the influence of both intracranial and extracranial hemodynamics and explore the feasibility of mapping dynamic cerebral autoregulation, with potential clinical relevance for evaluating cerebrovascular dysfunction.

Enhancing Safety in BBB Opening: A Statistical Approach to Cavitation Monitoring

NIM 04

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Focused ultrasound with microbubbles (MBs) can transiently and locally open the blood–brain barrier (BBB), facilitating the delivery of large molecules to the brain. To prevent harmful inertial cavitation, control systems track acoustic emissions via three metrics: stable cavitation dose (SCD, harmonics), instability dose (IUD, ultraharmonics), and inertial cavitation dose (ICD, broadband). Thresholds reported in the literature vary widely (4–10 dB), reflecting variability in MB acoustic responses across experimental conditions.

We examined the effects of MB concentration, flow rate, acoustic pressure (AP) on cavitation emissions in a realistic phantom. Sonovue MBs were administered at two clinically relevant concentrations ($30 \mu\text{L kg}^{-1}$ and 1.6 mL kg^{-1}) while simulating mouse and rat brain capillary flow (0.49 and 2.00 mL min^{-1}) in a microvessel. MBs were sonicated at 1.5 MHz across 25 APs (0.1 – 2.0 MPa) with 3 ms pulses, and acoustic emissions were recorded using a 1.5 MHz passive cavitation detector.

Multivariate mixed-model analysis showed significant effects of MB concentration, flow rate, and AP on both ultraharmonic and broadband signals; nevertheless, mean IUD and ICD values remained below 10 dB in all conditions. ICD and IUD distributions were compared with a reference pressure of 0.3 MPa (mechanical index = 0.4), where only stable cavitation is expected. Kolmogorov–Smirnov tests indicated significant distribution deviations at intermediate pressures (0.4 – 0.75 MPa ; $\text{MI} = 0.4$ – 0.61). Further analysis revealed that at least 20 pulses are required to reliably detect changes in ICD and IUD distributions, a finding confirmed in vivo in mice under identical conditions.

These results support an adaptive, real-time control system that acquires a 20-pulse baseline and then compares it with ICD/IUD values during pressure escalation, stopping escalation upon significant deviations. This strategy enables safe and efficient BBB opening, thereby improving therapeutic delivery.

The MYSTICALS study protocol: Microorganisms, Toxins and Immunity InterSeCTion In Amyotrophic LateraAL Sclerosis Frontotemporal Dementia - targeting disease heterogeneity

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Recent studies have looked at the role of the gut microbiota (GM) in a variety of neurological disorders. As far as the amyotrophic lateral sclerosis and frontotemporal dementia (ALS-FTD) spectrum of disease, previous animal and human studies demonstrated a possible role of GM in driving disease features and progression. ALS patients have a different bacterial GM with respect to healthy controls (HC). The fungal microbiota (mycobiota) component and mycotoxins have never been studied in the ALS-FTD spectrum of disease, nor have been the interactions among these kingdoms and immunity. Our aim is to assess gut and oral bacteriome, mycobiome, and immune-metabolic profile of ALS-FTD patients, their interconnection and relationship with exposomics, and their influence on disease progression and clinical features for patients stratification. We are performing a large case-control longitudinal study (100 ALS-FTD patients, 50 HC) setted in two time points (T0, T6) with biological samples, focused on gut and oral shotgun metagenomic profiling, free fatty acids and mycotoxin evaluation, neurodegenerative/neuroinflammatory biomarkers analysis and immune-metabolic status assessment. These data will be correlated with patients' clinical and exposomic features. Furthermore, we will evaluate the mycotoxins effect on cellular models to provide mechanistic insight into ALS-FTD pathogenesis. Specific patterns of oral and gut fungal dysbiosis and related toxins could influence immune-metabolic response in the host and, ultimately, trigger and/or increase the severity/type of ALS-FTD syndrome. Identification of metagenomic signatures specifically correlated with ALS-FTD would open novel avenues to understand and combat the heterogeneity of this disease. Defining systems biology of ALS-FTD could allow to stratify patients for specific interventions and guide towards new frontiers of personalized medicine.



Investigating therapeutic approaches in Congenital Hydrocephalus

NDV 14

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Congenital hydrocephalus (CH) is a complex neurodevelopmental disorder characterized by enlarged brain ventricles and reduced cognitive functions. We recently identified FLVCR1 as a gene implicated in congenital hydrocephalus. The full-length isoform, FLVCR1a, plays a key role in neurogenesis by modulating mitochondrial calcium homeostasis through its interaction with the IP3R3-VDAC complex at mitochondria-associated membranes. Loss of Flvcr1a in murine neural progenitor cells (NPCs) disrupts mitochondrial calcium uptake, compromises energy metabolism, and impairs neurogenic processes, ultimately leading to ventricular enlargement, a hallmark of CH pathology. (Bertino F., 2024) However, recent evidence has demonstrated that FLVCR1 functions as a high-affinity importer of choline. Besides its involvement in the regulation of membrane composition and neuronal communication, choline also plays an essential role in epigenetic regulation. Interestingly, mutations in the choline importers FLVCR1 and FLVCR2 have been implicated in the development of severe forms of CH. Considering such essential roles of choline and the importance of calcium in regulating mitochondrial metabolism, we sought to explore potential therapeutic interventions targeting these pathways. Specifically, we evaluated choline supplementation to restore substrate availability, along with treatments aimed at enhancing mitochondrial function, such as mitochondrial calcium uptake enhancers. Preliminary results indicate that these interventions, either alone or in combination, may exert beneficial effects on cellular function. However, further studies are required to better understand their mechanisms of action and to optimize treatment strategies for FLVCR1-related congenital hydrocephalus.

Disrupted CystatinB–TRiC/CCT complex interaction in EPM1: from molecular dysregulation to neural dysfunction

NDV 15

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EPM1 is the most common form of Progressive Myoclonus Epilepsy, caused by loss-of-function mutations in CYSTATIN B (CSTB) gene and characterized by myoclonus and neurological decline. Using cerebral organoids (COs) derived from human patient cells, we previously demonstrated the relevant role of CSTB in cortical development and synapse physiology. In addition, EPM1-COs exhibit a disrupted excitatory/inhibitory balance, likely driven by altered extracellular vesicle (EVs) signaling. These data indicate that both intrinsic and extrinsic mechanisms alter neural cell fate specification in EPM1. To further investigate how CSTB deficiency contributes to the EPM1 pathogenesis, we performed a co-Immunoprecipitation assay on dorsal- and ventral-patterned control COs to identify cortical region-specific CSTB interactors. In ventral COs, proteomic analysis revealed the subunits of the chaperonin TRiC/CCT complex as key CSTB interactors. TRiC/CCT complex is essential for folding cytoskeletal proteins and regulates endosomal and multivesicular body (MVB) formation.

In early-stage differentiation neurons derived from EPM1 patients, we observed MVB trafficking defects, as indicated by the mislocalization of RAB7, a marker of late endosome, which led to altered EVs production. Moreover, we detected abnormal F-actin suggesting altered cytoskeletal organization. Since TRiC/CCT complex also contributes to the elongation of newly synthesized protein, we assessed translational dynamics and revealed impairments in EPM1 mature neurons. To confirm that the disrupted CSTB-CCT complex interaction induces pathology-related changes, our experiments are directed to 1) the silencing of CCT components using CRISPR-I in a physiological context to verify if it recapitulates EPM1 phenotypes and 2) the overexpression of CSTB in EPM1 NPCs to rescue the CCT complex activity. These results will provide new insights into the molecular mechanisms underlying EPM1 epilepsy.

Dissecting the role of the synaptic adaptor protein p140Cap in human cortical development through brain organoids

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p140Cap is an adaptor protein encoded by the *SRCIN1* gene highly expressed in neurons. Initially identified as a SNAP25-binding protein, it was then found in excitatory postsynaptic densities and inhibitory presynaptic terminals. Mouse studies reveal its involvement in dendritic spine maturation and synaptic plasticity via microtubule and actin cytoskeleton reorganization. *p140Cap* knock-out mice show impaired long-term potentiation and depression, learning and memory deficits, and altered actin organisation of glutamatergic synapses. p140Cap interacts with proteins essential for excitatory and inhibitory synaptic transmission and cell–cell junctions, underscoring its role in synaptogenesis and synaptic plasticity. Its deletion also increases the number of inhibitory interneurons, suggesting a role in progenitor proliferation and interneuron migration. Co-expression data further associate *SRCIN1* with disorders such as schizophrenia, autism, and epilepsy.

The aim of this study is to define the role of p140Cap in human neurodevelopment and synaptic formation by employing cortical brain organoids derived from human induced pluripotent stem cells, in particular starting from a *SRCIN1* knock-out line alongside an isogenic control line. Organoids are examined across developmental stages to assess the impact of p140Cap loss on cellular composition and spatial organisation, by performing RNA expression levels analyses, morphometric and immunofluorescence studies to evaluate changes in neural progenitor proliferation and subsequent differentiation, and the overall cellular developmental processes. Additionally, we will investigate synaptic growth and circuit formation to determine whether p140Cap influences key processes such as synaptogenesis, spine formation, and neuronal connectivity, also in electrophysiological terms. This model will provide insight into the contribution of p140Cap to human cortical development and its potential link to neurodevelopmental disorders.

Enhancing human cerebellar organoids with enrichment of region-specific glia**NDV 17**

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Human brain organoids have recently advanced our understanding of species-specific features of neural development and pathology that cannot be recapitulated in non-human models. Recently, these advancements have extended to the modeling of the cerebellum. However, while cerebellar organoids show good levels of maturation, with the generation of the main progenitor and neuronal populations, glial cells are under-represented in these systems. Since glia is essential for the establishment of cerebellar cytoarchitecture and function, its absence is likely to hinder the ability of such models to recapitulate cerebellar development and pathological alterations.

We characterized endogenous glial populations of cerebellar organoids through transcriptomic and morphological analyses, highlighting limited maturation of astroglial cells and absence of oligodendroglia in the system. To increase the representation of glial cells in the model, we are testing the addition of hPSC-derived glial progenitors to cerebellar organoids via spontaneous migration or through a seeding strategy, showing integration of astroglia-like cells in the system. This enrichment technique will be compared to an established protocol developed to induce increased gliogenesis in cortical organoids. Additionally, we generated glial progenitors with specific cerebellar patterning, to be integrated in the organoids to investigate extrinsic and intrinsic determinants of region-specific properties of glial populations. To comprehensively evaluate the increase in glial lineages and consequent alterations in the transcriptomic profile of neighboring neuronal populations, we produced a preliminary single-nuclei RNAseq dataset comparing classical organoids to glia-enriched ones. The extent and modality of integration of the added glial component, together with its impact on the organoid cytoarchitecture and neuronal maturation, will be further evaluated through immunohistochemical and functional analyses.

A single cell atlas to unveil the diversity of mouse cerebellar astrocytes: insights into their molecular identities, development, and functions

NDV 18

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Astrocyte heterogeneity is essential for understanding the development and function of the central nervous system (CNS). The cerebellum (CRB), with its well-defined architecture, clear spatial organization, and distinct gross morphologies of major astrocyte types, provides an ideal framework for linking molecular profiles to specific astrocyte identities and functions. CRB astrocytes have been traditionally classified based on morphology and location. However, it remains unclear whether this classification reflects their molecular and functional heterogeneity, and our understanding of their developmental trajectories is limited. Through single-cell and spatial transcriptomics in postnatal and adult mouse CRB, we revealed huge astrocyte heterogeneity, defining the molecular profile of known types, identifying novel subtypes, and highlighting functional specializations such as vesicle-releasing and stem cell-like astrocytes persisting into adulthood. By developing a multimodal computational approach, we reconstructed their maturation trajectories, uncovered shared and subtype-specific gene regulatory networks, and traced embryonic clonal lineages, uncovering unexpected relationships with other cell types. We revealed a complex mechanism underlying the emergence of astrocyte heterogeneity postnatally, driven by divergence and convergence of cell fates as well as by early fate restriction. These findings position the CRB as a powerful model for studying astrocyte heterogeneity and offer new insights into the fundamental mechanisms governing astrocyte diversity across the CNS.

Loss of NR2F1 Impairs Mitochondrial Function in Mouse and Human Models of the Neurodevelopmental Disorder BBSOAS

NDV 19

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The Bosch-Boonstra-Schaaf optic atrophy syndrome (BBSOAS; OMIN#615722), is a rare neurodevelopmental disorder caused by mutations in the NR2F1 gene, a transcriptional regulator with key roles in brain development and function. Most NR2F1 pathological variants lead to haploinsufficiency or dominant negative effects, thereby compromising its transcriptional activity. Like many neurodevelopmental disorders, BBSOAS presents a broad clinical spectrum, whose symptoms are compatible with a mitochondria dysfunction in the brain. By combining in silico and in vivo approaches, our recent work uncovered a novel role for NR2F1 in mitochondrial regulation. We showed that NR2F1 controls a network of nuclear-encoded mitochondrial genes, influencing mitochondrial mass and morphology, pointing to mitochondrial dysfunctions in neural tissue as a potential key pathological mechanism in BBSOAS. Accordingly, Nr2f1-heterozygous mice, a validated disease model, display dysregulation of several nuclear-encoded mitochondrial genes and reduced levels of key mitochondrial proteins in the brain. However, how NR2F1 deficiency might lead to mitochondria-related functional defects in human neurons remains unknown. We are currently translating our findings to human iPSCs lines to further characterize the molecular and cellular mechanisms underlying mitochondrial impairment in the absence of NR2F1. We established a protocol to differentiate human iPSCs into a telencephalic cortical neurons, generating neural progenitors and neurons expressing key regional markers (i.e. PAX6; FOXG1). By means of super resolution imaging techniques, preliminary data are suggesting an increase in mitochondrial fragmentation in human iPSC-derived neurons devoid of NR2F1. Our findings highlight NR2F1 as an active regulator of mitochondrial gene expression and function in the brain, paving the way for further investigations into mitochondria-related mechanisms and new perspectives for targeted therapeutic strategies.

Modeling infantile neurological disorders linked to Gi/o-cAMP signaling pathway in *C. elegans*: insights into pathogenic mechanisms and therapeutic responses

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GNAO1 is part of a group of genes encoding proteins involved in postsynaptic signaling playing a key role in controlling signal flow through G-protein-coupled receptors and the cAMP. Variants in these genes cause infantile neurological disorders characterized by developmental delay, movement disorder (MD) and drug-resistant seizures. We have recently established *C. elegans* as a model for *GNAO1* encephalopathy, and highlighted the potential role of caffeine in controlling *GNAO1*-related dyskinesia. In this study, we characterized the phenotype and caffeine-response of CRISPR/Cas9 engineered worms carrying pathogenic variants (PVs) in several genes of the pathway. First, we analyzed a novel *GNAO1* variant (L23P) associated with parkinsonism rather than the more common hyperkinetic MD. Phenotypic analysis revealed that this variant behaves similarly to those linked to the canonical forms of the disease in terms of synaptic function and locomotion, suggesting that *GNAO1*-related disease is a continuous spectrum rather than distinct disorders. We then investigated the impact of a panel of PVs in *ADCY5*, *GNB1*, *PDE10A*, and *GNAL* genes. Notably, our findings indicate that PVs affecting adjacent residues in the G_β subunit of the heterotrimeric Go protein, encoded by *GNB1*, exhibit opposite responses to aldicarb, an acetylcholinesterase inhibitor, suggesting that gain- and loss-of-function variants in this gene cause a different spectrum of MDs. In addition, while hyperactive $G\alpha_{olf}$, a stimulatory G protein encoded by *GNAL*, resulted in aldicarb hypersensitivity and increased locomotion, *GNAL* haploinsufficiency was not pathogenic in worms. Phenotypic analysis of PVs in *ADCY5* (both caffeine-responsive and -unresponsive) is ongoing, as well as the analysis of caffeine response in all mutants. Our findings confirmed the utility of *C. elegans* as a powerful model for infantile neurological disorders, offering insights into disease mechanisms and a rapid platform for therapeutic screening.

Impact of Interleukin-17 on neuronal metabolism and synaptic functioning**NDV 21****Riccardo Grassi** ⁽¹⁾ - Genni Desiato ⁽²⁾ - Chiara Saulle ⁽³⁾ - Edoardo Fraviga ⁽¹⁾ - Davide Pozzi ⁽¹⁾

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Disruptions in the neurodevelopmental trajectory result in various neurological and neuropsychiatric disorders. Although specific genetic background is known to play a key role, exposure to environmental factors is now considered a critical risk factor that may affect the neurodevelopmental trajectory, and maternal inflammation has been causally linked to such disorders in the offspring. The pro-inflammatory cytokine Interleukin-17 (IL17) represents a crucial immune molecule since its transient elevation during pregnancy leads to an altered cortical cytoarchitecture and autistic-like behavior. In this project, we aim to investigate the direct mechanism by which IL17 affects neuronal development. We took advantage of primary cultures of cortical neurons treated with IL17 at early developmental stages to mimic a transient inflammatory response during gestation. We found that cortical neurons can respond to IL17 treatment by activating NFkB- and ERK-dependent signaling pathways. We also found that IL17 treatment increased neuronal metabolism, intracellular calcium and intrinsic excitability during early synaptic development, corresponding to 7 Days in Vitro (DIV). Moreover, the frequency of the miniature excitatory postsynaptic currents (mEPSCs) in cortical neurons was increased at mature stages (14 DIV), whilst no effect was reported on the miniature inhibitory postsynaptic currents (mIPSCs). Further studies will allow us to better elucidate the underlying molecular mechanism and its possible impact in an *in vivo* context.

Mechanisms and functions of RNF12/RLIM in human neurodevelopment and their disruption in intellectual disability

NDV 22

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Mutations in the gene encoding the RNF12/RLIM E3 ubiquitin ligase underlie Tonne-Kalscheuer syndrome (TOKAS), a syndromic form of X-linked intellectual disability characterised by global developmental delay, intellectual disability, abnormal gait, and behavioural issues. RNF12 comprises a nuclear localisation signal, basic region, nuclear export signal, RING domain and a predicted C-terminal PDZ-binding motif. RNF12 localises to the nucleus, where it engages chromatin and ubiquitylates transcription factor substrates such as ZFP42/REX1 to control gene expression. However, the pathogenic mechanisms of RNF12 TOKAS variants, and their impact on transcription during human neurodevelopment, remain incompletely defined. First, we aim to uncover potentially novel pathogenic mechanisms of recently reported RNF12 TOKAS variants. Of those, S622N is located in the predicted RNF12 PDZ-binding motif, whose function is not yet known. Using Biogrid database (<https://thebiogrid.org/>) and literature review, PDZ domain containing proteins LNX2 and PDLIM1 were identified as one of the probable interacting proteins of RNF12, both of which play role in neuronal signalling. *In silico* analysis using AlphaFold revealed that RNF12 S622N affects interaction with PDZ domains of predicted RNF12 interacting proteins LNX2 and PDLIM1. We will now investigate the biological function of the RNF12 PDZ binding motif and its disruption in TOKAS. Second, we will obtain a global picture of RNF12 in transcriptional regulation during human neural differentiation using a human induced pluripotent stem cell (hiPSC) model by performing RNA sequencing on wild-type and *RNF12/RLIM* knockout hiPSCs at different stages of neuronal differentiation. Together, this study will define the RNF12-dependent transcriptional program during human neurodevelopment and elucidate how its dysregulation by TOKAS variants contributes to the pathogenesis of intellectual disability.

NR2F1 Haploinsufficiency Impairs Hippocampal Oligodendrocyte lineage in BBSOAS mouse models

NDV 23

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Bosch-Boonstra-Schaaf Optic Atrophy Syndrome (BBSOAS) is a rare neurodevelopmental disorder caused by pathogenic variants in the *NR2F1* gene, encoding the transcription factor NR2F1. Clinically, BBSOAS manifests with intellectual disability, visual impairment, hypotonia, and white matter abnormalities, suggesting a potential contribution of oligodendroglial dysfunction to disease pathophysiology. However, NR2F1's role in oligodendrocytes remains poorly characterized. NR2F1 is known to orchestrate key aspects of neurodevelopment, including neural progenitor fate specification and cortical patterning, as well as adult neurogenesis. In this study, we investigated the function of NR2F1 in oligodendrocyte lineage cells within the adult dentate gyrus (DG) using *Nr2f1*-heterozygous (*Nr2f1*-Het) and *Nr2f1*-inducible conditional knockout mouse models targeting the *Ascl1*-lineage. By combining multiple immunofluorescence with confocal microscopy, we examined NR2F1 expression in relation to stage-specific oligodendroglial markers. We found that NR2F1 is expressed in Sox10+ oligodendrocyte lineage cells, where it's confined to oligodendrocyte precursor cells (OPCs) and downregulated in postmitotic oligodendrocytes (OLs). Adult *Nr2f1*-Het mice showed a significant reduction in Sox10+ oligodendrocytes, with preliminary data indicating an imbalance between OPCs and OLs, supporting a crucial role for NR2F1 in early oligodendrocyte differentiation. Consistently, preliminary data from conditional *Nr2f1* loss-of-function in *Ascl1*-lineage support its impact on proliferative behaviour and differentiation capacity of *Ascl1*+ OPCs. We are currently investigating new mouse models carrying patient-specific *NR2F1* mutations to further dissect possible defects in oligodendrocyte lineage in relation to BBSOAS. Future studies aim to identify downstream molecular targets of *Nr2f1* in oligodendrocytes and unravel new potential therapeutic targets to mitigate myelin defects in BBSOAS and related disorders.

Investigating the role of genetically regulated molecular traits in the phenotypic heterogeneity of autism spectrum disorder

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Autism spectrum disorder (ASD) shows wide phenotypic variation, with affected individuals ranging from high functioning adults to children with severe disabilities and behavioral symptoms. Previous studies parsed the genetic basis of such heterogeneity by mainly focusing on high-impact de novo variants and polygenic scores (PGSs). To further bridge the gap between genotype and phenotype, and to gain insight into the molecular mechanisms involved, we integrated these approaches with one based on intermediate molecular phenotypes (xWAS analysis), such as gene expression, splicing, and enhancer activity, whose genetically predicted levels can be used to understand the mechanisms of complex traits.

Factor analysis applied to phenotype data retrieved from the SPARK cohort identified six factors (Communication skills, Idiosyncratic speech, Social Interaction, Repetitive, Sensory-motor and Self-injurious behavior). xWAS analysis evaluated the association between factor scores and genetically regulated gene expression, splicing, and enhancer activity.

The analysis identified 32 significant genes and enhancers associated with ASD phenotypes. Several genes located in the 1q21.1 region, including *ANKRD35* and *ITGA10*, were found associated with self-injurious behavior. Other notable findings include *WASHC4*, associated with self-injurious and sensory-motor behaviors, and known to be involved in intellectual disability); while *PNMT* and *ANKS6*, previously implicated, respectively, in ADHD and psychomotor delay, were found associated with sensory-motor behavior in ASD patients.

Intermediate molecular phenotypes are a powerful tool for uncovering the genetic determinants of phenotypic heterogeneity in neuropsychological disorders, potentially leading to the development of personalized therapeutic strategies.

Genetic determinants of Social Cognition in Amyotrophic Lateral Sclerosis: a cross-sectional study

NDV 25

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Background: In recent decades, the genetic basis of Social Cognition (SC) has been increasingly studied (Skuse, 2011; O'Connor, 2008). SC impairments have been reported in several genetic and neurodegenerative conditions, including Frontotemporal Dementia (FTD) (Jiskoot, 2021) and Huntington's Disease (HD) (McGinn Gray, 1997). In ALS, a neurodegenerative disease with known genetic correlates, SC deficits—especially in Facial Emotion Recognition (FER) and Theory of Mind (ToM)—have been observed (Abrahams, 2011). This study aimed to explore SC alterations in genetically determined ALS.

Methods: We included 53 genetically mutated ALS patients and 53 matched non-mutated ALS patients from the Turin ALS Center (2019–2025). All underwent neurological and neuropsychological assessments of language, executive functions, fluency, memory, visuospatial abilities, and SC. SC was evaluated through the Ekman 60 Faces Test, the Reading the Mind in the Eyes Test, and the Story-Based Empathy Task (SET). Mutated status was defined by pathogenic variants in ALS-related genes (C9orf72, SOD1, TARDBP, FUS) or rarer genes (ERBB4, TAF15, TBK1, SQSTM1, SORD, ALS2). Groups were matched by sex, age, education, and cognitive status (Strong criteria, 2017).

Results: Mutated patients performed worse on the SET–Emotion Attribution task ($p = 0.023$), categorical fluency ($p = 0.0006$), executive functions ($p = 0.001$), and memory ($p = 0.008$). Notably, C9orf72 patients showed a specific impairment in recognizing disgust ($p = 0.01$) compared to matched non-mutated patients.

Conclusions: Our findings highlight specific cognitive deficits, including SC impairments, in genetically determined ALS—particularly in affective ToM and emotion recognition. A novel finding is the selective impairment in disgust recognition in C9orf72 patients, previously reported in HD (Brain, 1997), possibly implicating basal ganglia. SC deficits have also been observed in genetic FTD. These results emphasize the importance of SC assessment in ALS, even in non-demented cases, to improve understanding of genetic mechanisms and support diagnosis.

Modelling *Tbc1d24* loss of expression in-vivo to study epileptogenesis and test novel therapeutic strategies

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Numerous mutations in *TBC1D24* have been identified in epilepsy-associated neurodevelopmental disorders. The protein, TBC1D24 harbors two conserved domains, namely the TBC and the TLDC, and regulates neuronal development and membrane trafficking at synapses. The TLDC family of proteins has recently been proposed as regulators of the proton pump v-ATPase. In the brain, v-ATPase acidifies synaptic vesicles and endolysosomal organelles, regulating neurotransmitter quanta formation and neuronal degradative capacity. We have previously shown that TBC1D24 interacts with v-ATPase in the brain and that the loss of *Tbc1d24* altered pH homeostasis and autophagy in *in-vitro* neuronal culture.

To date, the molecular basis of seizure onset in TBC1D24-related pathology is largely unexplored. Here we employed a novel *Tbc1d24* conditional knockout (*Tbc1d24*-cKO) mouse model to study the seizures related to the loss of function of *Tbc1d24* and the pathophysiological mechanisms underlying the alteration of brain homeostasis. Western blot analysis from cerebral cortex extracts confirmed a reduction in TBC1D24 expression at day 36 after the induction with tamoxifen, with accumulation of the autophagy receptor p62. This suggests that TBC1D24 loss is accompanied by autophagic impairment *in-vivo*.

Furthermore, EEG/EMG recordings detected visible absence seizures that are followed by tonic-clonic seizures at day 36 after the tamoxifen treatment.

These findings, in addition to our previous investigations *in-vitro*, provide a robust pre-clinical model to explore the potential therapeutic effects of small compounds that act on intraorganelar pH to restore the pH-homeostasis and the balance of excitation/inhibition.

Piezoelectric Barium Titanate Nanoparticles Combined with Low-Intensity Pulsed Ultrasound Modulate Microglial Activation

NI 10

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Neuroinflammation plays a crucial role in the pathogenesis of various neurological disorders and is primarily mediated by microglia, the principal immune effector in the central nervous system. Innovative, non-invasive strategies to modulate microglial responses are fundamental for developing novel therapeutic approaches. Recently, barium titanate piezoelectric nanoparticles (piezoNPs) have emerged for their potentiality in biomedical applications due to their mechano-electric conversion properties, while low-intensity pulsed ultrasound (LIPUS) has shown promising results in modulate cerebral inflammatory processes. Our study focuses on evaluating the capability of the combined application of piezoNPs and LIPUS to modulate microglia-mediated neuroinflammation. The murine N9 microglial cell line was activated by lipopolysaccharide (LPS) and then treated with either piezoNPs or LIPUS alone (control groups), or a combination of both. Microglial activation was assessed by evaluating morphological changes and the expression of pro-inflammatory markers using western blot analysis and RT-qPCR. Our results showed that the combined treatment significantly reduced the expression of pro-inflammatory markers (e.g., TNF- α) compared to controls. Notably, the combination of piezoNPs and LIPUS was more effective than either treatment alone, suggesting a synergistic interaction likely mediated by the piezoelectric properties of the NPs under ultrasound stimulation. In conclusion, our findings highlight the potential of piezoNPs-mediated ultrasound stimulation as a novel, non-invasive strategy to modulate microglial activation and mitigate neuroinflammation. This approach could pave the way for novel therapeutic interventions in neuroinflammatory and neurodegenerative diseases, warranting further studies to elucidate the underlying molecular mechanisms.

Therapeutic effects of short-chain fatty acids on seizures and comorbidities in a mouse model of acquired epilepsy

NI 11

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Emerging evidence suggests that the gut-brain axis plays a role in seizure generation, although a causal relationship remains unproven. We recently identified gut structural alterations, inflammation, and reduced short-chain fatty acid (SCFA)-producing bacteria during epileptogenesis in rodent models of acquired epilepsy. Since SCFAs have anti-inflammatory and antioxidant properties in gut and brain, we hypothesized that their reduction contributes to epilepsy by exacerbating inflammation and oxidative stress, both implicated in epileptogenesis. We tested whether SCFAs supplementation (40 mM sodium butyrate, 26 mM sodium propionate, 67 mM sodium acetate) reduces seizures, cognitive deficits, and rescue gut alterations in a mouse model of acquired epilepsy. Adult male C57Bl6 mice were intra-amygdala injected with kainate to induce status epilepticus (SE), followed by drug-resistant seizures, neuropathology, cognitive deficits and gut dysbiosis/dysfunction. Five hours post-SE mice were administered SCFAs or vehicle (133 mM NaCl) in drinking water for 10 weeks. Sham mice were similarly treated. SE mice were EEG monitored (24/7) from SE onset for 10 weeks to quantify seizure, then mice were tested for cognitive deficits in the Barnes Maze, and subsequently sacrificed. SCFA-treated mice showed reduced seizure progression and clustering (≥ 3 seizures/24 h), improved memory retention and goal-directed search strategy during the probe trial, compared to vehicle-treated mice ($p < 0.05$). These improvements were associated with restored levels of specific SCFA-related metabolite in blood and preserved gut structure. Our findings show that SCFAs reduce epilepsy severity and cognitive impairment, suggesting they may be considered as adjunctive treatment for epilepsy management.

Role of astrocytes in the initiation and progression of epilepsy: the focus on TRPV4 channel

NI 12

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Epilepsy affects ~1% of the population worldwide. Despite the availability of >20 antiseizure drugs that target neurons, 30% of patients experience drug-resistant seizures, highlighting the need for new therapies. Research on new drugs has focused mainly on neurons; however, astrocytes are essential for brain homeostasis and may play a key role in epileptogenesis. In particular, the Ca²⁺-permeable Transient Receptor Potential Vanilloid 4 (TRPV4) ion channel is enriched in reactive astrocytes and has been linked to epileptogenesis.

We studied the dynamics of TRPV4 expression in reactive astrocytes during epileptogenesis, and its association with astrocytic dysfunction markers to shed light on its involvement in epileptogenesis. We used an adult mouse model of acquired epilepsy induced by intra-amygdala kainate to induce status epilepticus (SE) and subsequent spontaneous seizures. Sham mice were saline-injected.

The brain was processed for immunohistochemistry to quantify TRPV4 expression, together with IL-1 β (inflammatory marker) and the glutamate transporter GLT-1 (homeostatic cell marker), in GFAP-positive astrocytes in the epileptogenic cortico-hippocampal-thalamic (CHT) circuit. Sholl analysis assessed astrocyte morphological changes. TRPV4 staining was increased in hippocampal astrocytes 24-48h post-SE, reaching a plateau at 96h (before seizures develop) and persisting in chronic epilepsy (2 months), with a progressive spreading to extrahippocampal regions during disease development. IL-1 β was increased whereas GLT-1 was reduced in astrocytes 1 week post-SE in the CHT circuit, coinciding with epilepsy onset. Co-localization analysis showed that GLT-1 was reduced in TRPV4-positive astrocytes in epileptic vs sham mice.

Data show that TRPV4 is induced in reactive/dysfunctional astrocytes during epilepsy development. This provides the rationale for targeting TRPV4 to assess whether the dysfunctional astrocyte phenotype is corrected, and the impact on epileptogenesis.

Olive Oil Polyphenols as Modulators of Fibrinogen-Driven Neuroinflammation: Implications for Neurodegenerative Disease Prevention

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Oleuropein aglycone (OleA) and its main metabolite hydroxytyrosol (HT) are polyphenols found in extra virgin olive oil (EVOO) and olives, main food of the Mediterranean diet, known for their antioxidant and anti-inflammatory properties, these compounds show significant potential in counteracting pro-inflammatory signals in the brain, such as those triggered by fibrinogen, a blood-clotting protein that can activate microglia, the brain's resident immune cells. This activation leads to the production of inflammatory cytokines, contributing to neuroinflammatory conditions.

This study explored the molecular mechanisms by which EVOO polyphenols mitigate fibrinogen-induced damage. Using the C13NJ human microglia cell model, we assessed changes in neuroinflammatory markers using confocal microscopy following pre-treatment with these polyphenols. We also analysed the effects of the C13NJ conditioned medium on neuronal health in the SH-SY5Y cell model.

Our findings reveal that both polyphenols significantly inhibit the activation of TLR4, p-NF-κB and the release of pro-inflammatory factors, as assessed through conditioned medium treatments on the SH-SY5Y neuronal cell model. Furthermore, OleA and HT exert their effects by increasing TREM2 levels and acting as epigenetic modulators in terms of histone 4 lactylation, while also increasing the mitochondrial function.

The ability of OleA and HT to promote an anti-inflammatory microglial phenotype positions them as promising molecules for reducing neuroinflammation, protecting neuronal integrity, and supporting brain health. Given the chronic inflammation observed in neurodegenerative diseases, OleA and HT offer valuable potential for research and possible therapeutic interventions. Dietary or supplemental strategies incorporating these polyphenols could provide preventive approaches, as well as strategies aimed at mitigating inflammation before the onset of neurodegenerative diseases.

Study of Peripheral Nervous System involvement in early ALS using SOD1G93A mouse model

NI 14

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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by muscle weakness and atrophy, resulting from the degeneration of both upper and lower motor neurons. ALS is a terminal condition that leads to paralysis and ultimately death due to respiratory failure. In this study, we investigated the involvement of the peripheral nervous system during the early stages of the disease. We analyzed SOD1G93A mice at three time points representing different stages of disease progression: 60 days of age (pre-symptomatic stage), 90 days of age (early symptomatic stage), and 120 days of age (end-stage). We applied [18 F]-DPA-714-micro-PET, which binds to TSPO, to detect metabolic differences between SOD1G93A mice and wild-type littermates. Following imaging analysis, animals were euthanized, and tissues were collected for neuropathological examination. We focused on evaluating morphological alterations in peripheral nerves and quantifying macrophage density in both muscle and nerve using CD68 immunostaining. Our findings revealed a statistically significant increase in TSPO uptake in hindlimb muscles of SOD1G93A mice compared to wild-type controls at all examined time points. We also observed significant macrophage infiltration in the sciatic nerves of 90- and 120-day-old SOD1G93A mice compared to wild-type littermates. These results suggest an involvement of the peripheral nervous system in the early stages of ALS, including structural degeneration and nerve inflammation, which may contribute to disease progression. We are confident that this study will provide interesting insights into the mechanisms active during the early stages of the disease, focusing on the peripheric compartment.

TREM2 in Microglia: A Central Player in the Hypothalamic Control of Metabolic Stress?

NI 15

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Background: Adipose metabolic homeostasis relies on Triggering receptor expressed on myeloid cells 2 (Trem2) receptor-dependent mechanisms in lipid-associated macrophages (LAMs). Trem2-expressing LAMs protect against adipocyte hypertrophy and systemic metabolic dysfunction during obesity (Jaitin et al., 2019). However, whether Trem2-expressing microglia cells play a complementary role in the central nervous system, particularly within the hypothalamic region, remains unclear. Given the hypothalamus's central role in sensing and responding to systemic metabolic changes via its proximity to permeable interfaces (i.e., the blood-brain and cerebrospinal fluid-brain barriers), we hypothesized that microglial Trem2 regulates neurovascular interactions and barrier integrity in response to metabolic challenges.

Methods: Taking advantage of Trem2 knockout (Trem2-KO) mice, we are combining AgRP ELISA, C-Fos IF, Trem2 IHC, scRNA-sequencing, and spatial transcriptomics to profile changes across hypothalamic nuclei in a sex-specific manner. We aim to assess metabolic stress responses under fasting conditions, with a focus on hypothalamic barriers components (i.e., endothelial cells, tanycytes). Previous work from our lab demonstrated that Trem2 deficiency in microglia impairs neuronal bioenergetics during development (Tagliatti et al., 2024) with variants that are associated with neurodegenerative diseases, including Alzheimer's disease. Trem2 is essential for microglia-mediated synaptic refinement, but whether Trem2 contributes to shaping neuronal development remains unclear. Here, we demonstrate that Trem2 plays a key role in controlling the bioenergetic profile of pyramidal neurons during development. In the absence of Trem2, developing neurons in the hippocampal cornu ammonis (CA. Here, we extend these findings to hypothalamic circuits involved in endocrine and metabolic regulation.

Results: Both scRNA-seq data (CellChat analyses on DEG) and imaging cell-cell contact analyses revealed altered molecular crosstalk between microglia and other components of the neurovascular unit, including endothelial cells, in the absence of Trem2. Ongoing analyses are expected to refine our understanding of sex- and region-specific contributions of microglial Trem2 in CNS-metabolic axis regulation, potentially unveiling new therapeutic opportunities for metabolic and neurodegenerative diseases.

Conclusions: The absence of Trem2 could lead to disrupted neurovascular communication, compromising the hypothalamic capacity to sense and integrate systemic metabolic cues. These findings will provide mechanistic insights linking microglial function, barrier dynamics, and systemic energy homeostasis.

Enhancing anandamide signaling via FAAH inhibition resolves aberrant neuroinflammation in a presymptomatic Alzheimer's disease mouse Model

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Alzheimer's disease (AD) is marked by unresolved neuroinflammation driven by resident and infiltrated immune cells that undergo aberrant activation. Thus, enhancing endogenous resolution mechanisms represents a promising therapeutic approach in AD. Endocannabinoids (eCBs) can act as immunomodulatory agents, both by targeting immune cells and by engaging pro-resolving pathways, suggesting their therapeutic potential in AD.

We investigated whether acute inflammation elicits distinct neuroimmune responses in pre-symptomatic Tg2576 mice (4 months old), a transgenic AD model, compared to wild-type (WT) mice, and evaluated the ability of URB597, a selective inhibitor of fatty acid amide hydrolase (FAAH), the main enzyme degrading anandamide (AEA), to promote spontaneous resolution by enhancing endogenous AEA tone. Male Tg2576 and WT mice received intracerebroventricular injections of lipopolysaccharide (LPS, 10 µg/1 µL saline) or vehicle. After three days, brains were dissociated and analyzed by flow cytometry.

LPS induced infiltration of neutrophils, monocytes/macrophages, and microglia in both genotypes, with Tg2576 mice displaying a more pronounced immune cell influx. URB597 pretreatment reduced infiltration of neutrophils and monocytes/macrophages in both groups—more markedly in Tg2576—and lowered pro-inflammatory markers (CD40, CD86, TREM2) in microglia and macrophages, suggesting a shift toward immunomodulatory and resolution-associated phenotypes.

As AEA also induces resolution via pro-resolving lipid receptors, we tested URB597 in monocyte-derived macrophages from patients with mild cognitive impairment (MCI). These cells showed reduced expression of pro-resolving receptors and enzymes (GPR18, 5-lipoxygenase) and impaired efferocytosis, suggesting that URB597 may promote immune modulation via multiple pathways.

These findings support a role for AEA signaling in modulating the neuroinflammatory surge, and its resolution, in AD pathology.

Phenotypical analysis of innate immune system in neuropathy by antibodies anti-myelin associated glycoprotein

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Background: Myelin Associated Glycoprotein (MAG) is important in axonal maintenance. The complex carbohydrate on MAG includes the HNK-1 epitope, which defines an antigen shared between the immune and the nervous system expressed on Natural Killer cells. Disruption in the expression of inhibitory NK cell receptors has been described in Rituximab-treated patients with anti-MAG antibodies but few data are available on NK cell phenotype and function in these patients before starting and correlated with response to therapy

Methods: 10 MAG patients and 10 Healthy Donors were enrolled at the IRCCS Ospedale Policlinico San Martino (Genoa, Italy). Samples were longitudinally collected before treatment (T0). Peripheral blood mononuclear cells (PBMCs) isolated by Ficoll–Paque were analysed by multi-colour flow cytometry. Statistical analysis was performed using the Mann-Whitney U tests for unpaired comparison datasets. Tests were two-sided.

Results: Flow cytometric analysis of NK cells from patients enrolled at T0 showed an impaired expression of NKG2D, NKG2A and CD85j (**p<0.01). Circulating inflammatory CD34+CXCR4+D-NAM-1bright precursors showed a trend to increase compared to healthy donors.

Conclusion: Impaired expression of activating and inhibitory receptors reinforces and integrates previous data, and circulating inflammatory precursors represent recent BM migrants, possibly derived from endosteal niches following chronic inflammation with bone remodelling. Future perspectives will consider analysing the NK cell phenotype in MAG patients six months after the start of therapy and at the end of the treatment; the goal is to identify one or more biological markers correlated with the response to therapy or disease prognosis.

Sex-specific responses to inflammation in the dorsal and ventral hippocampus

NI 18

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The hippocampus is a brain region characterized by high levels of plasticity, due to its central role in learning, memory, and emotions. Moreover, it exhibits functional segregation and different levels of plasticity along its longitudinal axis. It was previously found that Long-Term Potentiation (LTP) differs between the Dorsal and Ventral Hippocampus (DH and VH) of male mice, while it is similar between these two poles in female mice. Microglial cells are known to play a key role in the modulation of neuronal transmission and plasticity and, in physiological conditions, they exhibit different features at the hippocampal poles depending on sex. Among others, microglial CX3CR1 receptor signalling appears to be particularly important in this context. Beyond the physiological state, microglia are also known to modulate neuronal plasticity in neuroinflammation-associated diseases. We investigated whether, under inflammatory conditions, microglia modulate neuronal plasticity at the two poles in a sex-specific manner. We used a mouse model of acute neuroinflammation based on lipopolysaccharide (LPS) injection in both wild-type (C57BL/6J) and Cx3cr1^{-/-} (KO) mice, the latter lacking the microglial CX3CR1 receptor. At 24 hours post-injection, we evaluated Long-Term Potentiation (LTP) by extracellular field recordings and the kynurenine pathway (KP), known to be involved in neuroinflammation, by metabolomics experiments. We found that LPS-induced neuroinflammation differentially modulates LTP and the levels of KP metabolites at the two poles in a sex-specific and strain-specific manner, demonstrating that male and female mice differentially respond to inflammatory challenge at hippocampal poles, and that microglia play an important role in shaping these differences through the CX3CR1 receptor.

Hydroxytyrosol of olive oil prevents oxysterol-induced neuroinflammation in Alzheimer's disease through sirtuin-1 induction.

NI 19

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Cholesterol oxidation products, known as oxysterols, are one of the main triggers of Alzheimer's disease (AD) contributing to neuroinflammation.

AD lacks current effective treatments, prompting exploration of promising preventative approaches. Growing experimental evidence suggests that the Mediterranean diet reduces risk of dementia. Of note, olive oil contains the potent polyphenol hydroxytyrosol (HXT) currently considered a bioactive compound able to improve cognitive function. However, to date little data are available regarding HXT anti-inflammatory effects in AD.

The aim of our study focuses on the ability of HXT in preventing neuroinflammation induced by an oxysterol mixture, whose composition represent oxysterol amounts previously quantified in severe AD brain samples, using human neuroblastoma SK-N-BE cells. We have also analyzed the sirtuin 1 (SIRT1)-dependent anti-inflammatory pathways through which HXT acts.

Our data show an increase of expression and synthesis of IL-1 β , IL-6, IL-8, TNF α , IFN γ , and MCP-1 inflammatory mediators induced by oxysterols; this increase was markedly prevented by HXT cell pre-treatment. It was also demonstrated that oxysterols trigger neuroinflammation via TLR4-mediated NF κ B activation. Moreover, mRNA overexpression and protein up-regulation of SIRT1 induced by HXT was observed. Through SIRT1 activation, HXT was able to inhibit the oxysterol-induced TLR4 up-regulation and the consequent NF κ B p65 translocation; using sirtinol, a specific SIRT1 inhibitor, the anti-inflammatory effects of HXT were not observed.

The obtained results show HXT's ability to mitigate oxysterol-induced neuroinflammation via SIRT1/TLR4/NF κ B pathway. These data suggest HXT as a potential nutraceutical, offering promise for preventing neuroinflammation and, consequently, neurodegeneration in AD.

Morphological Transformation and Metabolic Shift in Friedreich's Ataxia Cerebellar Microglia

NI 20

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Friedreich's ataxia (FRDA) is a neurodegenerative condition characterized by impaired coordination and muscle control. Mitochondrial frataxin (FXN) protein depletion is the primary cause of FRDA. This degenerative disease damages cerebellum, spinal cord and peripheral nerves. Here, we employed a multi-layered approach to study the metabolic and phenotypic changes in cerebellar immune cells, mostly represented by microglia. In our study, we took advantage by the use of two different FRDA FXN-lacking mouse models (KIKO and PVB). First of all, we performed a high-resolution microscopy assay to explore the distribution and morphology of microglia in several scans of cerebellar convolutions of KIKO mice. In all scans, we noticed significant changes in number and shape of microglial cells if compared to healthy mice. In more detail, we spotted an evident difference in microglia branches number, these branches are characterized by fragmented ramifications and an overall increased length. The mean cell occupancy is increased as well. Such shape is typical of microglial in an activation and reactive state. In order to confirm and deeply explore the phenotype of cerebellar microglia, we studied the transcriptomic profile of both FRDA models by the use of single-cell RNA sequencing analysis. We found an increase of the microglia cells number in KIKO and PVB mice either. Such cells are characterized by an inflammatory polarization (M1-like) and a defective metabolism both in the cellular and mitochondrial components. This metabolic failure leads to speculate a disrupted homeostatic function in cerebellar microglia, characterized by a shift towards glycolysis and itaconate production. Our findings highlighted a crucial role of microglia in FRDA pathogenesis, suggesting a metabolic mechanism that could reduce inflammation and mitigate its effect on the neural counterpart.

Generation and Characterization of a Knock-in Mouse Model for FLVCR1-Related Sensory Neuropathy

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FLVCR1 (feline leukemia virus subgroup C cellular receptor 1) encodes a plasma membrane choline/ethanolamine importer involved in intracellular heme homeostasis and mitochondrial calcium handling. Pathogenic mutations in *FLVCR1* cause a progressive sensory and autonomic neuropathy, with distal loss of pain, thermal and tactile perception, gait imbalance, retinitis pigmentosa, and proprioceptive ataxia due to posterior column involvement. Despite the defined clinical phenotype, the underlying mechanisms remain poorly understood. To address this issue, we generated a CRISPR-Cas9 knock-in mouse model carrying the patient-specific *FLVCR1* P221S mutation in heterozygosity (*FLVCR1*^{P221S/+}) and established a homozygous line (*FLVCR1*^{P221S/P221S}). Primary DRG (dorsal root ganglia) neurons from homozygous mice showed marked morphological defects, including reduced axonal length and smaller soma size, suggesting impaired neuronal growth or maintenance. To assess functional impact, behavioral testing revealed increased latency to a 50 °C thermal stimulus at both 7 and 10 months, consistent with reduced thermal sensitivity. A trend toward higher tactile thresholds in the von Frey test at the same time points indicated mild mechanical hyposensitivity. No motor deficits were observed in rotarod or grip strength tests. Given *FLVCR1*'s role in mitochondrial and heme metabolism, ongoing work includes metabolomic profiling of neuronal (DRG, sciatic nerve, cerebellum) and metabolic (liver, muscle) tissues. Additional assays underway include balance beam and cold plate testing (4 °C), alongside sciatic nerve osmium staining, skin innervation analysis, and DRG neuronal subtype mapping. In conclusion, our knock-in mouse model of *FLVCR1*-linked sensory neuropathy provides new insights into the morphological and functional consequences of the P221S mutation and provides a starting point for future studies aimed at clarifying disease mechanisms and identifying potential therapeutic targets.

CXCR4: an early injury response marker at the NMJ and a target for functional recovery in ALS

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Peripheral nerve regeneration relies on the activation of intrinsic growth programs and a permissive environment. In humans, recovery from nerve injury is poor, highlighting the need for strategies to improve outcomes after peripheral nerve injuries and in neurodegenerative diseases. The neuromuscular junction (NMJ), a key chemical synapse essential for motor control, is especially vulnerable to damage as it lacks a protective barrier. A key signalling axis between the chemokine CXCL12 α (from terminal Schwann cells) and its receptor CXCR4 (expressed by injured motor axons) supports recovery after a peripheral nerve damage. Inhibiting this axis delays repair, showing CXCR4 as a stress marker and a driver of regeneration. In Amyotrophic Lateral Sclerosis (ALS), early NMJ alterations occur before motor neuron (MN) death and symptoms onset. In SOD1G93A mice, we have identified an early window where the NMJ re-expresses CXCR4, confirming this synapse as an early site of disease. Chronic administration of the CXCR4 agonist NUCC-390 in SOD1G93A mice from early symptomatic stage slows motor functional decay and respiratory decline and reduces denervation. Additionally, this treatment shows neuroprotective effects promoting spinal MN survival and reducing plasma neurofilament elevation. Importantly, CXCR4 is commonly expressed in several ALS models, including hFUS+/+ and TD-P43Q331K mice, and SOD1G93A swine, and in human biopsies, highlighting this receptor as a common therapeutic target across different ALS forms. These findings suggest that: i) supporting peripheral nerve regeneration is a promising strategy for improving ALS therapeutic protocols; ii) targeting the periphery to stabilize the NMJ and promote regeneration helps counteract MN death; iii) NUCC-390 is a pro-regenerative factor with potential applications in other neurodegenerative conditions where NMJ dysfunction is an early pathological feature.

Generation of isogenic lines using CRISPR/Cas9 technology from TBCE mutated iPSCs

NDG 25

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Early progressive encephalopathy with distal spinal muscular atrophy and optic atrophy (PEA-MO) is a rare, severe autosomal recessive neurodegenerative disease caused by homozygous missense mutations in the *TBCE* gene (1q42.3). Clinical features include developmental delay, infantile hypotonia, motor regression, distal amyotrophy, and optic atrophy. The correct development of the central nervous system relies on the proper organisation and function of microtubules, essential to obtain a functionally integrated neuronal network. To achieve this, newly synthesised tubulin polypeptides require the assistance of five specific chaperones Tubulin Folding Cofactors (TBCA, TBCB, TCCC, TBCD, TBCE) to mature into functional α/β -tubulin dimers. Pathogenic mutations in *TBCE* impair microtubule organization, in particular reducing microtubule stability and leading to neurodegeneration. In this study, we reprogrammed and characterised patient-derived skin cells carrying the homozygous c.463T>A (p.Ile155Asn) mutation in *TBCE*, using a non-integrating episomal method. These iPSCs were differentiated into motor neurons and molecular and morphological analyses revealed altered neuronal differentiation compared to controls. To confirm the causative pathogenic role of the *TBCE* variant, we used CRISPR/Cas9 and *piggyBac* technologies to generate isogenic corrected lines. This combined approach allowed us to validate the mutation-specific effects and establish a valuable platform for investigating the molecular mechanisms of *TBCE*-related neurodegeneration.

Altered hematopoiesis in the skull bone marrow in a mouse model of Parkinson's disease.

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The skull bone marrow (BM) serves as a primary source for generating brain-infiltrating immune cells in experimental models of multiple sclerosis, ischemic stroke, and traumatic brain injury. The cerebrospinal fluid (CSF) accesses the skull BM allowing direct exposure to brain-derived factors, including neuropeptides. While neuroinflammation is recognized to contribute to neuronal damage in Parkinson's disease (PD), coinciding with dopamine signaling loss, the role of the skull BM in the generation of brain-infiltrating immune cells remains unclear. The goal of this study was to assess whether hematopoiesis in the skull bone marrow is altered in a mouse model of Parkinson's disease. We modelled Parkinson's disease in mice through the monolateral injection of 6-hydroxydopamine in the substantia nigra. BM cells were analyzed by multiparametric flow cytometry. Brain were processed for immunofluorescence or gene expression analysis. Quantification of hypothalamic AgRP peptide in blood samples of people with PD was quantified by ELISA. Our data indicate altered frequency of hematopoietic stem cells and mature immune cells in the skull BM but not in femoral BM and blood. Particularly, in the skull BM we observed an increased frequency of both undifferentiated, myeloid and lymphoid hematopoietic precursors, occurring 3 weeks after disease induction. These changes occurred in parallel with a significant reduction of neutrophils in the BM. A gene expression analysis of the hypothalamus indicated an altered expression of neuropeptides that are involved in the control of hematopoiesis. Interestingly, one of these neuropeptides, AgRP, was also increased in the serum of people with PD. These observation suggested that brain-derived factors, possibly including neuropeptides involved in the control of hematopoiesis, target the skull BM and alter hematopoiesis in PD.

Ultrastructural and Mitochondrial Alterations in a Mouse Model of Lafora Disease

NDG 27

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Lafora Disease (LD) is a rare neurodegenerative disorder characterized by the pathological accumulation of glycogen aggregates, known as Lafora bodies (LBs). It is caused by mutations in the EPM2A and EPM2B genes, encoding laforin and malin, respectively. The loss of functional laforin-malin complex disrupts glycogen metabolism, leading to the accumulation of LBs and contributing to the progression of neurodegeneration in LD. LBs are present in both neurons and astrocytes, with distinct morphological and spatial characteristics. While neuronal LBs are typically large and localized in somatic regions, astrocytic LBs are smaller, clustered, and similar to corpora amylacea observed in aging and other neurodegenerative conditions. However, the role of LBs in LD-associated neurodegeneration remains unclear, as does their impact on mitochondria, whose dysfunction is a hallmark of the disease. This study examined hippocampal samples from Lafora-knockin mice at three different disease stages using Serial Block-Face Scanning Electron Microscopy (Volutome, ZEISS). Volumes of $70 \times 70 \times 35 \mu\text{m}^3$ per sample were imaged at a voxel resolution of $5 \times 5 \times 50 \text{ nm}^3$ (x, y, z). Leveraging state-of-the-art deep learning algorithms, we analyzed astrocytic LBs and mitochondrial networks quantitatively. Preliminary findings at 5 months revealed that LBs appear to accumulate to a greater extent near blood vessels (61 % within $6 \mu\text{m}$, 67 out of 110 LBs). Moreover, LBs are associated with structurally compromised mitochondria exhibiting internal vesicle-like features. These insights provide a foundation for understanding how LBs contribute to mitochondrial dysfunction and the broader neurodegenerative processes in LD, advancing our knowledge of disease pathogenesis.

Transglutaminase 2 as a novel therapeutic target to study ER-mitochondria contact sites in Alzheimer disease using iPSC model

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by progressive cognitive decline, memory impairment, and behavioral changes. It is primarily associated with the accumulation of amyloid-beta (A β) plaques and tau tangles in the brain, which lead to synaptic dysfunction and neuronal loss especially in memory-related areas. The "amyloid cascade" is the key event in AD pathogenesis, and recent evidence showed that this could be related to alterations in the mitochondria-associated ER membranes (MAMs) compartment, that acts as a signaling hub, playing a crucial role in mitochondrial function. Recently, my group demonstrated that Transglutaminase 2 (TG2), a multifunctional enzyme that plays a key role in mitochondria homeostasis, regulates MAMs. Its presence in the ER-mitochondria contact sites is essential to maintain the homeostasis of this cellular regulatory site. Since TG2 dysfunction is closely associated with many neurodegenerative diseases, we are exploring the effects of TG2 on MAMs functionality, correlated to the analysis of AD phenotype. Since *in vitro* models properly recapitulating AD are lacking, we will use induced pluripotent stem cells (iPSC), which have become increasingly important in AD research as they allow us to study neurons, the cell type mostly affected by the pathology. We collected data showing A β peptide treatment led to increased level of superoxide anion associated with increased ER-mitochondria interactions, increased neuronal death and shorter neurite lengths. Interestingly, treatments with TG2 inhibitors ZDON and cystamine restored the number of ER-mitochondria contact sites. This was correlated to a partial improvement in cell viability, neuronal morphology and oxidative stress suggesting that inhibition of TG2 could improve AD pathogenesis through modulation of MAMs homeostasis. This study could provide the basis for a therapeutic strategy focused on targeting TG2 for the prevention or treatment of AD pathogenesis.

LRRK2 G2019S impairs GABAergic signaling and perturbs striatal E/I balance in Parkinson's Disease.

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Disruption of the excitatory/inhibitory (E/I) balance is a hallmark of several neurodegenerative disorders, including Parkinson's disease (PD). In Parkinson's disease (PD), alterations in striatal circuitry can lead to excessive excitatory input, ultimately resulting in excitotoxic neuronal damage. Among the genetic contributors to PD, the Gly2019Ser (G2019S) substitution in *Leucine-rich repeat kinase 2* (LRRK2) represents the most common mutation linked to familial and sporadic forms of the disease. While LRRK2 G2019S is known to affect glutamatergic transmission by regulating the EAAT2 glutamate transporter, its role in inhibitory signaling remains largely unexplored.

To investigate the possible involvement of LRRK2 on GABAergic neurotransmission, we used two-electrode voltage-clamp recordings in *Xenopus laevis* oocytes injected with striatal membranes from G2019S LRRK2 transgenic mice.

Electrophysiological analysis revealed a significant reduction in GABA-evoked currents amplitude in striatal membranes from G2019S LRRK2 mice, indicating impaired inhibitory transmission. This deficit is not associated to intrinsic dysfunction of GABA_A receptor, but rather to changes in receptor subunit composition and distribution, affecting both tonic and phasic GABA currents. Notably, GABA_A receptors containing the $\gamma 2$ subunit are functionally modulated by the G2019S mutation. In parallel, reduced levels of the synaptic scaffold protein gephyrin suggest that LRRK2 G2019S destabilizes the gephyrin-GABA_A receptor complex, leading to diminished surface expression of synaptic receptors and contributing to an altered glutamate/GABA ratio. The contribution of LRRK2 G2019S to E/I imbalance is further corroborated, as evidenced by increased activity of both AMPA and NMDA receptors observed under pathological conditions. In conclusion, our results uncover a novel role for LRRK2 in the regulation of GABAergic signaling, opening new perspectives for targeted therapeutic strategies.

Exploring the effect of microgravity on beta-amyloid aggregates in transgenic *C. elegans* lines: insights into gene-environment interactions in neurodegeneration

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With the increasing challenges posed by pollution, overpopulation, and climate change, long-term space travel may soon become a necessity. In this scenario, environmental stressors such as microgravity (μG) and cosmic radiation are expected to exert profound effects on human physiology. While space biomedicine has advanced, the molecular and genetic bases of disease processes, particularly neurodegenerative disorders, remain poorly understood under these conditions.

To investigate how space-like conditions influence the aggregation of beta-amyloid ($\text{A}\beta$), a hallmark of Alzheimer's disease, we employed *C. elegans*, a genetically tractable model organism. We used transgenic lines constitutively expressing human $\text{A}\beta_{1-42}$ in neuronal or muscle tissues. This allowed us to examine tissue-specific genetic responses and phenotypic consequences of $\text{A}\beta$ expression under both normal and simulated μG conditions.

Our data show that both neuronal and muscular expression of $\text{A}\beta$ lead to locomotion impairments compared to isogenic controls. However, only the muscle-expressing line exhibits reduced pharyngeal pumping associated with an unpredicted extension of lifespan, possibly due to caloric restriction. Conversely, neuronal $\text{A}\beta$ expression shortens lifespan. Immunofluorescence analysis confirmed β -amyloid deposition in target tissues, validating the model at the histological level. Notably, we implemented the NASA-developed Rotary Cell Culture System (RCCS) to expose animals to simulated μG from early adulthood to mid-life. This setup offers a unique opportunity to explore how altered gravity modulates gene expression, protein aggregation, and neurodegenerative phenotypes *in vivo*.

Our findings contribute to the understanding of gene-environment interactions relevant to neurodegeneration. By integrating a genetically defined model with a controlled space analog system, this study lays the groundwork for screening antibody-based therapies targeting protein aggregation, both in space and on Earth.

GBA2 as a potential therapeutic target in GBA1-PD**NDG 31****Zuzanna Kula** ⁽¹⁾ - Dan Sillence ⁽²⁾ - Frances Platt ⁽³⁾ - Danielle Te Vrugte ⁽³⁾ - Carlo Breda ⁽²⁾

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Parkinson's disease (PD) is a progressive neurodegenerative disorder for which aetiology is unclear. Amongst the genetic causes, the N370S and L444P mutations in GBA1 gene are the most common genetic risk factors for PD. GBA1 encodes glucocerebrosidase, a lysosomal enzyme that hydrolyses glucosylceramide (GlcCer) into ceramide and glucose at ~5 pH. GBA2 encodes a non-lysosomal enzyme located on the cytosolic side of the ER, which also hydrolyses GlcCer at a higher pH (~6). While the GlcCer's role remains unclear, it modulates v-ATPase function which is essential for lysosomal acidification. The traditional model suggests that misfolded GBA1 fails to reach the lysosome, causing GlcCer accumulation. However, clinical trials using GlcCer synthesis inhibitors worsened PD symptoms, suggesting GlcCer is crucial for cell function. We propose a new hypothesis in which misfolded mutated GBA1 remains in the cytoplasm/ER, where it hydrolyses GlcCer at neutral pH, duplicating GBA2's function and depleting cytosolic GlcCer pools. This may impair v-ATPase activity, raising lysosomal pH and disrupting dopamine packaging in synaptic vesicles, ultimately contributing to neuronal death. Using N370S and L444P Gaucher fibroblasts, we found that treatment with adamantyl GlcCer, a soluble GlcCer analogue, restored lysosomal pH and size. Furthermore, GBA2 inhibition with miglustat has shown similar rescue effects in various lysosomal storage disorders. Currently, we testing this hypothesis in *Drosophila melanogaster* overexpressing the human mutant GBA1 variants. These flies exhibit reduced climbing ability and shorter lifespan, especially when the N370S mutation is expressed in the central nervous system. Those flies are currently being treated with with miglustat and we aim to present its effects on locomotor activity, survival, and GlcCer levels. Our findings may highlight GBA2 inhibition as a promising therapy for GBA1-linked PD.

Exploring miRNA-mediated regulation of oligodendrocyte progenitor differentiation during brain aging

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Aging is associated with progressive morphological and functional brain alterations that contribute to cognitive, motor, and sensory decline. One of the earliest and most significant changes is the reduction of white matter volume, with detectable abnormalities emerging as early as midlife. In the central nervous system, white matter is primarily composed of myelin, a lipid structure crucial for efficient axonal conduction and neural communication. Mounting evidence indicates that early myelin loss and impaired oligodendrocyte (OL) function precede the appearance of neurological symptoms, suggesting that these events are central to the onset of age-related neurodegeneration.

In this study, we focused on oligodendrocyte progenitor cells (OPCs), a population that progressively loses its differentiation capacity with age, and we explored the role of microRNAs (miRNAs), small non-coding RNAs that regulate gene expression at a post-transcriptional level.

In particular, we isolated PDGFR α -positive OPCs from the brains of young (2-month-old), middle-aged (6-month-old), and aged (12-month-old) mice, followed by RNA extraction and comprehensive transcriptome and miRNome profiling. Differential expression analysis and miRNA-target enrichment revealed a specific subset of miRNAs altered during aging in OPCs, that potentially modulate genes such as Klotho, IGF-2, Claudin 1 and 2, and EPCAM, associated to aging processes, and OPC differentiation.

Functional experiments are underway to assess whether overexpression of these miRNAs in young OPCs recapitulates the age-related differentiation block.

In parallel, we are investigating whether inhibition of these age-associated miRNAs in vivo via targeted delivery of a viral vector expressing multiple miRNA inhibitors can restore differentiation ability and promote a rejuvenated phenotype in aged OPCs.

Funded by European Union - Next Generation EU, Missione 4 Componente 1.1 CUP G53D23004440006.

Tau protein regulates age-related cognitive decline and structural white matter abnormalities

NDG 33



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The global population aged 60 years or over grew from 382 million in 1980 to 962 million in 2017 and is estimated to reach 2 billion by 2050. During aging, there is a gradual accumulation of pathologies associated cognitive impairment and brain volume loss. With healthy aging and neurodegenerative processes, there is an accumulation of pathologic microtubule stabilizing protein tau which induces cognitive decline. Disentangling the role of tau in age-related cognitive decline could open new avenues for therapeutic intervention. Three age cohorts of male TauKO and WT mice were used: 3 months (m), 18m and 23m. Each group underwent *in vivo* cognitive and MRI analysis. *Ex vivo* they underwent either histopathological or biochemical analyses (IHC). IHC was performed using luxol fast blue to evaluate myelin staining intensity, a measure of the overall myelin content, and NeuN. Western blotting was performed against Tau5, MAP6, MAP7, acetylated and tyrosinated tubulin (aceTub, tyrTub). WT mice show a clear cognitive decline in Ymaze testing starting at 18m which continued to 23m. TauKO mice did not exhibit this decline across age. The volume of the corpus callosum (CC) was lower in 23m TauKO mice in MRI analysis. By 23m of age, WT mice have significantly more myelin than TauKO. TauKO mice demonstrate a thicker dentate gyrus at 18m compared to WT which indicates a greater quantity of projections to the CC. TauKO express higher levels of MAP6, aceTub and tyrTub than WT mice at 23m of age. Aged TauKO mice retain cognitive function unlike WT mice, which show age-related decline accompanied by increased white matter volume and myelin intensity at 23m. TauKO mice exhibit greater levels of aceTub and tyrTub in old age. As acetylation stabilizes microtubules and tyrosination reflects dynamic turnover, these results suggest that tau deletion promotes an adaptable microtubule network, supporting resilience to cognitive aging.

A human oligodendrocyte-based platform for dissecting myelin dysfunction and uncovering therapeutic targets in Niemann-Pick type C disease.

NDG 34



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Niemann–Pick type C (NPC) disease is a fatal lysosomal storage disorder caused by mutations in NPC1 (~95%) or NPC2 (~5%), which disrupt intracellular cholesterol trafficking and lead to unesterified cholesterol accumulation within late endosomal/lysosomal compartments. This buildup impairs endolysosomal function and gives rise to both visceral and neurological symptoms. Currently, no curative treatments are available for NPC. In the central nervous system, nearly 80% of cholesterol resides in myelin, where it is essential for myelin sheath assembly and compaction, ensuring proper axonal insulation. Defects in myelination, likely stemming from impaired oligodendrocyte (OL) maturation, have been documented in NPC patients and animal models, underscoring the importance of NPC transporters in OL biology. However, human-specific OL contributions to NPC pathogenesis remain unexplored. In this study, we generated an NPC-like phenotype in human induced pluripotent stem cell–derived oligodendrocytes (hOLs) by treating them with U18666A, a pharmacological NPC1 inhibitor. Despite high U18666A levels induced massive hOL apoptosis, lower concentrations faithfully reproduced key NPC hallmarks, including cholesterol accumulation and autophagy dysregulation. NPC-like hOLs also exhibit reduced cell area and branching complexity, indicative of maturation arrest. Notably, rapamycin, a known mTOR inhibitor and autophagy enhancer, partially ameliorated these defects. Comprehensive RNA sequencing and sphingolipidomic profiling of NPC-like hOLs revealed novel pathogenic pathways amenable to therapeutic intervention.

The research was supported by Italian Ministry of University and Research (MUR), DM 737/2021, “My first SEED Grant” (University of Milan); PRIN—Progetti di Ricerca di Interesse Nazionale (n.2022AJ3FHA); and European Union—NextGenerationEU (PNRR M4C2-Investment 1.4- Projects CN00000041 and PE00000006).

Impaired mitophagy and mitochondrial dynamics in RTD iPSC-Derived Motor Neurons

NDG 35

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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. Additionally, we demonstrated an abnormal activation of the autophagic process by WB experiments for LC3 and p62. Since the clinical response to RF therapy in RTD patients is highly variable, we proposed to use the neurotrophic factor NGF (nerve growth factor) as a potential effective therapy. 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.

Analysis of Neurofilament-light chain concentration in cerebrospinal fluid of an ALS patient carrying a novel SOD1 p.A90del mutation, treated with Tofersen and that developed myelitis

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Neurofilaments (Nf) are the major constituent proteins of the neuronal cytoskeleton. Axonal damage causes the release of neurofilaments in the extracellular ambient, where they can reach cerebrospinal fluid (CSF) and, to a lesser extent, blood. Thus, an increase in Nf levels in biofluids is a sign of neuronal damage and elevated concentrations of Neurofilament-Light chain (NfL) and phospho-NfL have been found in patients with ALS. Tofersen or QALSODY® is an antisense oligonucleotide (ASO) that interferes with the production of SOD1 protein, recently approved by the Food and Drug Administration and the European Medicines Agency for treatment of ALS forms relying on *SOD1* mutations. During the placebo-controlled phase I-III trial, patients treated with Tofersen exhibited a robust decrease of NfL in CSF, plasma and serum compared to the placebo group. In this study, we measured the NfL levels in the CSF of a patient treated with Tofersen. The subject carried a *SOD1* mutation (p.A90del), not previously described. Since some *SOD1* mutations, although found in individuals with ALS, are not considered pathogenic (i.e. variant of uncertain significance, VUS), a possible decrease in NfL levels after treatment may suggest the variant pathogenicity. We measured a drastic decrease (70-80% from baseline) of NfL levels in patient's CSF starting from day 57 of treatment with Tofersen, suggesting the pathogenicity of the *SOD1* p.A90del mutation. After 466 days of treatment, the patient developed the symptoms of myelitis, which is a possible Tofersen side effect. This was accompanied by a slight increase of the NfL levels. Treatment was then halted and restarted after the myelitis resolution. This is the first case to our knowledge, restarting the drug assumption after fully recovering from myelitis symptoms. Notably, NfL levels in CSF decrease again once treatment was restarted, reaching pre- myelitis values after 29 days.

NfL levels during therapy with the antisense oligonucleotide Tofersen in an ALS patient carrying a SOD1 variant and the C9orf72 pathogenic expansion

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Genetic forms of ALS account for about 5-12% of ALS cases and superoxide dismutase 1 (SOD1) gene is responsible for 2-3% of these forms. Mutations in *SOD1* cause the protein to adopt abnormal conformations or misfolding, which promote protein aggregation in brain tissue and neuronal damage. Tofersen is a treatment for *SOD1*-relying ALS recently approved by the Food And Drug Administration and European Medicine Agency. This is an intrathecally delivered antisense oligonucleotide that targets *SOD1* messenger RNA, leading to its degradation via RNase H and subsequently reducing the production of the SOD1 protein. A Phase 3 randomized trial has shown that Tofersen leads to a decrease in the overall levels of SOD1 protein in cerebrospinal fluid (CSF), serving as an indirect indicator of target engagement, as well as a reduction in plasma and CSF neurofilament light chain (NfL) concentrations, which is a marker of axonal damage and neurodegeneration. In this study we evaluate the trend of NfL levels in CSF and plasma samples from an ALS patient, treated with Tofersen, carrying variants in two different genes: *SOD1* (p.N20S) and pathogenic repeat expansion in *C9orf72*. The trend was compared to that of patients harboring well-known pathogenic *SOD1* mutations (p.A96G, p.L145F, p.G148C). The measurement was carried out using the SIMOA (Single Molecule Array) technology, a highly sensitive technique able to detect the amount of sample in the order of femtograms. Our analysis showed a clear decrease in NfL levels for patients carrying known pathogenic *SOD1* variants, in line with the data from the clinical trial. In contrast, a reduction of NfL levels was not detected in the *SOD1/C9orf72* patient even after 281 days of treatment. These findings suggest that in our patient the driving force of ALS pathophysiology is the presence of *C9orf72* pathogenic expansion. This seems to be confirmed by the presence of a cognitive and behavioural deficit typical of *C9orf72* subjects. The existing literature indicates that the significance of the *SOD1* p.N20S is still unknown. Our data suggest that its pathogenic role, if any, is limited in the presence of the *C9orf72* expansion.

Cerebrospinal fluid and plasma HDL (dys)function in Multiple Sclerosis**NDG 38**

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Multiple sclerosis (MS) is a multifactorial neurodegenerative disease in which cholesterol plays a key role. Cerebral cholesterol transport is essential to provide neurons with cholesterol to maintain their physiological functions. It is mediated by lipoproteins similar to plasma HDL that have been identified in human cerebrospinal fluid (CSF). Their function is to promote efflux of cholesterol from astrocytes through mechanisms mediated by ABCA1 and ABCG1 transporters. Dysregulation of cholesterol transport in the central nervous system has been associated with neurodegenerative disorders, however, whether dysfunctions occur in MS has not yet clarified. This study aimed at identifying possible disturbances in HDL function in MS by measuring CSF and serum HDL cholesterol efflux capacity (CSF and serum HDL-CEC).

We conducted an observational study including CSF and serum from 45 MS patients and 14 age- and sex-matched controls. Cerebral and serum HDL-CEC were evaluated in central and peripheral cell models using a radioisotopic technique.

CSF HDL-CEC from astrocytes was significantly lower in MS patients compared to control subjects (-32%, $p=0.0003$), with an impairment of the ABCG1-mediated efflux (-18%, $p=0.0196$). No significant differences were observed between the groups for serum HDL-CEC mediated by ABCA1 and ABCG1. However, stratification of MS subjects by severity of pathology based on the presence of oligoclonal bands (OCB), pointed out a significantly lower CSF HDL-CEC from astrocytes (-21%, $p=0.0374$) and a lower serum HDL-CEC specifically mediated by ABCA1 (-27%, $p=0.0086$) in MS OCB+ subjects.

Overall, our results show that CSF HDL-CEC, the first step of cerebral cholesterol transport, is dysfunctional in MS, suggesting cerebral HDL as a potential pharmacological target. In addition, the observation that serum HDL-CEC mediated by ABCA1 is lower in MS subjects OCB+ may put the premises to study serum HDL-CEC as a potential peripheral biomarker of the disease.

Correlation between oxidative stress and mitochondrial dysfunction in Alzheimer's disease

NDG 39

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Alzheimer's disease (AD) is a neurodegenerative disease characterized by a decline in memory and cognition that impairs daily behavior. Both genetic and environmental risk factors play a role in the manifestation of AD, but the main risk factor is aging. The mechanism of AD pathology is still elusive, but a pivotal role is played by mitochondrial dysfunction and oxidative stress, two of the main hallmarks of aging itself.

Thus, we aimed to investigate the correlation between these two aspects in skin fibroblasts' cultures from AD patients compared to no AD subjects. Cells were treated with 1mM hydrogen peroxide in complete culture medium at different time points (30 minutes, 1 hour, 8 hours and 24 hours) to simulate both acute and chronic exposure to oxidative stress. Then, cell viability, 8-Oxo-2'-deoxyguanosine (8-oxo-dG) accumulation and co-localization with mitochondria, mitochondrial fission and fusion targets, and general DNA damage were analyzed.

Our preliminary results showed that AD and no-AD fibroblasts react differently to oxidative stress exposure, in particular considering 8-oxo-dG accumulation, that resulted higher in no-AD fibroblasts, and its co-localization with mitochondria and mitochondrial DNA (mtDNA), that resulted more evident in AD fibroblasts. Moreover, we observed a peculiar alteration in the fluorescence signal related to mitochondria staining in AD cells, an aspect that could be related to a prevalent interference in these cells with 8-oxo-dG signal, as well as the mitochondria depletion characteristic of early stages of neurodegeneration. Although other experiments and investigations are still ongoing, we already believe that this work may contribute to unveiling novel molecular aspects related to the interplay between oxidative stress and mitochondria in the context of AD pathology.

Evaluating the neuroprotective effects of the novel oestrogen receptor GPER1 in tau related dementia.

NDG 40

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Alzheimer's disease (AD) is a progressive disorder, characterised by memory loss and cognitive decline. Two major hallmarks in AD are the extracellular accumulation of amyloid plaques, containing toxic amyloid- β ($A\beta$) peptide and intracellular neurofibrillary tangles comprised of hyperphosphorylated tau. Oestrogens have reported protective actions in the brain and periphery and the novel plasma membrane associated-coupled oestrogen receptor 1 (GPER1), is implicated in the neuroprotective actions of oestrogen. To investigate the potential protective actions of GPER1 in AD, the effects of the highly selective GPER1 agonist, G1 were evaluated in a cellular model of tau related dementia. In this model, treatment of primary hippocampal neurons with amyloid β ($A\beta_{1-42}$) results in aberrant movement of tau from axons to dendrites and synapses. We have shown that G1 prevents $A\beta_{42}$ -driven targeting of tau to dendrites. These effects of G1 are due to GPER1 activation, as the protective effects of G1 were inhibited by the selective GPER1 antagonists, G36 and G15. The downstream signalling pathways, mediating the protective actions of GPER1 have been assessed using selective pharmacological tools. To verify the involvement of phosphoinositide 3-kinase two different inhibitors of PI3K, namely wortmannin (WM) and LY294002 (LY) were used. We found that treatment with either WM and LY blocked the ability of G1 to prevent tau mislocalisation to dendrites, thereby indicating that activation of PI3K is critical for the neuroprotective effects of GPER1. The role of GSK-3 β has also been assessed using two different inhibitors, TCS2002 (TCS) and SB21676 (SB). We have shown that treatment with either TCS or SB treatment mimics and occludes the protective effects of G1, indicating that inhibition of GSK-3 β plays a role. These findings increase our understanding of the neuroprotective role of GPER1 in the early pre-clinical stages of AD.

A map of transcriptomic profile of Different Brain areas and a longitudinal based study of mitochondrial DNA (mtDNA) methylation in whole blood and brain areas in Alzheimer's disease

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Aging is often characterized by a progressive loss of cognitive abilities due to the onset of Alzheimer Disease (AD). In this study, a Transcriptome Analysis and Gene Set Enrichment Analysis (GSEA) were performed on brain samples from the hippocampus (HI), temporal and parietal cortex (TC, PC), cingulate cortex (CG) and substantia nigra (SN) from the Abbiategrasso Brain Bank (ABB) of subjects with a clinical and neuropathological diagnosis of AD and healthy controls. According to the ABB protocol, the neuropathological diagnosis was based on a complete histological characterization, including all major proteinopathies and vascular scoring. Transcriptomic results showed a greater number of differentially expressed genes (DEGs) in TC (1571) and CG (1210) and a least amount of DEGs in HI (206), PC (109) and SN (60). Furthermore, GSEA revealed a difference between the group of early-affected brain areas (HI, TC) and the group of the subsequently involved areas (PC, CG, SN). Notably, in HI and TC there was a significant down-regulation of shared DEGs primarily involved in the synaptic transmission and in the development of the central nervous system, while in PC, CG and SN there was a significant down-regulation of genes primarily involved in the proper protein folding and trafficking and inflammation. In addition to assessing biological processes, we found a greater deregulation of the pathways involved in mitochondrial dysfunction. Since we hypothesised the presence of mitochondrial alterations in AD patients, we will assess the possible presence of methylation alterations in the D-loop regulatory region of the mitochondria and its relation to mtDNA copy number. In conclusion it is possible to draw a molecular map of pathology spreading in the brain that could unveil unexplored roads and possible shortcuts leading to a better understanding of AD pathogenesis. This could help to discover novel biological targets or possible biomarkers of disease.

Towards ultrasonic neuromodulation mediated by piezoelectric nanoparticles (NPs): a biophysical study on a neuronal cell model

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Neurological disorders affect millions worldwide. Current treatments, including extracellular electrical stimulation via neuro-chips or photo- and magneto-thermal materials delivering localized heat, are considered invasive, lacking the specificity of stimulation on single cells or brain regions, and risk of producing unpredictable cell damage. Recently, to overcome such limitations, sonogenetics has emerged as a promising technology for non-invasive and highly selective brain stimulation that exploits focused medical ultrasound (US), but it necessitates genetic modification to express mechanosensitive proteins. Thus, to prevent gene therapy, we propose a novel neuromodulation approach that combines mechanical stimulation from low-intensity US and mechano-electric transduction via piezoelectric nanoparticles (NPs) adsorbed onto the cell membrane, capable of inducing cell excitability. Exploiting this method, we look for higher selectivity, improving local amplification of mechanical stimulus using soft, deformable NPs-decorated PVA microbubbles (NPs-MBs) under biomedical-relevant US frequencies optimized for future in vivo applications. To this aim, a deep physicochemical characterization of piezoelectric NPs (size, z-potential) and biological analysis of their interaction with cells alone and in combination with US was conducted. Our data on cell viability and morphology, cell membrane permeability, and activation of neuronal inflammation pathways demonstrate an encouraging outcome between NPs and cells also when combined with US. Moreover, preliminary experiments with cells exposed to NP-MBs showed promising responses. These data prompt us to develop further this strategy toward novel neuromodulation strategies capable of inducing compensatory neural activity in dysfunctional brain areas (e.g., in epilepsy, in hyperexcitability conditions after brain injury, or other neuropathological disorders).

Small molecules inhibitors of Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) as potential pharmacological strategy in Alzheimer's Disease (AD)

NDG 59

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PCSK9, a key regulator of plasma cholesterol, is also expressed in the brain, where it may contribute to AD pathogenesis. We previously showed that PCSK9 enhances A β -induced neurotoxicity and inflammation in cerebral cells, while its genetic deletion in AD mice improves cognition and reduces A β burden. This study aims to investigate the effects of small lipophilic molecules PCSK9 inhibitors *in vitro* in human AD-relevant models and their tolerability and biodistribution in WT mice.

Four compounds - MR3 and MR546 (chemotype 1), MR533 and MR644 (chemotype 2) - were tested in human neuroblastoma IMR-32 cells and compared to a reference inhibitor, 7030B-C5. PCSK9 expression/secretion (WB, ELISA), LDLR expression (WB), and A β -induced neurotoxicity (MTT assay) were evaluated. Anti-inflammatory effects were assessed in LPS-stimulated THP-1 microglial-like cells via IL-6 secretion (ELISA). *In vivo*, MR3 and MR644 were orally administered (12.5–100 mg/kg, 5 days) in C57BL/6J mice to assess tolerability (ALT activity, body weight) and compound distribution (LC-MS/MS).

At 10 μ M, MR3, MR546, and 7030B-C5 significantly reduced PCSK9 expression (–44%, –19%, –34%, $p < 0.01$ vs basal) and secretion (–49%, –33%, –49%, $p < 0.05$ vs basal). MR533 and MR644 reduced secretion only (–8%, –46%, $p < 0.05$ vs basal) without affecting expression. All compounds increased LDLR expression (+20%, +9%, +44%, +26%, $p < 0.05$ vs basal) and significantly reduced A β -induced cytotoxicity, with the most pronounced effect for MR546, MR644 and 7030B-C5 ($p > 0.05$ vs basal). All MR compounds strongly reduced LPS-induced IL-6 secretion ($p < 0.001$). *In vivo*, MR3 and MR644 were well tolerated and dose-dependently detectable in plasma (1243–7051; 3531–7781 nM), liver (16771–112249; 16394–57642 pmol/g), and brain (1002–8499; 1241–7763 pmol/g).

PCSK9 inhibition with small molecules might exert a favorable effect on A β -induced neurotoxicity and neuroinflammation and represents a promising safe and brain-directed strategy for AD.

Longitudinal Tracking of Glioblastoma Clones Acquiring Immune-Evasive Properties

NO 09

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Glioblastomas are incurable brain tumors depicted by high intra-tumoral heterogeneity and by an immune-evasive phenotype. In a previous published work, we traced the clonal dynamics of glioblastoma evolution by overexpressing PDGFB oncogene into mouse brains and labelling each transformed cell with genetic barcodes. We observed a continuous clonal loss during the acquisition of a malignant phenotype, underlined by the modulation of the levels of c-Myc expression and their functional targets.

In this study, we further explore the evolution of glioblastoma by transplanting multiclonal, early-stage glioma cells into multiple immunodeficient NOD-SCID mice. This experimental design allowed us to track the clonal dynamics over serial transplants, revealing how early-stage glioma clones acquire immune-evasive features capable of initiating tertiary tumors in immune-competent hosts.

Clonal tracing via genetic barcoding revealed that, among the many clones initially present in early-stage gliomas, only a limited number were able to successfully engraft and expand in secondary tumors. Interestingly, secondary tumors derived from the same primary glioma exhibited partial clonal overlap, suggesting that the acquisition of immune-evasive traits is not entirely stochastic but may be partially predetermined at early stages.

Given that secondary and tertiary tumors are often composed of a few dominant clones, we leveraged this clonal purification to compare the transcriptomic profiles of individual clones across different tumor stages. To this end, we performed bulk RNA sequencing of secondary and tertiary tumors, while taking advantage of single-cell RNA-seq data from the corresponding primary tumors, where barcode detection enabled us to assign transcriptomes to individual clones. This integrative approach allowed us to reconstruct longitudinal gene expression trajectories for specific clones and to investigate how their functional states evolve during immune selection.

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

NO 10

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We have recently shown that during gliomagenesis, clonal populations diverge into subgroups with varying fitness levels, often leading to the predominance of one and extinction of others. Selection appears to persist even among clonally related cells in later tumor stages, suggesting that clonal divergence continues over time. The mechanisms behind this process are still unclear.

Preliminary data from PDGFB-induced murine gliomas reveal heritable, clone-specific gene expression patterns, which may provide the transcriptional heterogeneity needed to fuel clonal extinction. As gene expression is closely linked to chromatin accessibility, we hypothesize that specific epigenetic profiles act as a form of clonal memory, sustaining transcriptional differences and fitness variation among genetically identical subclones.

To explore these mechanisms, we have developed an optimized lentiviral lineage tracing library compatible with single-cell RNA sequencing. This vector includes a high-diversity genetic barcode embedded in a transcript scaffold, enabling simultaneous recovery of clonal and gene expression information using standard 3'-based scRNA-seq protocols.

We will first apply this tool to monoclonal murine glioma cultures, collecting samples at multiple timepoints to investigate whether subclonal competition emerges in vitro. Single-cell transcriptomics linked with clonal information will reveal transcriptional traits of emerging subclones and the extent of intra-clonal heterogeneity. Special focus will be given to Myc and its targets, linked to clonal dominance in our previous work.

Finally, integrating single-cell multiomics, we aim to correlate transcriptional and clonal identity with chromatin accessibility, testing whether stochastic epigenetic variability drives persistent clonal competition in glioma progression.

Our study presents a single-cell barcoding platform for combined lineage tracing and transcriptomics. Applied to monoclonal glioma cultures, it will dissect subclonal dynamics and test whether epigenetic heterogeneity acts as a non-genetic driver of clonal competition. This approach may reveal mechanisms underlying glioma evolution and identify targets to disrupt clonal selection.

The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and is involved in medulloblastoma tumorigenesis

NO 11

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Medulloblastoma (MB) is a lethal pediatric malignancy of the cerebellum. Its molecular and genetic heterogeneity make effective treatment challenging. Among MB molecular subgroups, Sonic Hedgehog (SHH) is the second most prevalent and is characterized by alterations in key regulators of the SHH pathway. However, the molecular mechanisms driving SHH-MB still need to be clarified to develop more successful therapies. We recently identified INF2, a formin that mediates cytoskeletal dynamics, as a negative regulator of SHH signaling. INF2 acts in opposition to mDia formin, previously shown to promote SHH pathway activation. We found that INF2 overexpression counteracts the positive effects of mDia on GLI1, the final SHH effector, by reducing both its transcriptional activity and expression. We demonstrated that the repressive effect of INF2 on SHH signaling is independent of its role in actin polymerization, and is instead directly associated with its interaction with mDia. Indeed, variants in INF2's Diaphanous Inhibitory Domain (E184K and R218Q) that disrupt INF2/mDia binding fail to decrease GLI1 protein levels compared to wild-type INF2. Moreover, we found that INF2 expression rises as SHH signaling declines during normal cerebellar development in mice, showing an inverse trend to mDia. Accordingly, INF2 silencing increases GLI1 expression and promotes the proliferation of granule cell progenitors (GCPs), the cells of origin of MB. Notably, INF2 protein levels are strongly reduced in both murine and human SHH-MBs, while mDia is upregulated. INF2 overexpression, but not that of INF2 variants unable to bind mDia, significantly inhibits SHH-MB cell proliferation by reducing GLI1 levels. These findings reveal INF2 as a negative regulator of SHH signaling and highlight cytoskeletal remodeling proteins as promising therapeutic targets in SHH-driven MB.

Modeling Glioblastoma-Microglia Crosstalk in a Patient-Derived 3D Assembloid System

NO 12

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Glioblastoma (GB) is the most prevalent and lethal primary malignant brain tumor in adults characterized by high invasiveness, cell heterogeneity and dynamic interactions within the tumor microenvironment (TME). Current preclinical models of GB appear insufficient to recapitulate the complex intercellular interactions occurring in the TME, limiting their utility in improving clinical outcomes. GB 3D in vitro cultures, such as tumoroids, provide a defined culture environment which allows to maintain histological features of the primary tumor and relevant degrees of cancer stem cells (CSCs) heterogeneity. Nonetheless, one of the main challenges remains to adequately integrate the key cellular constituents of the TME in 3D models. In this milieu, microglia, the primary resident immune cell component in the brain, is found to represent approximately 40% of the GB mass in vivo, underlying its crucial role in influencing GB behavior and response to therapy. For that purpose, we set up a reproducible protocol for the generation and the maintenance of a patient-derived GB tumoroid model that accurately recapitulates original tumor phenotypical and molecular characteristics. Consequently, we leveraged the assembloid technology to incorporate a human microglia cell line into the patient-derived GB tumoroid. This tumor-microglia assembloid model recapitulates key features of the GB in vivo behavior, including tumor growth dynamics and response to Temozolomide (TMZ), a standard chemotherapeutic agent and allowed us for the research of molecular mechanism involved in GB pathology. In this regard, we focused on the emerging role of Adenosine (ADO) system in microglia-GB cross-talk. Activation of ADO receptors, particularly $A_{2A}AR$, seems to stimulate Extracellular Vesicles (EVs) release in glial cells, a mechanism that facilitates intercellular communication. On the other hand, the EVs released by GB cells have a prominent pro-tumoral role by inhibiting the immune system. On this line, we targeted $A_{2A}AR$ by treating assembloids with a specific partial agonist for the ADO receptor. To this end, we consider this tumor-microglia 3D in vitro model to represent a powerful platform for investigating therapeutic strategy for GB.

AMBRA1 controls the Sonic Hedgehog signaling pathway and SHH-medulloblastoma**NO 13**

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The Sonic Hedgehog (SHH) signaling pathway plays a crucial role in embryonic development, and its dysregulation is implicated in several cancers, including medulloblastoma (MB), a highly heterogeneous and aggressive pediatric brain tumor. Here, we uncover a novel role for the pro-autophagic protein AMBRA1 in SHH-driven MB. Specifically, we identify AMBRA1 as a direct substrate and functional interactor of REN/KCTD11 (hereafter REN), the substrate receptor of the Cullin3-RING ubiquitin ligase complex (CRL3^{REN}), and a tumor suppressor frequently lost in ~30% of SHH-MB cases. Through proteomic and biochemical analyses, we demonstrate that REN binds AMBRA1 and promotes its polyubiquitylation and proteasome-dependent degradation. Loss of REN leads to aberrant accumulation of AMBRA1, which in turn acts as a positive regulator of the SHH pathway by enhancing the stability of GLI1, the final effector of SHH signaling. Mechanistically, AMBRA1 interacts with β TrCP, an adaptor protein mediating GLI1 degradation, and impairs its binding to GLI1. This event results in the accumulation of GLI1 and in the enhancement of SHH activity. Notably, AMBRA1 protein is significantly overexpressed in SHH-MB patient samples and correlates with poor prognosis. Moreover, genetic depletion of AMBRA1 strongly reduces SHH-MB tumor growth both *in vitro* and *in vivo*. These findings unveil a previously unrecognized AMBRA1/ β TrCP/REN axis as a key regulator of SHH signaling and MB progression. Our study identifies AMBRA1 as a novel substrate of the CRL3^{REN} complex and highlights its potential as a promising therapeutic target in SHH-dependent medulloblastoma.

PX-478-mediated HIF-1 α inhibition as a possible mechanism to revert GBM chemoresistance: molecular and biochemical studies**NO 14**

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Glioblastoma (GBM) is the most common and aggressive astrocytic brain tumour, characterised by rapid progression and poor prognosis, despite standard treatment with Temozolomide (TMZ) and radiotherapy. TMZ is an alkylating agent that methylates DNA, disrupting gene transcription. The methylation status of the MGMT promoter serves as a biomarker for TMZ responsiveness¹. However, additional molecules are emerging, such as Hypoxia-Inducible Factor-1 α (HIF-1 α), a transcription factor that regulates genes involved in the hypoxic response, tumour invasion, and genomic instability. TMZ has also been shown to trigger chaperone-mediated autophagy (CMA) through mitochondrial stress, and HIF-1 α levels are influenced by CMA activity². Resistant cells typically express higher basal levels, which may contribute to their resistance mechanisms. Previous work has demonstrated significant differences in cell viability between TMZ-sensitive and TMZ-resistant models, which may be explained by varying basal levels of HIF-1 α . PX-478 is a small-molecule inhibitor of HIF-1 α that disrupts cancer cell adaptation to hypoxia³. Therefore, we assessed the effects of PX-478, both alone and in combination with TMZ, in a range of commercially available TMZ-resistant GBM cell lines through cellular, molecular and biochemical assays. We found that pharmacological inhibition with PX-478 restores TMZ sensitivity in resistant GBM models, triggering ferroptosis, CMA, and apoptotic processes. Furthermore, the treatment can induce changes in cell cycle progression and oxidative state.

Future applications of the project will focus on the use of PX-478 in optical imaging studies in orthotopic glioma models. Through a combined approach using a chemoresistant cell line engineered to express the luciferase reporter gene and fluorescent probes to highlight key processes in tumour progression, the efficacy of PX-478 will also be validated in a preclinical setting.

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In vitro modeling of early glioma progression using neural organoids**NO 15****Chiara Riviera**⁽¹⁾ - Irene Appolloni⁽²⁾ - Davide Ceresa⁽²⁾ - Francesca Piaggio⁽¹⁾ - Daniela Marubbi⁽²⁾ - Paolo Malatesta⁽²⁾⁽¹⁾ IRCCS Ospedale Policlinico San Martino, UO Neuro-oncologia e Mutagenesi, Genova, Italy - ⁽²⁾ Università di Genova, Dipartimento di Medicina Sperimentale, Genova, Italy

Early stages of glioma progression, following the first molecular lesion, are still largely unknown. In vivo murine models pose challenges due to limited access to early stages and complex manipulation. Neural organoids (NO) offer an accessible, manipulable, and translatable in vitro alternative. We aim to model glioma progression using NO derived from murine embryonic stem cell line E14Tg2A, used to create embryoid bodies (EB) that are differentiated into unguided neural organoids. NO display neural rosettes composed of neural progenitors (Sox2+), neurons (MAP2+ and NeuN+) and astrocytes (GFAP+). Glioma was modeled via two strategies: 1) co-culture or transplantation of mouse high-grade glioma primary cells (mHGG); 2) Platelet-derived growth factor B (PDGF-B) overexpression, that induces glioma of different grades from murine embryonic neural progenitors in vivo. For the first approach, tumor take rate is 95% for co-culture (103/108 NO) and 90% for transplantation (27/30 NO). After 30 days, 74.8±24.8% of mHGG are ki67+ and 80.6±8.2% are Sox2+, confirming their proliferative and stem-like nature. Infiltration into NO increased over time, based on the median distance from the organoid edge (10 days: 77.2±22.9 mm; 40 days: 99.6±20.3 mm), indicating the preservation of invasive potential. In the genetic approach, PDGF-B is overexpressed via piggyBac electroporation, leading to tumor-like masses of proliferating cells expressing Sox2, Nestin, and PDGFR α , closely resembling the in vivo phenotype. The co-culture model suits translational applications with patient-derived cells, while the PDGF-B model provides a controlled system to dissect early molecular events following the first oncogenic hit. Thus, these NO-based approaches offer a powerful platform to study glioma progression, early-stage tumor dynamics and potential therapeutic interventions.

Genome-Wide ATAC-seq Profiling Reveals the Impact of MEOX2 on Chromatin Accessibility in Glioblastoma Stem Cells

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Glioblastoma (GBM) is a highly aggressive brain tumor, thought to arise from glioblastoma stem cells (GSCs), driving tumor growth, therapy resistance, and recurrence. The homeobox transcription factor MEOX2 has emerged as an oncogenic regulator in GBM. Its expression is elevated in gliomas compared to normal brain tissue, correlates with poor patient survival, and is particularly enriched in GSCs. Our transcriptomic analysis of patient-derived GSC lines confirms that MEOX2 is significantly overexpressed in GSCs relative to non-stem glioblastoma cells and is essential for GSC self-renewal and viability, as siRNA knockdown (KD) impairs these traits and induces apoptosis and gene expression changes. However, the mechanisms underlying MEOX2 function remain unclear. We hypothesized that MEOX2 targets distinct genomic regions in GSCs, influencing chromatin accessibility and gene regulation. We performed ATAC-Seq on patient-derived GSC following MEOX2 knockdown. DNA was sequenced and analyzed using standard bioinformatics tools within the nf-core/atacseq pipeline. Peak calling and differential accessibility analysis identified significantly altered chromatin regions ($FDR < 0.05$, $\log_2FC \geq 0.7$). ATAC-Seq This revealed widespread changes in chromatin accessibility, particularly at distal intergenic and intronic regions, suggesting a role as a general chromatin regulator involved in stemness maintenance rather than a classical promoter-bound transcription factor. Among repressed genes, several (e.g., LOXL2, NEAT1, SDC1) showed reduced accessibility within known super-enhancers, suggesting MEOX2 maintains accessibility at key oncogenic loci. Conversely, KD increased accessibility at genes like PCDH10, ENPP2, and MMP2—implicating potential anti-tumor responses and compensatory mechanisms. These results highlight MEOX2 as a key regulator of the chromatin landscape in GSCs and suggest that targeting its activity may provide novel therapeutic opportunities in GBM.

Succisa Pratensis: A Natural Enhancer of Chemotherapy in Glioblastoma**NO 17**

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Glioblastoma multiforme (GBM) is an aggressive brain tumor with a poor prognosis and strong resistance to standard therapies, including Temozolomide (TMZ) ^{1,2}. New adjuvant approaches to conventional therapies are being studied to overcome current limitations. Biodiversity offers a valuable source of natural compounds with anticancer potential ³. In this study, we evaluated the effects of *Succisa Pratensis* extract on TMZ-resistant GBM cells as adjuvant strategy, focusing on its ability to modulate the Pregnane X Receptor (PXR) activity, a key regulator of drug resistance. The efficacy of *S. Pratensis* extract, alone and in combination with TMZ, was tested on a panel of TMZ-resistant GBM cell lines. A multidisciplinary *in vitro* approach was used to assess cell proliferation, viability, colony formation, and migration. LC-MS analysis was performed to characterize the extract and identify bioactive metabolites. PXR modulation was investigated through qPCR, immunofluorescence, and molecular docking to evaluate the binding affinity of selected metabolites to the receptor.

The combined treatment with *S. Pratensis* and TMZ significantly reduced proliferation, colony formation, and migration of GBM cells. A more in-depth study of drug resistance, gene expression and immunofluorescence revealed increased PXR levels after treatment, even though its target genes were not upregulated. Docking analysis identified specific secondary metabolites with high affinity for the PXR ligand-binding domain, suggesting an inhibitory effect on receptor activity and a role in enhancing TMZ sensitivity.

These results suggest that *S. Pratensis* metabolites could represent promising adjuvant agents for GBM therapy. Further studies on preclinical models and patient-derived cells are needed to validate their potential in overcoming TMZ resistance and improving clinical outcomes.

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Unraveling the role of emotional memory in anxiety-related behaviors in rats**NP 12****Felice Ciciarelli** ⁽¹⁾ - Camilla Giglio ⁽¹⁾ - Annamaria Renna ⁽¹⁾ - Luisella Milano ⁽¹⁾ - Benedetto Sacchetti ⁽¹⁾⁽¹⁾ Department of Neuroscience, University of Turin, C.so Raffaello, 30, 10125, Turin, Italy

In the event of threatening stimuli, animals must pursue appropriate behaviors to overcome the threat. Normal fear and anxiety are emotional responses to these dangers. The first one induces an immediate defense behavior, the second one a lasting state of alert also in absence of threats. Brain areas involved in fear and anxiety neuronal circuits have been, at least in part, already established. Situations of acute or sustained stress can induce modifications in such brain areas, i.e prefrontal and cingulate cortex, through neuroplasticity mechanisms. This can lead to anxiety- and stress-related disorders often associated with generalized fear, for example post-traumatic stress disorders (PTSD). Anxiety-like behavior in rodents can be assessed through ethologically relevant assays designed to measure approach versus avoidance behavior and defensive responses. The mechanisms by which anxiety-related behaviors are maintained after a traumatic experience are still not fully understood.

Oligodendroglia-specific ablation of Citron Kinase is associated with cortical hypomyelination and cognitive impairment which are mitigated by PTEN co-deletion

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Although extensive evidence correlates myelination and cognition, the mechanisms linking these two processes are not yet fully understood. Here, we addressed this issue in a mouse model of delayed myelination and regionalized hypomyelination, i.e. a conditional oligodendrocyte-specific Citron-kinase (Cit-k) mouse mutant (Sox10Cre::Cit-k fl/fl mice).

Cit-k is a serine-threonine kinase involved in DNA repair and cytoskeletal dynamics, whose loss-of-function results in microcephaly and severe CNS hypomyelination in both humans and mice. Cit-k loss cell-autonomously induces forebrain oligodendrocyte progenitor cells to either undergo cell death or enter a senescent state, leading to diffuse impairment of myelin deposition at juvenile stages. This myelin defect is only partly compensated in adult mice, where a severe hypomyelination persists in the cerebral cortex.

To assess whether this region-specific hypomyelination was associated with functional alterations, we characterized the behavioral phenotype of the conditional mutants in adulthood. While gross motor, sensory and emotional aspects appeared unaltered, Sox10Cre::Cit-k fl/fl mice displayed impaired working, short term and associative fear memories. Ongoing experiments are aimed at unveiling alterations in cortico-cortical and cortico-hippocampal functional connectivity underlying the observed cognitive impairment. Of note, co-deletion of PTEN in Cit-k deficient oligodendroglia partly restored cortical myelination and mitigated mouse memory defects.

Overall, our data indicate that the regionalized hypomyelination resulting from the oligodendroglia-specific deletion of Cit-k is associated with specific cognitive impairments, in line with the idea that myelin alterations compromise network activity and contributes to functional impairment. Further experiments will investigate the mechanistic substrates of PTEN-dependent restoration of myelin and cognitive functions.

Importin alpha3 controls age-dependent axonal branching via a ribosomal protein

NP 14

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Importin alpha3 (Impα3, Kpna4) knockout (KO) mice exhibit diminished pain perception. We analyzed RNAseq data from lumbar dorsal root ganglia (DRG) of wild-type (WT) and impα3 KO mice 7 days post-spared nerve injury (SNI). Among the differentially expressed genes (DEG), Rps3a1 was significantly up-regulated in the post-injury KO. Rps3a, a 40S ribosomal protein, is highly expressed in sensory neurons as well as in iPSC-derived sensory neurons. Using advanced fluorescence imaging and ImageJ analysis we detected Rps3a protein significantly increased in both axons and branch points in the KO compared to WT. Analysis of the axonal outgrowth of cultured primary DRGs from young, adult and old males revealed increased branching in old WT as well as increased Rps3a expression. In contrast, cultured DRG neurons of Impα3 old KO exhibited significant decreased neurite growth and branching together with a decreased Rps3a expression in both branch points and axons. Moreover, Proximity Ligation Assay (PLA) reveals local protein synthesis of Rps3a in the axons of Impα3 KO highlighting its role in the regulation axonal morphology. Importantly, Rps3a transcription is regulated by cFOS, a transcription factor transported by Impα3. We propose that Impα3 loss leads to reduced cFOS translocation and Rps3a expression, impairing branching. Connectivity Map analysis highlighted PI3K inhibitors as potential modulators, aligning with our observation that PI3K and cFOS inhibitors significantly reduced Rps3a levels and axonal branching in DRG cultures and iPSC-derived sensory neurons. These insights suggest that targeting cFOS and Rps3a could offer new therapeutic approaches for pain management. In the future, we plan to perform behavioral experiments following sciatic nerve crush lesion to assess the degree of recovery and regeneration in Impα3 KO animals.

Decoding the Neural Mechanisms Linking Emotion and Social Behavior in Autism Spectrum Disorder

NP 15

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by core behavioral abnormalities, including social deficits, verbal impairments, and intellectual disabilities. Individuals with ASD also exhibit difficulties in emotion recognition and an altered ability to attribute affective meaning to sensory stimuli. In particular, assigning emotional significance to socially relevant cues is challenging for autistic individuals.

The research aims to investigate the neural mechanisms underlying these altered emotional-associative processes and their relationship with social deficits in ASD. As a first step, we are exploring whether and how the neuronal circuits responsible for assigning emotional incentive value to sensory stimuli contribute to social behaviors in wild-type (WT) mice. Specifically, the focus is on olfactory stimuli involved in both social interactions and positive experiences, with an emphasis on the role of the piriform cortex (PPC).

To this end, a viral approach enabling inducible activity-dependent tagging of neurons will be employed to assess whether there is an overlap between the neuronal networks engaged in appetitive experiences and those involved in social behavior. Additionally, chemogenetic silencing will help determine whether those common neurons are necessary for social interactions.

The underlying hypothesis is that specific neurons in the PPC, activated during appetitive memory formation, are not only engaged but also functionally required for social behavior, and that disruption of these shared neural networks may underlie the deficits observed in ASD mouse models.

Cortical dysregulations of GABA signaling and metabolism in Spinal Muscular Atrophy: results from SMAΔ7 mice

NP 16

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Spinal Muscular Atrophy (SMA) is a neuromuscular disease due to the lack of Survival Motor Neuron (SMN) protein, characterized by lower motor neuron (MN) degeneration and muscle atrophy. Moreover, evidence shows that motor cortex (CRTX) morphofunctional abnormalities in SMA patients, suggesting altered maturation and maladaptive plasticity. Given limited access to patient samples, preclinical models are mandatory to clarify motor cortical involvement and underlying mechanisms in SMA pathogenesis. We previously described a significant vulnerability of upper MN in SMA mice. Here we dissected cortical inhibitory GABAergic signaling, metabolism, and interneuron (IN) function in the sensorimotor (SM) CRTX and in primary neuron-astrocyte co-cultures of a severe SMA mouse model in comparison with WT controls. Bioinformatic and biochemical analyses revealed stage-specific alterations in GABAergic pathways and metabolite profiles during SMA progression, particularly at late disease stage. Therein, using imaging and molecular approaches, we identified in SMA SM-CRTX: (i) GABA⁺ neuron loss (-38%) (ii) impaired GABAergic synthesis (GAD65/67 enzymes); (iii) impaired motor CRTX PV⁺ interneuron morphology and density (-30%), with a concurrent reduction of GABAergic synapses in layers II/III (-28%) and V (-38%). Electrophysiological characterizations confirmed decreased inhibitory neurotransmission onto layer V pyramidal neurons. Further *in vitro* and molecular analyses highlighted the pivotal role of SMN in the neuronal-astrocyte interactions regulating GABA metabolism, release and reuptake by influencing astrocytic sodium-dependent solute carrier transporter expressions (SNAT5: -45.11 %; GAT3: +29 %). Overall, here we propose a mechanistic model for SMN deficiency-induced GABAergic pathway dysregulation in SMA cortical motor network, advancing our disease understanding and therapy design.

Evaluating potential Immune System dysregulation in Type 1 Narcolepsy patients

NP 17

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Background: Type 1 Narcolepsy (NT1) is a rare chronic central nervous system disorder causing excessive daytime sleepiness and cataplexy due to selective orexin neuron loss. Despite typical adolescent-onset, diagnosis averages 8 years post-symptom. A strong HLA DQB1*06:02 link suggests autoimmunity, with implicated CD4⁺ T lymphocyte activation and a humoral response, though the exact mechanism is unclear. This study investigated autoantibodies (Abs) targeting lateral hypothalamic nuclei and neuronal synaptic/onconeural antigens (Ag) in NT1 immune dysregulation. **Methods:** Serum from 26 NT1 patients (30.35±16.37 years, 38% female) (collected ~10 years post-symptom onset) and 34 healthy controls (HC) was analyzed. All sera underwent indirect immunofluorescence (IIF) on primate hypothalamic sections (Euroimmun) for IgG/IgM Abs. IIF also detected IgG Abs against neuronal synaptic Ag on Fixed Cell-Based Assay (Euroimmun) and onconeural IgG Abs on primate cerebellum (only 15 samples). Orexin, TRib2 and HCRT2 levels were quantified by ELISA (MBS). **Results:** Hypothalamic IIF showed positive reactivity in 96% of NT1 subjects (one uncertain, none negative). No HC showed positive reactivity (two uncertain). NT1 patients exhibited significantly higher positive immunoreactivity vs HC ($p<0.001$). No NT1 serum samples tested positive for Abs against neuronal synaptic/onconeural Ag. Serum orexin level was lower in NT1 (19.8 pg/ml) vs HC (62.5 pg/ml); ELISA found no statistically significant differences in TRib2 and HCRT2 levels. **Conclusions:** Preliminary results suggest hypothalamic Abs in NT1. Lack of reactivity to synaptic/onconeural Abs may indicate specificity towards hypothalamic neurons. Identifying Abs is challenging due to time since disease onset, which can reduce inflammation/Ag expression. Future studies are needed, especially in pediatric cohorts closer to onset, to identify specific hypothalamic Ag and better define the immune system's role in NT1 pathogenesis.

This work was supported by grants from Italian Ministry of Health - 5 x 1000 funds 2024

Sex-age dimorphism in Importin alpha3 KO mice**NP 18**

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Your pain is unique. Clinical data show that nociception varies by age and sex, yet its neurogenetic basis remains poorly understood. A longstanding research bias toward young male subjects has left women and the elderly underserved, limiting the development of therapies tailored to diverse demographics. Meanwhile, modern pain management relies heavily on brain-targeting drugs such as opioids, contributing to addiction epidemics. In this project, we aim to map the neurogenetic basis of age- and sex-specific nociception in the peripheral nervous system. Previous work identified importin $\alpha 3$ (IMP $\alpha 3$, KPNA4) as a key regulator of chronic pain in sensory neurons, acting as a gatekeeper of pain-related transcription. Using IMP $\alpha 3$ knockout (KO) models, we studied neurite outgrowth associated with pain sensitivity across age and sex. We measured axonal outgrowth in dorsal root ganglion (DRG) neurons and found that, while aging wild-type sensory neurons tend to exhibit increased branching, neurons from IMP $\alpha 3$ KO mice branch significantly less. Further, we will investigate the role of KPNA4 in aging. To this end, we will map behavioral, neural, and genetic variation in nociception across age and sex using *Caenorhabditis elegans* as an *in vivo* model, reducing experimental timelines from years to weeks. By uncovering the molecular pathways that modulate nociceptive processing according to sex and age, this project will identify sensory-specific drug targets. These findings will pave the way for personalized pain therapies that are effective across a broad range of patient populations. Through translational research and collaboration with the pharmaceutical industry, these targets can be efficiently developed into clinical applications—transforming the lives of millions underserved by traditional analgesics.

Evidence for Food-related Pathological Decision-making in a Mouse Model of Binge Eating Disorder

NP 19

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Rising numbers of binge eating disorder (BED) cases and excessive associated economic costs, together with the absence of efficient treatment strategies, highlight the importance of research in this area. To date, numerous studies have investigated the role of aberrant motivation in compulsive, maladaptive feeding behaviors. However, while the majority of them considered the role of neurobiological adaptations in dopaminergic pathways that result in excessive incentive salience assigned to an object (highly palatable food), there is a lack of studies exploring the role of pathological decision-making that may drive individuals towards rewarding objects in the first place. In this research, two types of environmental stressors — food-related and non-food-related — are explored in their ability to model compulsive behavior towards palatable food in mice, and assessed from the perspective of pathological decision-making. Results from the behavioral experiments suggest that while both types of stressors, paired with the availability of highly palatable food, can produce aberrant motivation towards highly palatable food, when evaluated from the perspective of pathological decision-making, only food-restricted mice displayed compulsive seeking and consumption of palatable food. These findings were subsequently supported by data obtained from cortisol concentration analysis in subjects. This study was supported by the Ministry of Health of the Czech Republic, grant no. NU23-04-00381.

Keywords: binge eating disorder, pathological decision-making, environmental stressors, cortisol levels, palatable food

Cortical immature neuron density in macaques (Rhesus monkey) and its place in the phylogenetic variation

NP 20

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Considering brain structural plasticity, neurogenic processes vary across species, anatomical regions, and ages. Beside stem cell-driven adult neurogenesis, a form of “neurogenesis without division” has been recently shown, involving “immature” or “dormant” neurons in the cortical layer II (cortical immature neurons, cINs). These are prenatally generated cells in “arrested maturation” retaining the ability to resume maturation at different life stages and to functionally integrate into pre-existing circuits. Through immunocytochemical detection of the cINs, by expression of the cytoskeletal protein doublecortin, and quantitative analysis of their density conducted in nine mammalian species varying in brain size, gyrencephaly, and lifespan, we showed their striking abundance in large-brained mammals with respect to rodents. Phylogenetic analyses revealed covariation with brain size and neocortical expansion, suggesting that a trade-off has occurred during evolution between different types of plasticity. Here, using the same approach, we characterized and quantified the cINs in macaques (Rhesus monkey; a primate with human-like brain anatomy, phylogenetics, and cognitive functions). First, we studied the cIN density in 4 young adult macaques (7-10 years) to understand their position in the phylogenetic variation. Our results place them among the gyrencephalic species and confirm the covariance between cIN density and both increased brain size and neocortical expansion. Then, we used 10 middle-aged macaques (23 years) to quantify the cIN density across 16 neuroanatomically defined cortical regions in search for possible regional variation. Significant differences were observed, with higher presence in the temporal cortex and lower in the prefrontal cortex. Overall, it is confirmed that cINs follow an evolutionary trend in mammals, involving the entire cortical mantle in gyrencephalic species, yet, with regional differences.

Paw posture is a robust indicator of injury, pain, and age**NP 21**

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Research on motor function, regeneration, and pain often involves nerve injury, but assessing sensory and motor function in vivo is challenging due to behavioral variability and potential discomfort. In this study, we propose a keypoint-segmentation-based tool that accurately classifies and quantifies paw posture using state-of-the-art machine vision algorithms. Our tool infers a virtual skeleton from photographs of paws taken with consumer cameras during routine animal care procedures, avoiding additional discomfort. Using this approach, we demonstrate that injury history can be accurately inferred from the spread angle of the hind paw toes after sciatic nerve injury. Additionally, we show that affected paws regain motor function when the nucleocytoplasmic transport factor protein *kpna4* is depleted in motor neurons following sciatic nerve injury. Conversely, *kpna4* null mice exhibit a reduced opening angle, resembling an age-related decline in toe spread observed in wild-type mice. In paws experiencing acute pain, we detect transient poses that can classify pain states. These findings suggest that static posture metrics, such as toe spread angle, reliably indicate motor function. Beyond injury research, our tool offers a versatile solution for pose estimation in advanced behavioral analysis.

Molecular diversity of adult mouse cerebellar astrocytes

NP 22

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In the mature cerebellum astrocytes with different morphologies occupy distinct cerebellar territories. This enables their classification into 4 distinct types based on their morphology and topographical localization. Such a well-defined classification offers an excellent platform to challenge the concept of astrocyte heterogeneity as well as to attempt cross-correlating the multilevel expression of such diversity.

Through single-cell RNA sequencing of the adult mouse cerebellum, we shed light to an unprecedented degree of transcriptional heterogeneity within cerebellar astrocytes and uncovered the transcriptional profiles of nine distinct astrocyte subtypes, whose regional segregation was unveiled by spatial transcriptomics.

Many of these subtypes showed strong enrichment in one cerebellar layer, enabling us to confirm the correspondence between morphologically and topographically defined cell classes and transcriptional heterogeneity. Of note, among the four identified BG subtypes, two showed complementary distributions along the anteroposterior axis. Some subtypes instead showed enrichment in multiple layers. Among them, a subtype localized in the Purkinje cell layer and in the white matter showed transcriptional features similar to progenitors. Another subgroup of cells, spanning both the cerebellar cortex and white matter, showed an enrichment in terms suggesting their involvement in vesicular gliotransmission and synaptic communication. While these clusters highlighted specialized functional traits, the overall transcriptional heterogeneity among all nine profiles underscored a predominant broad functional divergence between BG and non-BG astrocytes. BG transcriptional signature confirmed their specialization in interacting with Purkinje cells and modulating their synaptic inputs, while the other non-BG astrocytes displayed enrichment for genes that pointed to their novel potential roles in energy homeostasis via regulation of blood supply, glucose and glycogen metabolism and in neuroprotection. These findings set the stage for investigating cerebellar astrocyte heterogeneity in diseased conditions and for comparative studies between mouse and human cerebellum.

Brain metabolic connectivity in ALS due to C9ORF72 hexanucleotide expansion: a 2-[¹⁸F]FDG-PET study

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This study aimed to investigate brain metabolic connectivity, as assessed via 2-[¹⁸F]FDG-PET, in Amyotrophic Lateral Sclerosis (ALS) patients carrying the C9ORF72 expansion (C9-ALS). For this purpose, we compared brain metabolism of C9-ALS and patients without mutations of the main ALS-related genes (ctrl-ALS) through the two-sample t-test model of SPM12 software. Metabolic clusters showing a significant difference between the two groups were used as seed regions for an interregional correlation analysis (IRCA) in each group to evaluate metabolic connectivity. As compared to ctrl-ALS, C9-ALS showed a relative hypometabolism in bilateral thalamus and left precentral and postcentral gyri, and a relative hypermetabolism in bilateral cerebellum and brainstem. In the IRCA, the positive correlation between the thalamic seed region with the cingulate cortex, including its anterior part, resulted broader in C9-ALS than in ctrl-ALS. A negative correlation of the thalamic seed region with sensorimotor cortex was found only in C9-ALS. In the IRCA based on the cerebellar/brainstem cluster, the positive correlation with the seed region substantially represented autocorrelation in both groups. Otherwise, negative correlation, mainly including frontal cortices, resulted more extensive in C9-ALS compared to ctrl-ALS. To conclude, as compared to ctrl-ALS, C9-ALS showed a relatively lower metabolism in the thalami, a relatively higher metabolism in the brainstem and the cerebellum and seemed to show a predominant involvement of the salience network, which is related to cognitive and behavioural control. The cerebellum might be recruited to cope with cognitive impairment to a greater extent in C9-ALS than in ctrl-ALS.

Rubik: a fluorescent reporter to label and manipulate specific neuronal populations

NIM 07

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Genetic manipulation technologies mostly rely on site-specific recombinases to generate conditional transgenic animal models for studying gene functions in specific tissues and time windows. However, detecting and visualizing combinatorial gene expression patterns when multiple recombinases are used is still challenging. To overcome this limitation, we developed Rubik, a fluorescent reporter that monitors Cre and Flp activity by distinguishing all possible recombination outcomes through four distinct fluorophores: blue for Cre, green for Flp, yellow for Cre followed by Flp, and red for Flp followed by Cre. Additionally, the two combinatorial configurations co-express an excitatory or inhibitory optogenetic tool, enabling photo-stimulation or photo-inhibition based on recombinase order, adding functional control over labeled cells. We validated Rubik by generating a stable knock-in HeLa cell line via CRISPR-Cas9. Our results confirmed efficient recombination with either Cre or Flp individually, as well as in combination. To enhance the temporal resolution of recombinase action, we implemented a double-inducible recombination system combining the widely used tamoxifen-inducible Cre recombinase with a newly optimized trimethoprim-inducible Flp recombinase. We triggered specific recombination in Rubik-expressing cells by using both recombinases and their respective drugs. Altogether, Rubik provides a powerful tool for dissecting complex gene expression dynamics. Its application in knock-in mouse models could offer precise anatomical and functional mapping of specific neuronal populations, holding strong potential for neuroscience research and circuit-level investigations in health and disease.

Functional transcranial Doppler (fTCD) neurofeedback for voluntary modulation of cerebral blood flow**NIM 08**[Rosita Rabbito](#)⁽¹⁾ - Caterina Guiot⁽¹⁾ - Silvestro Roatta⁽¹⁾⁽¹⁾ *Università degli Studi di Torino, Dipartimento di Neuroscienze, Torino, Italy*

Functional transcranial Doppler (fTCD) ultrasound allows the detection of cerebral blood flow between hemispheres during mental tasks. This study investigates the effectiveness of real-time neurofeedback in enhancing individuals' ability to modulate lateralization of mental activity. Fourteen participants underwent bilateral monitoring of cerebral blood flow velocity (CBFV) in the middle cerebral arteries during four sessions, each including 15 min of unsupervised training followed by a 15 min test (12 trials of 35 s task – 30 s rest, 6 for each direction). A ball displayed on a screen moved right or left depending on the normalized CBFV difference between hemispheres (LI), thus providing visual feedback of lateralization. Participants were encouraged to explore mental strategies to control the ball's direction freely during training. In the test phase, they followed randomized cues (left/right) for 35 s. The performance was evaluated using receiver operating characteristic (ROC) analysis. The accuracy improved significantly from 0.51 to 0.65 and the area under the ROC increased from 0.55 to 0.69, indicating enhanced lateralization control. To expand the system's potential, we tested a second version of the task in which the ball could also move vertically, according to the sum of the two CBFVs. The goal was to increase the transfer information through the system. Ten participants completed this 4-directions protocol, with shorter training (10 min) and testing sessions (4x4 attempts to move in the different directions for 15 s, with 15-s rest in-between). Preliminary results showed some performance improvement, though less pronounced than in the left/right-only task. This may be due to the increased difficulty of managing four directions instead of two, and to the reduced training time.

Sulcal morphometry and cortical morphology in children and adolescents with different APOE genotypes

NIM 09

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Despite well-documented findings in adults, exploring how APOE genotypes influence brain structure and cognitive function in children is critical to understanding the developmental trajectories that may predispose individuals to later cognitive decline. A total of 10,527 participants with baseline neuroimaging and genetic data were included from the Adolescent Brain Cognitive Development Study. T1-weighted brain images were processed with Freesurfer and BrainVISA framework to extract sulcal width, depth, surface area, and length, for 123 sulci, and cortical thickness and surface area, for 68 regions across the whole brain. We extracted genomic data to determine six possible APOE genotypes using PLINK 2, $\epsilon 2/\epsilon 2$, $\epsilon 3/\epsilon 3$, $\epsilon 4/\epsilon 4$, $\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, and $\epsilon 3/\epsilon 4$. Cognitive performance, including attention, episodic memory, language, and processing speed was evaluated with the NIH Toolbox, a battery validated for children. Across the six APOE genotypes, eight sulcal and eleven cortical metrics differed significantly. Sulcal openings, particularly anterior intralingual, intermediate frontal, and intraparietal sulcus were consistently widest in $\epsilon 2$ carriers and narrowed step-wise with each additional $\epsilon 4$ allele, whereas depth and surface-area were region-specific and often maximised in heterozygotes. $\epsilon 2$ homozygosity tended to decrease thickness in multiple frontal, increase thickness in cingulate, and reduce temporal surface area. Regarding cognitive performance, in $\epsilon 4$ homozygotes, wider anterior intralingual openings were more strongly associated with superior language performance, whereas in $\epsilon 2$ homozygotes, wider fronto-parietal sulci showed stronger negative correlations with executive and reading skills. APOE genotype may shape brain folding and cortical patterns, and sulcal-based metrics emerge as promising neuroimaging markers for early detection of trajectories in individuals who might benefit from early intervention to reduce later-life brain injury and dementia risk.

3

CDKL5 deficiency disorder and gut dysfunctions: exploring the role of Cdkl5 in the organization of the intestinal epithelial barrier**NDV 27**

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disorder characterized by early-onset drug-resistant epileptic seizures, intellectual disability, motor and gastrointestinal (GI) impairment and caused by de novo mutations in the X-linked cyclin-dependent kinase-like 5 (*CDKL5*) gene. Given the high expression of CDKL5 in the brain and the main cognitive deficits of CDD patients, most of the research is focused on the protein role in the central nervous system. Despite 86% of patients reporting GI symptoms, eventually leading to gastrostomy with a great impact on the quality of life, the role of CDKL5 in the gut remains unexplored. To fill this gap, in this study we start to characterize the role of CDKL5 in the context of the intestinal epithelial barrier (IEB) as well as GI tract alteration caused by its absence. One of the well-known functions of CDKL5 is the regulation of microtubules (MTs) dynamics through its interaction with CLIP170, a MT-plus end binding protein, and IQGAP1, a scaffold protein implicated in MT organization and tight junction (TJ) formation. This interaction is essential for the correct generation of apico-basal MT array in polarized epithelial cells, fundamental for maintaining IEB morphology and function. Since we already showed that the CLIP170-IQGAP1 interaction is impaired in *Cdkl5*-silenced HeLa cells, here we show that MDCK cells silenced for CDKL5 present reduced expression of IQGAP1, accompanied by altered morphology, defective paracellular permeability and deregulation of Claudin4, a key component of the TJs. Of note, *in vivo* studies in a constitutive *Cdkl5* knock-out model reveal a reduction in GI length, along with a downregulation of IQGAP1 and E-Cadherin expression in the mucosal layer. Although preliminary, our intriguing results underline the role of CDKL5 in IEB organization, thus paving the way for a better understanding of GI alterations in CDD and giving novel insights into gut-brain axis regulation.

Functional characterization of the InSyn1-CDKL5 interaction for dystrophin/dystroglycan complex dependent inhibitory synapse formation**NDV 28**[Serena Baldin](#) ⁽¹⁾ - Giorgia Valetti ⁽¹⁾ - Giulia Di Marco ⁽¹⁾ - Charlotte Kilstrup-Nielsen ⁽¹⁾ - Isabella Barbiero ⁽¹⁾⁽¹⁾ University of Insubria, Department of Biotechnology and Life Sciences, Center of Neuroscience, Busto Arsizio, Italy

Severe and drug-resistant epileptic seizures are a common feature of an X-linked neurodevelopmental encephalopathy known as CDKL5 deficiency disorder (CDD), a disease that affects predominantly females in a ratio of 1:50 000 live births. The condition is caused by mutations in *CDKL5* gene that encodes for the homonymous protein, a serine-threonine kinase which has been largely demonstrated to regulate excitatory post-synapse stabilization and functioning. Nevertheless, the demonstration of CDKL5 expression in the inhibitory post-synaptic density (iPSD) highlights a potential role for CDKL5 also in this compartment. In this context, we have recently found that the kinase is able to interact *in vivo* and *in vitro* with the newly discovered and poorly characterized protein of the iPSD called inhibitory synaptic protein 1 (InSyn1), implicated in the formation of inhibitory synapses through the interaction with the dystrophin-dystroglycan complex (DGC). The latter was shown to be essential for the innervation of cholecystokinin-positive (CCK⁺) interneurons onto pyramidal neurons. Importantly, our findings in both *Cdkl5*-KO neurons and mice revealed that DGC clusters are altered. This alteration corresponds to a significant reduction in CCK⁺-dependent innervation *in vivo*. We further validated the homology-independent targeted integration (HITI) system for labeling endogenous InSyn1 with an exogenous tag. This system, which circumvents the lack of reliable antibodies, allowed us to better investigate InSyn1 behavior in the absence of CDKL5. Altogether, with this project we are exploring the impact of CDKL5 loss on the inhibitory compartment—an aspect that has been poorly investigated in the context of CDD. This represents an important step forward in understanding the epileptic seizures and cognitive deficits that characterize patients with CDKL5 deficiency disorder.

Negr1: a New Possible Convergent Hub for Autistic Spectrum Disorders**NDV 29**

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Autism spectrum disorders (ASDs) are a group of medical conditions encompassing diverse neurodevelopmental syndromes (e.g. Fragile X) caused by alterations in specific genes. Although all ASDs converge into common core behaviors (i.e. social deficits and repetitive behaviors), genetic variants in ASD are very heterogeneous. Nowadays there is no effective treatment for core behavior impairments in autism, and numerous key mechanisms remain unknown, although preliminary insights have been gained. Here, we describe a cleavable cell-adhesion molecule (Negr1) as causative of core ASD behaviors when up-regulated in Wild type (WT) animals, and we indicate Negr1 as a potential convergent molecule dysregulated in ASD. Indeed, up-regulation of Negr1 in the prefrontal cortex (PFC) was sufficient to induce ASD core behaviors in WT mice and brain morphological deficits. By specific overexpression of the soluble Negr1 form in WT animals we demonstrated that the soluble form of Negr1 is sufficient and necessary to cause social deficits in mice. Supporting the relevance of the latter finding, we also found that Negr1 is up-regulated in the PFC of five diverse mouse models of genetic ASD and in postmortem PFC samples of people with ASD and Fragile X. Altogether, our results on the causal link between Negr1 up-regulation and ASD core behaviors in mice together with Negr1 dysregulation in diverse ASD mouse models may explain how a wide variety of ASD genetic variants converge into a unique core group of impaired processes during brain development and of behavioral phenotypes. Our results also suggest regulation of Negr1 pathway and its cleavage as a new target for the design of future therapeutic approaches.

Targeting myelination deficits in CDKL5-KO mice with positive allosteric modulation of mGlu5 receptors

NDV 30

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Cyclin-dependent kinase-like 5 (CDKL5) gene is highly expressed in forebrain neurons where it encodes for a serine/threonine kinase involved in critical processes such as cytoskeleton dynamic, calcium channel properties, and synapse formation. Therefore, mutations of this gene cause a severe neurodevelopmental condition, CDKL5 Deficiency Disorder (CDD), characterized by early-onset drug-resistant epilepsy, intellectual disability, autistic-like traits, and sensory deficits, predominantly affecting girls. Despite neurons, glial cells also express CDKL5 with higher levels shown by myelinating oligodendrocytes (OLs) and oligodendrocyte precursor cells (OPCs). However, effect of CDKL5 deletion on myelination have been largely ignored. Recently, we reported that CDKL5-KO mice show atypical myelination in cerebral cortex, and its expression is critical for OPC maturation. Here, to investigate the mechanisms underlying CDKL5 regulation of myelination and OL maturation, we crossed *Cdkl5*^{flox} females with either *Emx1*Cre or *Sox10*Cre males to delete *Cdkl5* in forebrain neurons or oligodendroglia, respectively. Our results indicate that neuronal expression of CDKL5 is mostly important for typical myelination to occur. Then, we explored if impaired myelination consequences could be directly observed at the behavioral level by conducting both bar-test and adhesive-tape removal test in which KO mice revealed significant deficits. Finally, we treated both WT and *Cdkl5*-KO adult mice with RO68, a positive allosteric modulator of metabotropic glutamate receptor 5 (mGluR5), as a rescuing strategy. Remarkably, RO68 injection rescued both myelination defects and behavioral abnormalities in mutants. Our data indicate that myelination defects produced by CDKL5 loss are mainly non-cell autonomous and that mGluR5 signaling is crucially involved in these negative effects and represent a therapeutic target to revert myelination defects in CDD and potentially other autism spectrum disorders.

Anticodon-edited tRNAs: a novel strategy to suppress premature stop codons in Rett syndrome

NDV 31

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Mutations that either reduce or increase the function of the X-linked *MECP2* (methyl-CpG-binding protein 2) gene are linked to a group of typically severe neurological disorders. Specifically, *MECP2* deficiency is primarily associated with Rett syndrome (RTT), while *MECP2* gene duplication is the main cause of the *MECP2* duplication syndrome (MDS). Currently, there is no cure for these disorders. Although several studies have demonstrated that reintroducing the wild-type *MECP2* gene can restore normal function in *Mecp2*-deficient mouse models, gene replacement strategies for RTT carry significant risks, particularly due to the potential for overexpression, which can lead to symptoms associated with MDS. As a result, it is critically important to regulate MeCP2 protein levels tightly. In this context, a promising therapeutic approach—specifically for nonsense mutations, which account for approximately 35% of RTT cases—is the use of translational read-through (TR). In particular, the tRNA suppressor strategy involves anticodon-edited tRNAs (ACE-tRNAs) that recognize premature stop codons and insert the corresponding canonical amino acid, enabling the synthesis of the full-length protein. Here we demonstrate that ACE-tRNAs for arginine (ACE-tRNA^{arg}UGA) are restoring full-length MeCP2 levels in HEK cells co-transfected with constructs expressing the R168X/R255X or R270X MeCP2 pathogenic derivatives and ACE-tRNA^{arg}UGA. Notably, no readthrough of canonical STOP codons was observed. We are now investigating the therapeutic efficacy of this strategy in a genomic context using patient-derived iPSCs. First results will be reported.

TBC1D24 regulates presynaptic vesicle acidification and recycling in hippocampal neurons via V-ATPase modulation

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TBC1D24 is a gene mutated in a broad spectrum of neurodevelopmental disorders, from mild epilepsy to severe epileptic encephalopathy. TBC1D24 regulates neuronal development, synaptic vesicle trafficking and synaptic function. This protein uniquely contains the conserved TBC and TLDC domains, the latter of which has been recently linked to interaction with the vacuolar-type ATPase (V-ATPase), a proton pump essential for organelle acidification and neuronal homeostasis. In presynaptic terminals, V-ATPase is crucial for the acidification of synaptic vesicles (SVs), a prerequisite for the maintenance of vesicular pH and proper neurotransmitter loading. However, the contribution of TBC1D24 to V-ATPase function at presynaptic sites remains largely unexplored. To investigate this, we conducted live imaging experiments with Synapto-pHluorin probe to assess SV acidification and recycling at excitatory and inhibitory synapses. Using hippocampal neuronal primary cultures from *Tbc1d24* knockout mice, we performed electrical and chemical stimulation assays to evaluate synaptic vesicle dynamics. We observed a significant impairment in vesicle reacidification and endocytic recovery, indicating that TBC1D24 facilitates synaptic vesicle reacidification, and proper recycling at synaptic boutons. We also observed accumulation of enlarged vesicular structures accompanied by a reduction of total SVs. Future studies will be aimed at understanding the nature of the enlarged structure by comparative proteomics from isolated nerve terminals and determining how TBC1D24 differentially affects excitatory and inhibitory synapses. The project will allow us to better understand how synaptic subtype-specific alterations contribute to the pathophysiology of TBC1D24-related neurological disorders and may guide future therapeutic strategies targeting vesicular pH regulation and synaptic homeostasis.

Extracellular Vesicle-derived miRNAs as non-invasive biomarkers and therapeutic targets for CDKL5 Deficiency Disorder

NDV 33



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Emerging evidence suggests that extracellular vesicles (EVs), membrane-bound nanoparticles released by all cells that transport miRNAs, DNAs, RNAs and proteins, represent a promising source of biomarkers and provide otherwise elusive insights into disease's pathogenesis, including neurological disorders.

Here, we explored this possibility in a severe X-linked neurodevelopmental encephalopathy, CDKL5 deficiency disorder (CDD), that is caused by mutations in the CDKL5 gene and characterized by dramatic neurological dysfunctions. To this aim, we first performed miRNA profiling of salivary EVs from CDD patients (n = 26) and neurotypical controls (n = 30). Of 17 differentially expressed miRNAs (DEmiRNAs) identified, 5 DEmiRNAs are brain-enriched and linked to pathogenic processes such as autism and epilepsy, a result that has been validated in a one-year follow-up study. In parallel, we investigated the role of CDKL5 in EV-mediated cell-to-cell communication by isolating EVs from the culture medium of cortical neurons (NDEVs) of both CDKL5-KO and WT mice. We found that WT neurons treated for 48 hours with CDKL5-KO NDEVs displayed severe deficits in both neurotransmitter release and excitatory synaptic connectivity. Intriguingly, treating CDKL5-KO neurons with WT-NDEVs rescued excitatory synaptic defects shown by these cells. The miRNomic profiling of CDKL5-KO NDEVs highlighted several DEmiRNAs linked to pathways associated with neuronal organization, synaptic development, and function according to GO enrichment analyses. Thus, our findings indicate that EVs-derived miRNAs represent both accessible biomarkers and novel aetiological factors for CDD and disclose for the first time that CDKL5 influences EV-mediated neuron-to-neuron communication.

FLVCR1a Regulates Cortical Interneuron Development and Migration in the Embryonic Cortex

NDV 34



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FLVCR1 (feline leukemia virus subgroup C receptor 1) is a solute carrier protein implicated in choline and ethanolamine transport, highly expressed in the developing brain. Biallelic pathogenic variants in *FLVCR1* are associated with a broad spectrum of neurological disorders, including congenital hydrocephalus, microcephaly, developmental delay, epileptic encephalopathy, and adult-onset neurodegeneration. While *Flvcr1a* deletion in neural progenitor cells (NPCs) disrupts mitochondrial calcium homeostasis and energy metabolism leading to defective neurogenesis and congenital hydrocephalus, this does not fully account for the cognitive symptoms observed in patients.

Here, we explore the contribution of FLVCR1a to cortical interneuron development, as these GABAergic neurons are essential for the formation of neuronal networks. Using a conditional knockout mouse model lacking *Flvcr1a* in NPCs, we observed altered interneuron migration, characterized by reduced interneuron migration rates, shortened leading processes, and aberrant direction changes, along with altered morphology in vitro. Additionally, explants of medial ganglionic eminence (MGE) from mutant embryos displayed migration deficits compared to wild-type littermates.

To dissect the molecular mechanism, we investigated the FLVCR1a protein interactome and identified DOCK7, a Rac1 guanine nucleotide exchange factor, and CRAD, an inhibitor of F-actin capping proteins, as FLVCR1a interactors. Additionally, by using a FRET biosensor, we reported that FLVCR1a modulates Rac1 activity a key regulator of cytoskeletal organization during migration. These findings link FLVCR1a to the regulation of cytoskeletal dynamics and cell polarity in migrating interneurons.

These findings suggest that *FLVCR1a* deficiency disrupts cellular migration and morphology during development, likely through the modulation of cell bioenergetics and cytoskeletal dynamics. This study contributes to a deeper understanding of neurodevelopmental disorders linked to *FLVCR1a* mutations.

Investigating the pacemaking role of Hcn1 during cortical development

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Spontaneous patterns of neuronal activity emerge in the brain as early as the embryonic stages and - together with genetic programs and environmental cues - orchestrate the complex development of the cerebral cortex. Among these patterns, cortical calcium waves, are thought to play a key role in neurogenesis, cell migration, proliferation and maturation.

While pacemaker neurons have been proposed as initiators of these waves, the molecular regulators of this intrinsic activity in development remain poorly understood. Through an integrated single-cell molecular and functional analysis, we identified the voltage gated ion channel Hcn1 as a prominent candidate for modulating intrinsic activity. While HCN1 is known to be involved in the pathogenesis of epilepsy, its role in governing early network dynamics at the subpopulation level during cortical development is not well characterized.

Here, we show that Hcn1 critically shapes the developing cerebral cortex both at morphological and functional level. Using a subtype-specific conditional knockout mouse model, we dissected the contribution of Hcn1 in defining developing cortical neurons identity through transcriptomic and morphological analyses. Two-photon calcium imaging recordings in cortical brain slices, and in vivo from early postnatal mice, reveal that Hcn1 deletion drives severe impairments in early cortical dynamics and structure.

These findings highlight the importance of ion channel composition in defining the fate of developing cortical neuron subtypes as well as their integration in the newly formed cortical networks. Hcn1 emerges as a key determinant of early cortical activity and architecture, potentially contributing to disease when mutated.

Given that mutations in HCN1 channel are implicated in different forms of epilepsies, our study also lays the groundwork for developing precision medicine-based strategies that aim to specifically target ion channelopathies in neurodevelopmental disorders.

NDV 35



Functional characterization of GAT1 variants involved in SLC6A1-related neurodevelopmental disorder: not all mutations act alike

NDV 36



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The excitation/inhibition (E/I) balance is essential for brain function and is regulated by glutamate and γ -aminobutyric acid (GABA) signaling. Extracellular GABA is tightly controlled by transporters, with GAT1—encoded by *SLC6A1* and highly expressed in GABAergic neurons—playing a key role in maintaining E/I balance and controlling neurodevelopment.

SLC6A1-related neurodevelopmental disorder (SLC6A1-NDD) is a rare genetic condition caused by pathogenic variants in the *SLC6A1* gene. This disorder is characterized by developmental delay, motor and cognitive impairment, and epileptic encephalopathy, hypothesizing different phenotypes between variants. Loss-of-function mutations in *SLC6A1*, including over 100 known GAT1 variants, impair protein folding and GABA uptake, disrupting GABAergic signaling and contributing to NDD.

This study investigates the function of five common GAT1 mutants. Functional activity and membrane expression of the GAT1 mutants were assessed by heterologous expression in *Xenopus laevis* oocytes, via cRNA injection, two-electrode voltage clamp recordings, and immunolocalization. Biophysical characterization of mutants reveals that each mutation differentially impacts protein functionality. A288V and A357V exhibit transport efficiencies 75 and 5 times lower than wild type, respectively. The amino acid substitution reduced GABA and increased sodium affinity, suggesting altered ion-substrate coupling. In S295L and A288M GABA transport is completely abolished, while in A305T, GABA is translocated only if present at very high concentration. Immunolocalization confirmed a reduced membrane localization of GAT1 for functional mutants, while the S295L and A288M mutants failed to reach the plasma membrane and were instead retained intracellularly, suggesting defective trafficking.

These findings highlight that each mutation exerts a distinct impact on GAT1 function, underlining the need for mutation-specific approaches in understanding and treating SLC6A1-NDD.

CDKL2 as a potential compensatory kinase for CDKL5: common targets and neuronal functions?

NDV 37

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Mutations in the cyclin-dependent kinase like 5 (*CDKL5*) gene cause CDKL5 deficiency disorder (CDD), a neurodevelopmental condition characterized by early-onset epileptic encephalopathy, intellectual disability, and drug-resistant seizures. No cure exists for CDD patients, and treatment remains mainly symptomatic. Therefore, more studies are needed to develop disease-modifying therapeutic strategies. Recently, the microtubule binding protein EB2, a CDKL5 target, was found to be phosphorylated also by CDKL2, another member of the CDKL family. This suggests that CDKL5 and CDKL2 may share a common molecular network, placing CDKL2 as a potential therapeutic target in CDD. However, since CDKL2 functions in the brain remain poorly understood, we decided to investigate CDKL2 expression levels and its potential roles in this area. Interestingly, we found that CDKL2 expression is upregulated in adult *Cdkl5*-knockout mice compared to the wildtype controls, while no changes were observed in juvenile mice. This suggests a compensatory role of CDKL2 in the absence of CDKL5 during adulthood. To explore the functional overlap between CDKL2 and CDKL5 we considered our recent data showing the interaction of CDKL5 with the postsynaptic inhibitory scaffolding complex, composed of gephyrin and collybistin (CB). Notably, CDKL5 interacts with and activates CB, promoting gephyrin recruitment to the cell membrane. Hence, we investigated whether CDKL2 exerts a similar function. We found that CDKL2, independently of its kinase activity, coprecipitates with CB, suggesting a potential role analogous to that of CDKL5 in CB activation. In conclusion, although preliminary, our results indicate that CDKL2 may play a compensatory role in the absence of CDKL5, sharing components of a molecular network that includes microtubule binding proteins and regulators of neuronal inhibition. Further studies are needed to determine whether CDKL2 can effectively be considered for therapeutic intervention in CDD.

Characterization of astrocyte phenotype during epileptogenesis in a mouse model of acquired epilepsy

NI 21



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Drug-resistant seizures affect about 30% of people with epilepsy who do not respond to anti-seizure medications that target neurons, representing a major clinical challenge. Astrocytes are essential for maintaining brain homeostasis. Increasing evidence supports that deregulation of astrocyte homeostatic functions may impact neurons and contribute to epileptogenesis. However, the temporal and spatial dynamics of changes in astrocyte phenotype during epileptogenesis remain to be elucidated.

We studied specific astrocyte's markers to track their dysfunctional changes during epileptogenesis in an adult mouse model of acquired epilepsy. Status epilepticus (SE) was induced by intra-amygdala kainate, followed after 1 week by spontaneous seizures. SE mice were EEG recorded (24/7) to monitor seizures, and sacrificed at critical times of disease development post-SE: before (24 h, 96 h) and at disease onset (1 week) and during chronic epilepsy (3 months). Controls were saline-injected mice. Brain was processed for quantitative immunohistochemistry/immunofluorescence to examine astrocyte reactivity (GFAP), the glutamate transporter (Glt-1) and cellular senescence markers (β -galactosidase and IL-1 β) in the epileptogenic cortico-hippocampal-thalamic circuit. GFAP immunoreactivity increased in reactive astrocytes with distinct temporal and regional patterns, reflecting their progressive recruitment into the epileptogenic network. Glt-1 expression was reduced in GFAP-positive cells within the network, suggesting impaired glutamate clearance. IL-1 β and β -galactosidase were induced in GFAP-positive astrocytes, as assessed 1 week post-SE, indicating inflammation and cellular senescence.

Data show that astrocytes undergo a shift towards a dysfunctional/senescent phenotype during epileptogenesis, which may play a critical role in hyperexcitability and disease progression. Targeting these astrocyte's dysfunctions may represent a novel strategy to control drug-resistant seizures.

Impairment of spatial memory in chronic CNS inflammation is associated with hippocampal microgliosis and inefficient neuronal recruitment

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Emerging pre-clinical and clinical evidence suggests a critical role of microglia in driving cognitive impairment in conditions of chronic central nervous system (CNS) inflammation, such as multiple sclerosis (MS). However, the precise mechanisms by which microglia influence neuronal activity and cognitive function in MS remain unclear. Here, we investigated the relationship between microglia, neuronal activation, and cognition using the experimental autoimmune encephalomyelitis (EAE) mouse model of MS. We first examined which types of memory were affected by employing a battery of behavioural tests. Following cognitive assessments, we conducted a comprehensive whole-brain histopathology analysis of the CNS using the Aligning Big Brains and Atlases (ABBA) and Brain Analysis (BraiAn) pipelines for automatic multi-marker quantification. We found that working memory was not significantly impaired in EAE mice compared to healthy controls up to 50 days post-immunization (dpi). Recognition memory showed a declining trend in EAE mice, although it did not reach statistical significance relative to healthy controls at 50 dpi. In contrast, testing of spatial reference memory revealed that EAE mice had significantly decreased learning abilities during the training phase and spent significantly less time in the target quadrant at 50 dpi compared to healthy controls. Histological mapping of the whole brains of EAE and healthy control mice revealed increased microgliosis in EAE, particularly within the hippocampal formation, in which EAE mice also displayed significantly lower neuronal c-FOS expression. Further analysis demonstrated an inverse correlation between high microgliosis in the hippocampal region and levels of neuronal activation, which was significantly linked to poorer cognitive performance. In conclusion, our study indicates that chronic inflammation in EAE mice is associated with deficits in spatial reference memory, possibly driven by sustained microglial activation.

Short-term exposure to particulate matter triggers selective molecular and behavioral alterations in a mouse model of Multiple Sclerosis

NI 23



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Multiple Sclerosis (MS) is a chronic disease of the Central Nervous System (CNS), where neuroinflammation and autoimmune response against myelin lead to functional impairments and psychiatric symptoms. Exposure to peaks of air pollution – and in particular of particulate matter (PM) – has been associated with increased hospitalizations for MS onset and relapses, pointing to persons with MS (or predisposed to develop MS) as a PM-vulnerable cohort.

With the aim to disclose the biological substrate of such vulnerability, and to understand whether individuals primed to develop autoimmunity (as it occurs in MS and in the experimental autoimmune encephalomyelitis - EAE - animal model of MS) respond differently to PM, we firstly characterized plasmatic extracellular vesicles (EVs) and their microRNA (miRNA) cargo in healthy and pre-symptomatic EAE mice after exposure to PM10, compared to unexposed healthy and EAE mice. The response of EAE mice to PM10 did not differ in terms of EV number or source compared to that of healthy mice. Remarkable differences existed instead in the identity of deregulated EV-associated miRNAs, which in EAE mice were predicted to target several MS-relevant biological processes and nervous system-, immune- and inflammation-related pathways, possibly contributing to disease worsening.

To validate our findings, we monitored EAE pathological course. PM10 exposure in the pre-symptomatic phase did not modify EAE course, as assessed by clinical and neuropathological analyses. Yet, in PM-exposed EAE mice we observed selective behavioral alterations - i.e. increased disinhibited, risk-taking and novelty-seeking behaviors - compatible with neurotransmitter imbalances linked with the detected EV-packaged miRNA deregulation.

Overall, these data show a selective vulnerability of immunologically primed mice toward the effects of PM, detected at molecular and behavioral levels as early as in the pre-symptomatic stages of the pathology.

Activation of Somatostatin-Expressing AgRP Neurons contribute to the pathogenesis of experimental autoimmune encephalomyelitis

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The hypothalamus regulates homeostatic functions, including sleep, energy metabolism and hematopoiesis, the process of maturation of immune cells in the bone marrow (BM). In multiple sclerosis (MS) these processes, in particular hematopoiesis, are altered. We have previously demonstrated that Agouti-related peptide (AgRP) neurons within the hypothalamus regulate myeloid hematopoiesis in physiological conditions and are over-activated in experimental autoimmune encephalomyelitis (EAE). The aim of this study was to understand whether over-activation of AgRP neurons is relevant for the development of EAE and to characterize activated AgRP neurons. To assess the contribution of AgRP neurons activation on EAE we induced the disease in transgenic mice in which AgRP neurons can be selectively inhibited by administration of CNO. To characterize AgRP neurons activated in EAE at single cell level, we performed a RNA-scope analysis of the hypothalamus. Inhibition of AgRP neurons through daily administration of CNO restored the normal activity of AgRP neurons, their connectivity within the hypothalamus and ameliorated the EAE course. A flow cytometry analysis of the skull BM revealed that these neuronal changes were associated with a reduced frequency of myeloid cells, particularly of neutrophils. A single cell analysis of 3000 AgRP neurons revealed that a specific sub-population characterized by the expression of somatostatin (SST) is strongly and specifically activated in EAE. Most of these neurons lie within a defined region of the arcuate nucleus, very close to the median eminence (ME), a circumventricular organ with a specialized blood-brain barrier that allows access to peripheral signals. Our data demonstrate for the first time that the over-activation of SST-positive AgRP neurons contribute to the pathogenesis of EAE. The localization of these neurons close to the ME supports that activation of AgRP neurons in EAE is induced by peripheral signals.

Minimally Invasive Neuromodulation: Biocompatibility Assessment of Wireless Sub-100- μ m μ Bots in the Mouse Hippocampus

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Neurological disorders affect over a billion people globally, with ictal conditions—such as epileptic seizures, panic attacks, and psychosis—posing therapeutic challenges due to their unpredictable and patient-specific nature. A critical barrier for chronic neurotechnology is the Foreign Body Response (FBR), which impairs device performance and biocompatibility. The CROSS-BRAIN project addresses this issue developing fully implantable wireless microdevices (μ Bots, 100 μ m per side), designed to reduce FBR and enabling adaptive neuromodulation at the single-neuron scale.

To assess the biocompatibility of 100- μ m neural implants, we evaluated the brain's FBR to silicon dummy μ Bots chronically implanted in the hippocampal CA1 region of wild-type mice. Devices were released using a PEG-assisted tungsten shuttle, and accurate positioning was confirmed via synchrotron-based X-ray phase-contrast tomography and micro-CT imaging.

In acute experiments (3 mice), no hemorrhages were observed (0/3), suggesting minimal mechanical damage. In chronic conditions (2-week implantation, 3 mice), immunofluorescence was used to evaluate microglial (Iba1), astrocytic (GFAP), and neuronal (NeuN) responses. Around the μ Bot, GFAP and Iba1 remained at baseline, suggesting negligible glial activation. In contrast, increased levels of both markers were found at the entry site, likely due to insertion-related effects. NeuN staining revealed no significant neuronal loss in either region.

These results indicate that miniaturized implants can be chronically tolerated in deep brain regions, with minimal immune activation and preserved neuronal integrity. The absence of skull-mounted components further enhances the minimally invasive nature of this strategy. The promising biocompatibility and precise implantation of μ Bots support their potential for chronic personalized neuromodulation therapies. Ongoing studies will extend implantation periods (e.g., 2 months) to assess long-term immune dynamics and tissue integration.

Sex differences in CSF levels of FGF-2 and Anosmi-1 as novel bio-signatures in Multiple Sclerosis progression.

NI 26



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Background: Relapsing-Remitting Multiple Sclerosis (RRMS) is a chronic demyelinating disorder of the Central Nervous System with a higher prevalence in females. The disease frequently begins with a clinically isolated syndrome (CIS) which can evolve to a clinically definite MS. Fibroblast Growth Factor-2 (FGF-2), a pleiotropic mitogenic and angiogenic protein, has been implicated in spontaneous remyelination. Anosmi-1, a protein involved in axonal guidance, neural migration, different aspects of oligodendroglialogenesis and overexpressed in brain human samples of MS, may also reflect inflammatory processes in MS.

Objectives: To evaluate whether cerebrospinal fluid (CSF) levels of FGF-2 and Anosmin-1 (particularly its 55 kDa band) can serve as biomarkers for disease evolution and inflammatory activity in CIS and RRMS and correlate with clinical clustering.

Methods: We analysed CSF samples from male and female patients with CIS (either converting to clinically definite MS [CIS-CDMS] or not [CIS-CIS]), RRMS, and RRMS in relapse (RRMS-R). Levels of FGF-2 (ELISA) and Anosmin-1 (Western Blot) were quantified. Dimensionality reduction via UMAP was used to explore clustering of biomarker profiles based on several demographic and paraclinical categorical parameters (Sex, Disease Stage, MRI, Oligoclonal Bands -OCB-).

Results: In women, higher FGF-2 and Anosmin-1 55 kDa levels distinguished CIS-CDMS from CIS-CIS cases. FGF-2 levels correlated with shorter time to conversion and higher hazard ratios, supporting its role as a predictor of disease progression. Anosmin-1 55 kDa also delineated a pro-inflammatory profile associated with conversion.

Anosmin-1 consistently increased during relapse in both sexes, suggesting its association with inflammatory states. In men, Anosmin-1 levels were elevated during peri-relapse (CIS-CIS, CIS-CDMS) and relapse (RRMS-R) phases but did not correlate with conversion. Its low levels in the stable phase of the disease (RRMS) could be regarded as a putative remission signature.

UMAP analysis revealed sex as the primary driver of biomarker clustering, reinforcing the role of sexual dimorphism in MS pathogenesis. Combined disease and sex classification (DiseaseMW) enhanced data stratification.

Conclusions: These results position FGF-2 and Anosmin-1 55 kDa as promising sex-specific biomarkers in MS: predictive of conversion in women and reflective of relapse dynamics in both sexes. Additionally, in men Anosmin-1 may act as a marker of remission or as shows increased levels only in (peri)inflammatory phases. Our findings reinforce the critical need for sex-stratified biomarker frameworks in early MS.

C3 and CD14 modulate diffuse but not focal neuroinflammation in TBI associated with polytrauma

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Traumatic brain injury (TBI), often embedded within polytrauma involving thoracic insult, skeletal fractures, and hemorrhagic shock, initiates multifaceted neuroinflammatory responses. Employing a murine polytrauma model integrating all aforementioned components, we interrogated early molecular events within distinct neuroanatomical compartments. Particular focus was given to complement component C3 and the TLR co-receptor CD14, both central to innate immune surveillance and DAMP-mediated signaling. A cytokine array performed 3 hours post-injury revealed robust upregulation of TNF- α , subsequently corroborated by quantitative PCR and in situ hybridization. Notably, TNF- α expression transcended focal injury loci, encompassing contralateral and diffuse cortical regions.

Genetic ablation of C3 and/or CD14 led to significant attenuation of TNF- α and CCL2 transcription specifically in diffuse, but not focal, domains, suggesting region-specific immunomodulatory functions. In situ hybridization localized TNF- α mRNA proximal to cerebral vasculature, as well as in astrocytes and microglia, implicating the neurovascular interface in early cytokine propagation. Moreover, the KO of C3 and CD14 caused a strong activation of astrocytes and a deactivation of microglia, suggesting a partial role of the complement pathway in focal immunity regulation. Astrocytic activation, as evidenced by increased GFAP mRNA, was prominent in both focal and diffuse areas post-injury; however, this was selectively suppressed in diffuse zones of C3/CD14-deficient mice. Concurrently, anti-inflammatory astrocytic markers Emp1 and S100A10 were selectively upregulated in knockout models, indicative of a phenotypic transition. These data reveal a spatial dichotomy in neuroimmune responses post-polytrauma and highlight C3 and CD14 as key effectors of diffuse neuroinflammation. The targeted inhibition may represent a rational therapeutic strategy to mitigate secondary cerebral injury and improve outcomes in polytrauma-associated TBI.

NI 27



EGFR activation correlates with intracranial pressure and survival in a mixed intracranial bleeding porcine model

NI 28

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The pig model is an advanced system for studying human brain trauma due to its anatomical similarities with the human brain, such as gyrencephalic structure and white-to-gray matter ratio. Ischemia, a feature in acute intracranial hemorrhage cases, occurs when brain tissue compression obstructs vasculature, reducing cerebral blood flow. This study investigates the role of receptor tyrosine kinases in the injury response and clinical outcomes, focusing on their potential as targets for edema and reperfusion control after injury. We developed a sustained, resuscitated pig model of acute mixed intracranial hemorrhage with ICP, providing a robust system for examination of brain injury. Multimodal brain monitoring and neurological assessments offered valuable insights into the progression. Macroscopic postmortem analysis, transcriptional evaluations combined with WB and protein arrays, allowed the assessment of RTK pathway activation. Our findings showed that 44-54 hours post-injury, animals exhibited signs of hypoxia, neuroinflammation, and extensive tissue damage. Elevated HIF1- α expression in the ipsilateral hemisphere confirmed local hypoperfusion. Inflammatory markers such as TNF- α , CD68, and MMP-9 were upregulated in both hemispheres, reflecting a generalized neuroinflammatory response. Gene expression analysis revealed increased markers of vascular, astrocytic, and neuroimmune activation, particularly related to endothelial integrity and astrocyte activation. RTK expression analysis showed increased levels of VEGFR1, VEGFR2, Tie-2, EGFR, and Axl in the injured cortex, with activation of EGFR/ErbB4 and HGFR/Met pathways. Hierarchical clustering of astrocytic markers revealed distinct patterns of activation, highlighting the relationship between ICP severity and astrocyte response. Elevated EGFR phosphorylation correlated with astrocyte activation and ICP severity, survival, and Glasgow Coma Scale outcomes. These findings suggest that modulating EGFR signaling may offer a therapeutic approach for managing ICP and improving outcomes in brain injury.

Interleukin-9 Modulates Microglial Activation and Inflammatory Signaling in an Alzheimer's Disease Mouse Model

NI 29

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Interleukin-9 (IL-9) is a pleiotropic cytokine primarily known for its role in immune regulation, particularly in allergic inflammation and T cell-mediated responses. Recent studies have begun to explore the involvement of interleukin-9 (IL-9) in neuroinflammation and neurodegeneration, particularly in disorders such as amyotrophic lateral sclerosis (ALS), yet its role in Alzheimer's disease (AD) remains largely unexplored. In a recent *in vivo* study, we demonstrated that chronic administration of palmitoylethanolamide (PEA) in TG2576 transgenic mice, a well-established AD model, not only exerted neuroprotective effects but also led to a significant increase in hippocampal IL-9 levels. Based on these findings, the present study aims to investigate the direct role of IL-9 in modulating microglial activation in the context of neuroinflammation. To this end, primary microglial cells were isolated from both wild-type (WT) and TG2576 mice, chronically treated with IL-9, and subsequently challenged with lipopolysaccharide (LPS). Flow cytometric and gene expression analyses revealed that IL-9 reduced the expression of the activation markers CD40 and CD86 in both WT and TG microglia. Additionally, IL-9 downregulated the gene expression of toll-like receptor 4 (TLR4), the primary receptor for LPS, suggesting an attenuation of pro-inflammatory signaling pathways. Interestingly, IL-9 treatment upregulated GPR18 expression, a receptor involved in inflammation resolution, in both genotypes. Notably, an increase in CX3CR1 expression, the receptor for fractalkine implicated in neuron–microglia communication, was observed only in TG microglia. These findings provide novel evidence for the immunomodulatory properties of IL-9 in AD-related neuroinflammation and suggest that this cytokine may contribute to reshaping microglial responses in a genotype-dependent manner. Further investigation is warranted to clarify IL-9's potential as a therapeutic target in Alzheimer's disease.

Investigating the Impact of Chronic Palmitoylethanolamide on Microglial Immunophenotype and Functional Responses in Neuroinflammatory Contexts

NI 30

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Neuroinflammation plays a crucial role in the pathophysiology of Alzheimer's disease (AD), with microglial activation being a central component in the progression of neurodegeneration. Palmitoylethanolamide (PEA), an endogenous lipid mediator, has shown anti-inflammatory and neuroprotective properties in various models of central nervous system disorders. This study investigates the *in vitro* effects of chronic PEA treatment on microglial cells isolated from both wild-type (WT) and TG2576 transgenic (TG) mice, a model of Alzheimer's disease. Building on our previous findings demonstrating the beneficial role of PEA in modulating microglial morphology *in vivo* specifically promoting a less ramified phenotype in TG mice, we sought to explore the molecular and immunological impact of PEA under inflammatory conditions. Cells underwent a chronic PEA treatment followed by LPS stimulation. Flow cytometric analysis of the immunophenotype revealed that PEA significantly reduced CD40 and CD86 expression in WT microglia, whereas in TG cells only CD40 expression was downregulated. PEA also modulated phagocytic activity in WT microglia and selectively enhanced the gene expression of GPR18, a receptor implicated in inflammation resolution, exclusively in the WT group. Notably, PEA upregulated the expression of CX3CR1, the fractalkine receptor, in both WT and TG microglia, indicating a potential role in maintaining neuron-microglia communication. Additionally, PEA downregulated the expression of TLR4, the LPS receptor, in both genotypes, suggesting a broader anti-inflammatory effect. In conclusion, our data support the immunomodulatory potential of PEA on microglial activation states, with differential effects depending on the genotype. These findings underline the relevance of PEA in modulating microglial responses and inflammatory pathways, highlighting its therapeutic promise for neuroinflammatory and neurodegenerative conditions such as Alzheimer's disease.

Integrating different approaches for establishing a multi scale functional validation platform for RNA-based drugs in the CNS (MULTIVAL)

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The blood–brain barrier (BBB) is a major obstacle to delivering RNA-based therapeutics to the central nervous system. Functionalized lipoplexes have emerged as promising carriers capable of exploiting adsorptive- and receptor-mediated transcytosis to traverse the BBB. Within the MULTIVAL platform - Spoke 9 at the National Center for Gene Therapy and RNA Technology (CN3) - we evaluated the transport efficiency and underlying mechanisms of DPPC–Cholesterol liposomes across an in vitro BBB model.

Different cationic DPPC–Cholesterol liposomes were functionalized and labeled with a NBD fluorophore. Uptake kinetics were first screened in primary murine brain microvascular endothelial cells (BMECs) at 37 °C versus 4 °C to distinguish energy-dependent endocytosis from passive diffusion. Next, we assessed liposomes' internalization by resveratrol insertion in a static transwell configuration, quantifying it both by HPLC and fluorescence measurements. Finally, potential transcytosis pathways were probed via immunofluorescence colocalization studies of clathrin and caveolin.

Different DPPC–Chol liposomes exhibited robust uptake by BMECs at 37 °C, with negligible internalization at 4 °C, confirming an active, endocytosis-mediated mechanism. In the static transwell system, functionalized liposomes successfully traversed the endothelial monolayer without compromising barrier integrity.

Our in vitro results demonstrate that DPPC–Cholesterol-based liposomes could mediate energy-dependent transport of RNA cargo across BBB models. This platform offers a robust preclinical screening tool to optimize lipoplex composition and functionalization for the development of CNS-targeted RNA therapeutics.

Mutant TAU (P301L) enhances global protein translation in differentiated SH-SY5Y cells**NDG 44**Giovanni Luca Cipriano ⁽¹⁾ - Alessia Floramo ⁽¹⁾ - Veronica Argento ⁽¹⁾ - Salvatore Oddo ⁽²⁾ - [Osvaldo Artimagnella](#) ⁽¹⁾⁽¹⁾ IRCCS, IRCCS Centro Neurolesi Bonino-Pulejo, Messina, Italy - ⁽²⁾ University of Messina, Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Messina, Italy

Alterations in the rate and fidelity of messenger RNA (mRNA) translation have been linked to aging and various neurodegenerative diseases. The intracellular accumulation of hyperphosphorylated Tau protein is a hallmark of Alzheimer's disease (AD) and other tauopathies. Recent studies have shown that Tau interacts with ribosomal proteins, influencing translational selectivity. Interestingly, under pathological conditions, Tau has been reported to both suppress and enhance global translation, reducing the process in adult mice, though increasing it at early disease stages in *Drosophila*. To investigate the potential impact of TAU on translation in human neurons, we employed an *in vitro* model of AD using retinoic acid (RA)-differentiated SH-SY5Y cells overexpressing a mutant form of TAU (P301L; SH-TAU) compared to wild-type cells (SH-wt). We assessed global protein synthesis by measuring the rate of incorporation of puromycin on nascent proteome via Western blot assay. Our results showed that SH-TAU cells exhibited increased puromycin incorporation relative to SH-wt cells, indicating enhanced global translation driven by pathological TAU. To explore the underlying molecular mechanisms, we investigated the involvement of the AKT/mTOR signaling pathway. Notably, pharmacological inhibition of mTOR with rapamycin partially reversed the translation increase observed in SH-TAU cells. Given that dysregulated translation can affect critical processes such as synaptic plasticity and memory formation, our findings suggest that Tau-driven translational alterations may directly contribute to disease progression in *tauopathies*, highlighting novel avenues for therapeutic intervention.

Neuronal Loss of the Alzheimer's disease Risk Factor CALM Perturbs Synaptic Plasticity and Exacerbates Amyloid Pathology

NDG 45

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AMPA-type glutamate receptors (AMPA) mediate fast excitatory neurotransmission, and their surface levels play a crucial role in determining synaptic strength during learning. However, these levels are often altered in neurological disorders, including Alzheimer's disease (AD). We previously found that CALM, a highly validated genetic risk factor for Alzheimer's disease, regulates synaptic plasticity by selectively facilitating the endocytosis of ubiquitinated GluA1-homomeric Ca²⁺-permeable (CP) AMPARs. This mechanism relies on ubiquitin recognition, but is independent of clathrin. Here, using a combination of in vitro amyloid beta (Aβ) aggregation assays, mouse genetics, electrophysiological recordings and proteomics, we report that CALM directly bind Aβ42 preventing its aggregation. Moreover, we show that neuronal loss of CALM leads to increased levels of Aβ species, accelerates Aβ pathology in an AD mouse model, and impairs neurotransmission. Finally, TMT-labelling mass spectrometry elucidates the proteomic changes at synaptic resolution, shedding light on the role of CALM during AD progression.

Exploring Cognitive and Behavioral Reactions to Monoclonal Anti- β -Amyloid Antibody Treatment in Wild-Type Mice

NDG 46

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Alzheimer's disease (AD) is one of the leading causes of dementia and mortality worldwide, with its prevalence projected to triple every 20 years due to increased life expectancy (1). By 2030, the global cost of AD is expected to exceed \$2 trillion, placing a heavy burden on healthcare systems. Since treatment in late stages is complex and resource-intensive, early detection and intervention are essential. Among emerging therapeutic strategies, monoclonal antibody (mAb) treatments targeting soluble aggregated forms of beta-amyloid (A β), particularly protofibrils, have shown promising potential in modifying disease progression. AD is characterized by cognitive and behavioral impairments, including deficits in memory, learning, emotional regulation, and judgment, as well as psychotic and affective symptoms such as hallucinations, agitation, and apathy (2,3). In this study, we assessed the baseline cognitive and behavioral profiles of wild-type (WT) mice treated with either monoclonal antibodies or vehicle, at different ages (3-6 and 12 months old). The purpose is to evaluate the effects of the treatment in healthy animals, providing a foundational comparison for future studies in transgenic AD models.

A battery of behavioral tests was used, including: Novel Object Recognition (NOR) to evaluate recognition memory, Y-Maze Test to assess spatial working and reference memory, Light-Dark Box Test (LD) to measure anxiety-like behavior, and Fear Conditioning to investigate associative learning. These results will contribute to understanding the baseline behavioral impact of monoclonal antibody treatments and support the design of future preclinical studies in pathological models of Alzheimer's disease (4,5).

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α -Synuclein/ α -Tubulin interplay in Parkinson's disease**NDG 47**

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Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder, primarily characterized by the loss of dopaminergic neurons in the *substantia nigra* and the formation of α -Synuclein (α -Syn)-positive cytoplasmic inclusions, termed Lewy bodies. Since its identification as the first autosomal dominant gene for PD, α -Syn is known to play a key role in PD's pathogenesis. Particularly relevant is its interplay with acetylated- α -tubulin, a post-translationally modified major microtubule component linked to microtubule physical properties. Defective regulation of α -tubulin acetylation emerged to be related to α -Syn pathology *in vitro* and *in vivo*. Focusing on *post-mortem* human brains of PD patients at late stage of pathology (Braak stage 6), we have already described in *substantia nigra* that changes in acetylated α -tubulin localization are linked with the formation of α -Syn aggregates, suggesting that α -tubulin acetylation could trigger α -Syn aggregation. The overall role of acetylated α -tubulin in the disease is further supported by the increase of acetylated- α -tubulin positive neurons we found also in the Inferior Olivary Nucleus, a brainstem region not affected by α -Syn pathology but whose involvement in PD is emerging. To further investigate α -Syn/acetylated- α -tubulin interplay during PD progression, we moved to the SYN120 tg mice, a PD mouse model which expresses a mutant, truncated form of α -Syn under the rat tyrosine hydroxylase promoter and is characterized by the formation of pathological inclusions. Thanks to immunofluorescence, Proximity Ligation Assay, and confocal analyses, we are describing the evolution of α -Syn/acetylated- α -tubulin interplay in mice at early and late stages of the diseases. Our data supports the idea that changes in acetylated α -tubulin are involved in PD and are linked to α -Syn pathology.

This work was supported by "Fondazione Pezzoli per la Malattia di Parkinson" ("5x1000" funding).

Development of a chemically aged cerebral organoid model to investigate the pathogenesis of fatal familial insomnia

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Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N-129M *PRNP* mutation. The disease manifests at approximately 50 years of age and progresses rapidly, with more than 50% of patients dying within one year from onset.

To investigate the pathological mechanisms and to reproduce the conditions observed in patients, scientists have recently turned to iPSCs to generate human cerebral organoids (hCOs), which consist of self-organizing, neuronal lineage tissue, which effectively replicates the human cerebral environment. However, so far, hCOs modelling FFI only displayed subtle abnormalities, such as altered neurotransmitter levels, astrogliosis, and oxidative stress, lacking key pathological features, particularly the conversion of mutant PrP into a self-replicating prion isoform. To overcome these limitations, we aim to develop a more accurate and reliable iPSC-derived hCO model to investigate FFI pathogenesis.

We collected peripheral blood mononuclear cells from FFI mutation carriers and non-carrier relatives, and obtained 4 FFI iPSC lines, 3 iPSC lines from non-carrier individuals, and 2 CRISPR-Cas9 corrected lines, which served as rigorous co-isogenic controls. iPSCs were induced towards neural cell fate to produce neural progenitor cells, which were utilized to generate hCOs. To closely replicate adult brain characteristics and model the neurodegenerative condition typical of FFI patients, hCOs underwent chemically induced aging using the neddylation inhibitor Pevonedistat, a small molecule that has been shown to induce multiple hallmarks of aging in neurons. hCOs are being analysed at different time points for the presence of pathological PrP by histological and biochemical methods, including assays of detergent-insolubility, protease-resistance and seeding competency.

Endocannabinoid-driven modulation of progranulin expression in a model of senescent Tg2576-derived microglia

NDG 49

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Senescent microglia exhibit a chronic pro-inflammatory profile and impaired lysosomal clearance, contributing to neuroinflammation and age-related brain dysfunction. Progranulin (PGRN), a glycoprotein involved in lysosomal homeostasis and inflammation modulation, is downregulated in senescent microglia, correlating with intracellular accumulation of undegraded substrates such as lipofuscin granules.

In a model of stress-induced microglial senescence using primary cells from wild-type and Tg2576 mice, we observed a consistent reduction in PGRN expression. Notably, several PGRN-related receptors and trafficking molecules also showed altered expression, suggesting a broader disruption of the progranulin signaling axis.

Treatment with URB597, a selective fatty acid amide hydrolase (FAAH) inhibitor that enhances endocannabinoid tone, counteracted the modulation of senescence markers and restored PGRN expression in transgenic microglia. This molecular improvement was accompanied by reduced lipofuscin accumulation, suggesting a functional recovery of lysosomal degradation capacity.

Taken together, our findings indicate that enhancing endogenous anandamide signaling via URB597 can re-establish progranulin expression and improve cellular clearance mechanisms. These results support the existence of a functional link between endocannabinoid tone and the PGRN-lysosome axis, with potential implications for limiting age-associated neuroinflammatory phenotypes.

Evaluation of 6 monoclonal antibodies against Ser129-phosphorylated α -synuclein: Critical role of proteinase K antigen retrieval and superior sensitivity of the D1R1R clone in human skin biopsies

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Synucleinopathies, including Parkinson's disease (PD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), are characterized by intracellular inclusions of misfolded α -synuclein. The phosphorylation of α -synuclein at serine 129 (pS129- α -syn), found in ~90% of pathological inclusions versus ~4% in its native form, represents a promising biomarker. Skin biopsy has emerged as a minimally invasive tool for in vivo detection of pathological aggregates via immunofluorescence, especially in autonomic nerve fibers. However, antibody selection and protocol standardization remain critical challenges. This study evaluated six monoclonal antibodies (EP1536Y, 81A, D1R1R, J18, BBF19, pSyn#64) using three antigen retrieval methods (none, 30% formic acid, and proteinase K) on skin biopsies from 43 patients with confirmed synucleinopathies (including 7 MSA) and 33 controls. Each sample was double-stained with PGP9.5 and scored blindly by two reviewers. Antibodies J18, BBF19, and pSyn#64 showed poor sensitivity and specificity across protocols. EP1536Y and 81A showed variable sensitivity, with 81A outperforming EP1536Y in several structures without PK. Proteinase K markedly enhanced detection, particularly in autonomic plexuses (+102% for EP1536Y, +104.6% for 81A). D1R1R combined with PK showed the highest overall sensitivity, exceeding EP1536Y+PK in plexuses (+29.03%), vessels (+21.95%), and fibers (+33.66%). These findings highlight the importance of selecting appropriate antibodies and retrieval methods. D1R1R+PK emerges as the most effective combination, supporting its use in standardized diagnostic workflows for reliable detection of pathological pS129- α -syn in skin biopsies.

Comparison between D-loop methylation level and mtDNA copy number in Duchenne and Becker muscular Dystrophies patients

NDG 51

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Muscular Dystrophies (MD) are a group of genetic disorders characterized by progressive muscle weakness and damage. The most common cause of MD is mutations in *DMD* gene, which encodes dystrophin. Duchenne muscular dystrophy (DMD) typically involves mutations that disrupt the protein reading frame, while Becker muscular dystrophy (BMD) is linked to in-frame deletions that preserve the reading frame. Mitochondria play a critical role in muscle function by producing ATP, and in DMD, muscle energy stores are severely affected. Epigenetic mechanisms, such as DNA methylation, regulate mitochondrial DNA (mtDNA) and contribute to various physiological processes, including mitochondrial function and inflammation. The term “mitoepigenetics” has been introduced to describe the role of epigenetic changes in controlling mtDNA transcription and replication. This study aimed to investigate mtDNA alterations, including methylation and copy number, in DMD and BMD patients. We compared the methylation profile of D-loop regulatory region and mtDNA copy number in 24 muscle biopsies (6 BMD, 12 DMD, and 6 controls) and 67 additional blood samples (18 BMD, 28 DMD, 21 controls). Methylation levels were measured by pyrosequencing analysis, and mtDNA copy number was assessed by RT-qPCR. Results showed lower D-loop methylation levels in muscle biopsies of DMD and BMD patients compared to controls, with opposite trend in blood samples. mtDNA copy number was significantly reduced in patients compared to controls in both tissues. These findings suggest differences in methylation and mtDNA copy number between patients and controls, offering insights into potential mechanisms of mitochondrial dysfunction and epigenetic control in MD.

Discovering MAP1B: a new phenotypic determinant in sporadic ALS patients**NDG 52**

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Amyotrophic Lateral Sclerosis (ALS) is a complex disease with strong variability. Among sporadic patients, 70% develop spinal form (sALS), causing muscle weakness, atrophy, and 30% bulbar form (bALS), affecting swallowing and speech muscles. Our aim was to perform two RNA-seq studies on total RNAs and miRNAs in peripheral blood mononuclear cells (PBMCs) from 40 sALS patients, 10 bALS patients and 19 healthy controls (HC). We performed computational research for DE miRNAs' target and crossed results with DE genes from RNA-seq. We identified 3 DE miRNAs, downregulated, which had their target among DE genes emerged. We evidenced a single target gene (MAP1B), upregulated, common to the 3 miRNAs, largely involved in neuronal maturation, differentiation and survival. Only targets of miR-92a-3p revealed involvement in muscle contraction. The same miRNA targets TDP-43, largely involved in ALS pathogenesis. MAP1B is a component of Lewy Bodies, identified in bALS. Deregulation of miR-92a-3p and MAP1B may cause protein accumulation in motor neurons (MNs), leading to progressively faster MNs degeneration in sporadic ALS phenotype. Validation of results showed an increasing MAP1B expression which start with the lowest level detected in HCs and progressively increase from sALS to bALS, which had the highest expression of MAP1B. Same increasing trend was observed in a follow-up cohort of both phenotypes, after 1 and 2 years from the diagnosis. Same results were obtained on Rat model, which recapitulate sALS phenotype from subclinal form to end-stage, to bALS phenotype. We also validated the impact of miR-92a-3p and MAP1B expression in SH-SY5Y and neurons, showing the reduction of MAP1B as consequence of miRNA O/E and ectopic accumulation as consequence of MAP1B overexpression. MAP1B arises as a new factor that may explain different progression rate and severity degree in patients who do not share common genetic background.

Cerebral organoids as a model of FrontoTemporal Dementia (FTD)**NDG 53****Clelia Ferrero** ⁽¹⁾ - Paolo Pacca ⁽²⁾ - Alessandro Vercelli ⁽²⁾ - Marina Boido ⁽²⁾

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Frontotemporal Dementia (FTD) is a progressive neurodegenerative disorder involving the degeneration of the frontal and temporal lobes, leading to severe impairments in personality, behavior, and language. We are investigating the potential of cerebral organoids derived from human induced pluripotent stem cells (hiPSCs) to model FTD pathophysiology, with a focus on the C9ORF72 mutation—one of the most common genetic causes of both FTD and amyotrophic lateral sclerosis. Organoids were generated from hiPSCs obtained from both healthy individuals and FTD patients. Their development was analyzed at 30, 60, 90 and 120 days in vitro (DIV) through morphometric analysis and immunohistochemical staining of key neural markers. SOX2 and Ki67 were used to assess stemness and proliferation, PAX6 as a neural progenitor marker, and TBR1, CTIP2, SATB2, and MAP2 to evaluate neuronal differentiation and cortical organization. Preliminary results demonstrate time-dependent organoid growth, with diameters increasing from ~400 µm at early stages to over 2 mm by day 90. Marker expression analysis reveals a progressive shift from progenitor to neuronal identity. In both healthy and FTD organoids, SOX2+ cells expression declined from ~50% to 30%, and Ki67 from ~30% to under 10%, indicating reduced proliferation over time. SATB2 expression, associated with upper-layer cortical neurons, increased significantly between day 60 and 90, reaching ~30% in controls at 90 days. Notably, SATB2 expression is absent in FTD organoids, suggesting disrupted cortical layer formation. Although preliminary, this study highlights the value of cerebral organoids as a relevant and dynamic model for investigating FTD mechanisms and for developing therapeutic strategies, offering a promising alternative to traditional animal models.

Increased levels of 24-hydroxycholesterol reduce okadaic acid-induced tau hyperphosphorylation and neuronal morphological degeneration

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Multiple findings underline a link between altered brain cholesterol metabolism and Alzheimer's disease (AD) pathogenesis. Physiologically, excess brain cholesterol is mainly converted into 24-hydroxycholesterol (24-OHC) by the neuron-specific enzyme CYP46A1. In a previous study, we observed in autopsy specimens from human AD brains that 24-OHC and, in parallel, CYP46A1 expression decrease at advanced stages, suggesting a possible cause-and-effect relationship between these reductions and AD progression. Based on this data, we recently investigated whether maintaining high levels of 24-OHC, by its exogenous administration or CYP46A1 overexpression, can counteract tau hyperphosphorylation and accumulation of prefibrillar tau oligomers. To create an AD-like *in vitro* model exhibiting tauopathy, we utilized okadaic acid (OKA), a chemical compound that induces tau hyperphosphorylation. Our data show that in 24-OHC-treated primary neurons derived from wild-type mice, and in neurons from CYP46A1 overexpressing mice (CYP46Tg), elevated oxysterol levels effectively prevented tau hyperphosphorylation and oligomerization. Furthermore, the decrease in dendritic arborization induced by OKA was prevented, maintaining the organization and stability of the neuronal cytoskeleton. While hypothesized underlying molecular mechanisms (GSK3 β , CDK5, ERK1/2, and PP2A) did not appear to be involved, the protective effect of 24-OHC remains evident. The data highlight the positive impacts of 24-OHC and the need to prevent its reduction in the brain. This can be achieved either through the exogenous administration of 24-OHC using suitable technologies or by maintaining elevated levels and the activity of the enzyme CYP46A1. These therapeutic approaches could be useful to prevent or slow AD progression.

Cortical Dendritic Remodeling in a Murine Model of Oxaliplatin-Induced Neurotoxicity

NDG 55

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Oxaliplatin (OHP) is a platinum-based chemotherapeutic widely used in clinical practice and well known for inducing peripheral neurotoxicity. Despite its limited ability to cross the blood-brain barrier, patients often report persistent cognitive symptoms, referred to as “chemo-fog”, suggesting possible central effects. This study investigates OHP-induced morphological alterations in pyramidal neurons of the mouse cortex, aiming to better understand the structural correlates of chemotherapy-related cognitive impairment.

Adult male Balb/c mice received intravenous injections of oxaliplatin (7 mg/kg/week) for 8 weeks. Golgi-Cox staining was performed on brain tissue, and morphometric analyses were carried out on layer V pyramidal neurons in the somatosensory and prefrontal cortices using Neurolucida software.

At the end of treatment (T0), both cortical areas showed reduced average length and branching of basal dendrites, without a change in their total number. In the somatosensory cortex, spine number appeared preserved over a fixed dendritic length, although a shift toward immature spine types was observed. In the prefrontal cortex, spine number decreased and mature forms (mushroom-type) were less represented.

After 4 weeks of follow-up (T1), the somatosensory cortex exhibited a decrease in the total number of basal dendrites, with persistent spine immaturity. In the prefrontal cortex, dendrite number and branching were further reduced, accompanied by sustained loss of mature spines.

These findings indicate that chronic OHP treatment induces early dendritic simplification and long-term synaptic alterations in cortical neurons, despite its peripheral route of action. Further studies are needed to uncover the underlying mechanisms and their behavioral implications.

Supported by PRIN 2022 project 2022ZL4JP8 “Understanding and targeting CHEMOtherapy-related neuroTOXicity (CHEMOTOX)”.

Restoring Cognitive Function in 3xTg-AD Mice by Targeting the Prefrontal Cortex–Raphe Circuit via transcranial Alternating Current Stimulation (tACS)

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Alzheimer's disease (AD) progression is associated with impairments in various neurotransmitter systems. Alterations in the serotonergic (5-HT) system have been linked to cognitive dysfunction in AD. Notably, treatments with selective serotonin reuptake inhibitors have been demonstrated to improve cognitive and memory skills in individuals with AD. In rodents, the dorsal raphe nucleus (DRN) is the main 5-HT source in the brain and extensively innervates the prefrontal cortex (PFC), which, in turn, sends reciprocal projections back to the DRN. Beyond its well-known role in mood regulation, the DRN–PFC circuit is also critically involved in cognitive functions. Building on this, we examined the activity of the DRN and its crosstalk with the PFC in the 3xTg-AD mouse model at 4 and 8 months, i.e. before and after the onset of cognitive symptoms. Immunohistochemical staining was used to identify 5-HT neurons through TPH2 expression. Then, single-unit activity from DRN 5-HT neurons was recorded in anesthetized mice, whereas local field potential (LFP) was recorded in the DRN and the PFC while mice performed the novel object recognition (NOR) test. At 8 months, 3xTg-AD mice exhibited a reduced number of TPH2+ neurons, an altered 5-HT firing activity and a decreased DRN-PFC coherence in the low-beta range (peak at 14Hz) during exploration of the novel object, compared to both age-matched wild-type mice and younger 3xTg-AD mice. Our results indicate that dysfunctions of DRN 5-HT neurons parallels AD progression, supporting the role of 5-HT neurons in AD-related cognitive decline. Furthermore, based on the possibility of modulating brain circuits' activity by non-invasive techniques, we applied 14Hz transcranial alternating current stimulation (tACS) over the PFC in 8-month-old 3xTg-AD mice. tACS resulted in the activation of the PFC-DRN circuit and in a significant improvement in the NOR test performance, offering a promising avenue for future therapeutic exploration.

Multimodal study of brain reconfigurations in an experimental model of multiple sclerosis

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Multiple Sclerosis (MS) is a neurodegenerative disease marked by neuroinflammation, demyelination, and progressive neurological dysfunction. Cerebral cortex is severely affected since the earliest stages of MS, impacting physical and cognitive ability. Evidence shows that the disruption of functional brain network connectivity is a central contributor to cognitive impairment in MS. However, evidence in MS patients remains conflicting, with reports of both increased and decreased functional network connectivity, and a clear link between network disruptions and disease progression and severity remains elusive, likely reflecting the complex relationship between structural and functional damages. Human neuroimaging studies face challenges due to heterogeneous symptoms and treatments, and limited chances to explore underlying mechanisms. These limitations may be overcome using experimental models.

The primary aim of this study is to identify network connectivity signatures of experimental MS progression and severity using resting-state fMRI (rsfMRI). To explore the complex interaction between multidimensional structural and functional MS signatures, connectivity measures will be correlated with concurrent anatomical, metabolic, transcriptomic and molecular data collected in the same animals.

We identified alterations in the connectivity of specific brain regions in Experimental Autoimmune Encephalomyelitis (EAE) mice. Some of these alterations worsened with clinical severity, while others remained stable. This suggests the presence of both maladaptive and compensatory mechanisms. Proton Magnetic Resonance Spectroscopy was used to quantify metabolite concentrations, revealing elevated phosphocholine in affected areas, indicating disrupted membrane turnover and myelination.

Next, we will explore the relationship between MRI data and concurrent transcriptomic and electrophysiological alterations to provide a comprehensive picture of disease severity and progression.

The role of Extracellular vesicles in the spreading of tauopathies

NDG 58

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Synaptic dysfunction is an early mechanism in Alzheimer's disease (AD) that progressively affects larger areas of the brain over time. However, the initiation and propagation of this alteration remain unknown. Both amyloid- β (A β) and tau are key pathological hallmarks of AD, playing a significant role in synaptic impairment. We have previously investigated the biological properties of microglia-derived inflammatory extracellular vesicles (EVs) carrying A β . We showed that A β released by microglia, in association with EVs (A β -EVs), alters dendritic spine morphology at the site of neuron interaction *in vitro*. Then, injecting EVs into the mouse brain, to evaluate their impact on synaptic plasticity and memory, we confirmed that A β synaptotoxicity is worsened when associated with microglia-derived EVs. Notably, the propagation of A β effects in the brain is mediated by EVs movement along neuronal processes, following a diffusion pattern similar to that observed in human AD brains. Our aim is to extend these observations studying the effects of different forms of the tau protein: we used either wild-type oligomeric tau (for AD) or the mutated tau (P301S, typical of frontotemporal dementia, FTD). Using *in vitro* electrophysiology, we show that 1 h after Tau-EV injection into the mouse entorhinal cortex (EC), long-term potentiation (LTP) is impaired in the EC but not in the dentate gyrus (DG), its main target region, while 24 h later it was altered also in the DG, revealing a spreading of LTP deficit between the two regions. Similar results were obtained when P301S tau was used observing a spreading of the effect from the prefrontal cortex to the somatosensory cortex. The development of synaptic dysfunction is associated with a progressive memory impairment, as assessed by behavioral tasks. These findings aim to establish a novel mechanism of disease progression mediated by microglia-derived EVs, providing new insights into the pathophysiology of tauopathies.

Investigating the interaction between α -synuclein and Connexin 32 channels in the context of Parkinson's disease

NDG 60

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Connexins are transmembrane proteins that form examers called connexons or hemichannels (HCs). Two HCs from adjacent cells can dock end-to-end to form a gap junction channel (GJC), enabling direct exchange of ions and second messengers between cells. HCs opening is regulated by shifts of intracellular and extracellular calcium with the contribution of calmodulin (CaM). Emerging evidence suggests that connexin channels expression and function is altered in Parkinson's disease (PD). A hallmark of PD is the progressive accumulation of α -synuclein (α -syn) fibrils and death of dopaminergic neurons in the midbrain region.

Connexin 32 (Cx32), mainly expressed by oligodendrocytes in the central nervous system (CNS), has been reported to be upregulated and to colocalize with oligomers of α -syn in *in vitro* and *in vivo* models. However, a molecular pathway describing the interplay of α -syn and Cx32 in PD pathogenesis and progression is still lacking.

In this work, we investigated α -syn and Cx32 interaction by an *in vitro* model (HEK TReX™ cells) stably expressing Cx32 upon a doxycycline-inducible promoter. Exogenous administration of pre-formed fibrils (PFF) of α -syn stimulated its accumulation with HEK cells expressing Cx32 at a three-times higher rate than control cells. The quantification was performed by immunofluorescence and confirmed by Western Blot.

Our hypothesis is based on oligodendrocytes exchanging α -syn with astrocytes after internalization through annular GJs, with or without involving CaM. It is known that CaM binds to α -syn inducing α -syn fibrillization, we interrogate if α -syn fibrils – Cx32 interaction could be direct through high affinity residuals or mediated by CaM interaction.

Our bioinformatic analysis highlighted that α -syn PFFs have a strong interaction with Cx32 compared to the monomeric or oligomeric forms.

The potential role of PCSK9 in the pathogenesis of Alzheimer's disease

NDG 61

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder, mainly caused by the accumulation of β -amyloid peptides (A β) and hyperphosphorylated tau protein (p-tau) in the brain. Among the various contributing factors, dysregulation of cholesterol metabolism has emerged as a critical player in AD pathogenesis. Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) is a master regulator of cholesterol homeostasis, exerting its function by promoting the degradation of low-density lipoprotein receptor (LDLR), LDL receptor-related protein 1 (LRP1), and apolipoprotein E receptor 2 (APOER2). Moreover, increasing evidence supports the involvement of PCSK9 in key pathological processes in AD, including oxidative stress and inflammation. This study investigates the molecular mechanisms in the brain through which PCSK9 influences AD onset and progression. A prevention of lipoprotein receptors (LDLRs) degradation was observed in SK-N-BE neuroblastoma cells overexpressing PCSK9, using a specific PCSK9 inhibitor. To confirm the pathogenic role of PCSK9 in cerebral cholesterol dyshomeostasis, cells treated with a recombinant gain-of-function PCSK9 variant (D374Y) showed decreased levels of LDLRs. Additionally, *ex-vivo* studies were conducted on *post-mortem* tissue samples from the frontal, occipital, and temporal cortices of AD patients at various stages of disease and from control subjects. According to the hypothesis that PCSK9 may play a critical role in AD, we found a positive association between PCSK9 and LDLRs gene expression, markedly in frontal cortex. Furthermore, biological fluids from AD patients were analyzed to assess correlations between PCSK9 and lipid metabolites in plasma, and PCSK9 and neurodegeneration biomarkers (A β , p-tau and t-tau) in cerebrospinal fluids. These data may pave the way for the identification of a new diagnostic biomarker and therapeutic target for AD.

CAP2 as a novel regulator of mitochondrial dynamics in neurons**NDG 62**

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Mitochondrial plasticity is the ability of these organelles to adapt their morphology, position, and function in response to energy demand. These events rely on a dynamic balance between mitochondrial fusion and fission, which are tightly regulated by actin polymerization and actin-binding proteins such as cofilin and INF2. Elevated intracellular Ca^{2+} activates INF2, promoting F-actin assembly at endoplasmic reticulum (ER)-mitochondria contact sites, where Drp1 is recruited to initiate fission. Cofilin also regulates fission by modulating Drp1 distribution and activity. Cofilin and INF2 share a common protein partner: cyclase-associated protein 2 (CAP2), a synaptic actin-binding protein implicated in synapse plasticity and Alzheimer’s disease (AD) synaptic failure.

Here, we characterized the contribution of CAP2 in regulating mitochondrial dynamics. In neuronal cultures transduced with a viral vector expressing an shRNA targeting CAP2, we observed alterations in mitochondrial morphology and network. These changes were accompanied by disruptions in mitochondrial dynamics, as demonstrated by fluorescence recovery after photobleaching (FRAP) experiments. CAP2 deletion led to a marked increase in the levels of its protein partners INF2 and cofilin, promoting cofilin activation. These findings suggest a disruption in mitochondrial fission process, as evidenced by reduced Drp1 levels and changes in its phosphorylation, which are critical for proper fission. Furthermore, our study provided evidence of a dramatic decrease in the total area covered by the mitochondrial network triggered by the absence of CAP2 due to an impairment of mitobiogenesis pathway. Overall, these findings disclose a critical role of CAP2 in controlling mitochondria dynamics in neurons. Considering that CAP2 expression is reduced in AD patients, our results provide a potential cellular mechanism implicated in neuronal mitochondria vulnerability in AD.

Multiparametric approach to the identification of novel prognostic biomarker for high grade gliomas

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Glioblastoma multiforme (GBM) is the most aggressive and lethal primary brain tumor in adults, marked by strong infiltration, necrosis, and poor therapeutic response. Despite the current standard of care, surgery followed by radiotherapy and chemotherapy, the prognosis remains inauspicious, with an average survival of 12–15 months. Among the key molecular pathways involved, Receptor Tyrosine Kinase AXL (RTK-AXL), activated by its ligand Gas6, plays a pivotal role in GBM proliferation, invasion, and immune evasion. Upon activation, AXL is phosphorylated and triggers the PI3K/Akt cascade, promoting cancer cell proliferation, apoptosis and therapy resistance. Concurrently, AXL ectodomain (ECD) can be cleaved by metalloproteases ADAM10/17, in a process of binding regulation called “shedding” that generates a soluble form of the protein, sAXL, which can be released in the extracellular spaces and fluids. We leveraged the SB28 GFP+ murine model, a novel GBM in vivo model obtained by implanting SB28 GFP+ cells into the striatum of immunocompetent C57BL/6 mice, which closely replicates GBM pathophysiology. The model was fully characterized through MRI (Magnetic Resonance Imaging) to assess tumor growth longitudinally over time. Immunofluorescence and histological data confirmed necrosis, tumor microenvironment changes and tumor cells spreading in the striatum and cortex. Confocal fluorescence images revealed a progressive increase of AXL expression over-time, within the tumor core (where the signal co-localized with SB28 GFP+ cells) and at tumor borders (where AXL co-localized with reactive astrocytes and likely immune cells). In the contralateral healthy hemisphere, physiological AXL expression was reported in neurons. Molecular analysis via RT-qPCR and immunofluorescence on SB28, C6, and patient-derived human GSCs (Glioblastoma Stem Cells) cell lines confirmed the expression of AXL, Gas6, and ADAM10/17 RNA. Ongoing experiments are focusing on enhancing prognostic accuracy in glioblastoma, developing a multiparametric strategy that combines longitudinal MRI data with molecular profiling of cerebrospinal fluid and peripheral blood (serum and plasma) biomarkers, with the goal of refining prognostic prediction and disease monitoring in GBM models. In parallel, considering the leading AXL's role, our work aims to elucidate the functional role of the Gas6/AXL/ADAM axis in GBM pathogenesis and to evaluate the efficacy of new pharmacological agents targeting this signaling cascade as potential therapeutic interventions, translatable to clinical practice, and to be combined with standard therapy.

NO 18



BRAINTOX: Uremic toxins in chemotherapy response in the brain**NO 19**

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Glioblastoma is a highly aggressive and rapidly growing brain tumor with limited effective treatment options. Major challenges in understanding and treating it include its cellular heterogeneity and the tumor's ability to undergo metabolic reprogramming. Using untargeted metabolomics, we recently identified an unexpected increased concentration of microbiome-derived uremic toxins, p-cresyl sulphate and indoxyl sulphate, in glioblastoma tissue. These toxins are typically excreted via the kidneys and are not known to accumulate in organs. They have been associated with chronic kidney disease and neurological disorders. We hypothesize that p-cresyl sulphate and indoxyl sulphate are actively transported into glioblastoma cells via OAT1 and OAT3 transporters, contributing to metabolic reprogramming and affecting drug response.

To investigate this, we first screened a panel of 37 drugs in the U87MG glioblastoma cell line. Additionally, we assessed the toxicity of both uremic toxins in the same cell line. Histological characterization of patient tumor tissue was also performed using hematoxylin and eosin staining to evaluate intratumoral heterogeneity. Preliminary data from our group, which assessed the response of patient-derived cells to a panel of 80 drugs, revealed an inverse correlation between the concentration of these uremic toxins and the response to seven tested drugs. Drug screening in U87MG showed comparable effects. The toxicity assessment of the uremic toxins demonstrated a reduction in cell viability already 24h after treatment.

Ongoing experiments aim to compare drug responses in the U87MG cell alone versus those co-treated with uremic toxins. Furthermore, we are investigating OAT1 and OAT3 gene expression to better understand the mechanism of uremic toxins uptake in glioblastoma cells. This study provides new insights into how microbiome-derived metabolites may influence glioblastoma metabolism and drug response.

Liquid Biopsy in Glioblastoma: Leveraging Plasma Extracellular Vesicles for Tumor Diagnosis, Real-Time Monitoring, and Patient Stratification

NO 20



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Despite recent advances, the therapies available for glioblastoma (GBM) still fall short of delivering a cure. Meaningful progress now rests on precision-medicine approaches that depend on robust biomarkers to diagnose, characterize, and monitor the tumor while supporting patient stratification. Unfortunately, an effective liquid biopsy able to fulfill these roles for GBM has yet to be realized. This study tests plasma extracellular vesicles (EVs) as non-invasive GBM biomarkers. EVs were isolated from plasma by SEC. EVs concentration and mean diameter were quantified with TRPS. Baseline EV levels from 62 pre-operative GBM patients were compared with two control groups: 79 healthy volunteers and 50 patients harboring other brain malignancies that radiologically mimic GBM. Serial plasma samples for longitudinal EV analysis were collected from 44 of the GBM patients. Surface-marker profiling and proteomic characterization of EVs were performed by mass spectrometry in a subset of plasma specimens (27 GBM patients and 38 healthy controls).

Plasma EV concentration and mean size significantly differ between GBM patients and controls. Following surgery, both parameters become comparable to healthy controls but increase again upon tumor recurrence.

EV size and concentration effectively distinguish GBM from control groups, achieving near-perfect AUC values, indicative of high sensitivity and specificity.

EV size is a predictive biomarker for response to radio- and chemotherapy.

Longitudinal monitoring of EV concentration reflects GBM progression, even preceding radiological evidence on MRI.

Proteomic analysis identified over 2,000 proteins across all samples, with 117 upregulated in GBM patients and enriched in complement cascade pathways.

Monitoring circulating EVs offers a highly sensitive and specific, non-invasive tool for the differential diagnosis of GBM, patient stratification, and longitudinal follow-up.

Targeting pediatric CNS tumors with NKG2D-CAR T cells: A preclinical success**NO 21**

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Pediatric malignant CNS tumors are the most common solid tumors in children and the leading cause of cancer-related death, highlighting the urgent need for new therapies. In this regard, CAR T cells have emerged as a new pillar of treatment for these fatal tumors. The interactions between NKG2D receptor on immune effector cells and NKG2D ligands on tumor cells are essential for tumor immunosurveillance. The aim of this study was to analyze at the preclinical level/in preclinical models the capacity of NKG2D-CAR T cells to target high-grade pediatric CNS tumor cells. *In vitro*, we found NKG2D CAR T cells induced lysis of CNS pediatric tumors cell lines from different entities, being a glioblastoma (SJ-GBM2) and a medulloblastoma (HTB186) tumor cell lines the most sensitive to NKG2D CAR T cells cytotoxicity. Furthermore, NKG2D CAR T cells demonstrated ability to penetrate and eliminate glioblastoma and medulloblastoma cells when cultured as tumor-spheres, indicating their ability to target three-dimensional tumor architectures. *In vivo*, the therapeutic impact of NKG2D CAR T cells was evaluated in orthotopic murine models of human glioblastoma and medulloblastoma using SJ-GBM2 and HTB186 tumor cells as targets. We observed that an only intracranial infusion of NKG2D CAR T cells reduced tumor growth in both models, although this reduction was more patent and durable in the medulloblastoma model. Additionally, for both GBM and MB models, those mice treated with NKG2D CAR T cells showed increased survival compared with those mice treated with untransduced T cells or those left untreated. Although the data here presented are still preliminary, our findings suggest that NKG2D-CAR T cells could represent a promising therapeutic strategy for pediatric CNS tumors, opening new possibilities in cancer immunotherapy.

Studying STAG2-driven medulloblastoma by *in vitro* and *in vivo* models

NO 22

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Medulloblastoma (MB) is a cerebellar embryonal tumor likely caused by impaired neural progenitor differentiation, however, the underlying genetics, molecular mechanisms, and predisposing factors leading to MB onset are still unclear. Recently, somatic alterations in cohesin genes have emerged as oncogenic drivers in hematologic and solid tumors. The cohesin complex's role in cerebellum development, gene expression regulation, and DNA repair suggests this structure as a possible candidate in MB onset. Among cohesins, STAG2 is the most frequently affected subunit in cancers, and its loss of function is believed to promote tumorigenesis by altering 3D genome architecture and transcription. We studied *in vitro* the impact of STAG2 silencing on a cell model and, by cerebellum organoids, on cell differentiation. In addition, we investigated tumor-related phenotypic features in the *in vivo* fly model, obtained by SA1 (STAG2 ortholog) silencing. Specifically, *in vitro*, STAG2-deficient cells present increased DNA damage, while STAG2-silenced cerebellum organoids exhibit a deregulated gene expression related to development. *In vivo*, we showed a defective neuroblast differentiation during larval development, and mass formation within adult fly brains, with a lowered life expectancy consistent with the malignant phenotype. These models aim to elucidate molecular mechanisms underlying STAG2-driven MB pathogenesis but also represent a basis for testing targeted therapies, such as PARP inhibitors (PARPi). This treatment has been recently implemented in clinical trials for cohesin-mutated tumors, demonstrating enhanced efficacy in models with defective DNA repair. The use of PARPi in our STAG2-depleted *in vitro* and *in vivo* models resulted in increased apoptosis and rescue of life expectancy, respectively, supporting the potential of PARPi as a new patient-specific therapy for MB.

The Impact of Healthy Fecal Material Transplantation on Glioma

NO 23

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Glioblastoma (GB) has been identified as the most lethal type of brain tumor, characterized by a low lifespan of 12.1 to 14.6 months after diagnosis, and a 5-year survival rate of 5%. Glioblastoma bearing patients show different gut microbiota (GM) compared to healthy individuals. Recently, the important role of gut-brain axis in shaping the glioblastoma has made it a popular target to modulate. Among several approaches, fecal material transplantation (FMT) is one of the most direct ways to modulate the GM composition. FMT is a procedure of transplanting fecal material from a healthy donor to a diseased individual, after pre-treatment of broad spectrum antibiotics (ABX) to ensure that new GM get established. To assess if FMT from healthy mice to glioma bearing mice can eventually influence the tumor development, we treated mice orthotopically injected with murine glioma cells GL261 with terminal stool of healthy age-matched mice or vehicle (PBS) through oral gavage. Treatments were performed three times a week for two weeks with or without ABX pre-treatment. Data showed that FMT from healthy mice reduced tumor growth in both groups pre-treated or not with ABX compared to the mice receiving only PBS. Only in ABX+FMT group an increased number of circulating cytotoxic CD8⁺ T and NK cells expressing the degranulation marker CD107⁺ measured by FACS has been observed; the higher number of CD107⁺ NK cells were also found in the tumoral hemisphere. Furthermore, the ability of NK cells isolated from the spleens of all groups to directly kill glioma cells has been evaluated through viability assay. At a higher co-culture NK:GL261 ratio, NK cells derived from the spleens of ABX+FMT group exhibited increased cytotoxicity. These data showed that mice receiving a novel GM after the ABX pre-treatment experienced important immune response that could result in a long lasting anti-tumor effect on glioma growth, suggesting a new possible adjuvant therapeutic approach for patients.

Magnetite Nanodiscs in a Sinusoidal Magnetic Field Induce Non-Thermal Mechanical Ablation of Glioblastoma Cells In Vitro

NO 24



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Glioblastoma (GBM) is an aggressive brain tumor with a poor prognosis and limited treatment options, primarily due to the risks associated with surgical resection and collateral damage from thermal ablation. To address these challenges, we explored a novel strategy employing anisotropic magnetic nanodiscs (MNDs) for the mechanical ablation of GBM cells using externally applied magnetic fields (MF). We synthesised magnetite nanoparticles coated with either caffeic acid (CA) or poly-methylaluminoxane (PMAO) and assessed their distribution around GL261 GBM cells at different time points. Cultures were incubated with 25 µg/ml MNDs and, after 24 hours, subjected to oscillating magnetic fields (50 mT) at either 10 Hz or 15 Hz. The exposure consisted of five cycles, each comprising one minute on and two minutes off. Throughout the process, internal coil and surface temperatures were monitored to confirm that conditions remained non-thermal. The presence of MNDs throughout the culture was revealed by morphological observations and Perls assay. The impact on cell proliferation and viability was evaluated using BrdU incorporation and crystal violet assays within 48 h after treatment. We found that both the frequency of the MF and the type of nanoparticle coating play crucial roles in influencing nanoparticle aggregation as well as the exerted biological effects on GBM cells. Both tested MNDs were able to affect the proliferation and survival of the GBM cells, with a more pronounced response observed with CA-coated MNDs. In the CA group, a decrease in cell proliferation was observed up to 24 h after exposure to MF, with a greater effect at 10 Hz. Additionally, cell viability decreased compared to controls up to 48 h post-treatment. These results highlight CA-coated MNDs in 50 mT MF at 10 Hz as a promising strategy for further in vivo studies and underscore the potential of mechanically active, radiosensitive nanoagents as a multimodal therapeutic strategy for GBM.

Deciphering and Targeting miR675-5p derived MOONLIT sequences: Unraveling Hypoxia-Driven Resistance in Glioma

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Gliomas are the most common central nervous system tumors, with high-grade forms, especially Glioblastoma Multiforme (GBM), being resistant to current therapies involving Temozolomide (TMZ). One of the players in the resistance is the hypoxic tumor microenvironment, which fosters tumor progression by promoting cancer cell stemness and angiogenesis via Hypoxia-Inducible Factor 1- α (HIF-1 α) activation¹. miR675-5p is a hypoxamiR able to mimic hypoxia by upregulating HIF-1 α and its downstream effectors². While inhibition of miR675-5p has been shown to reduce tumor growth and decrease HIF-1 α signaling *in vitro* in resistant cells, its precise role in overcoming TMZ resistance remains unclear. The aims of the study are i) to identify direct targets of miR675-5p through bioinformatics approaches and validate them experimentally reconstructing the regulatory networks of miR675-5p; ii) to evaluate the therapeutic potential of chemically modified inhibitors³ (MOONLITs) to sequester mature miR675-5p, attenuating its activity; iii) to assess the intracellular localization of Cy5-labeled inhibitors via confocal microscopy. Preliminary results showed that: i) 16 putative target genes of miR675-5p were identified and functional assays demonstrated that miR675-5p regulates most of candidate targets. However, the extent to which these genes contribute to HIF-1 α signaling remains under investigation. ii) Cells treated with MOONLITs exhibit decreased viability and lower HIF-1 α activity compared to those treated with the traditional inhibitor. iii) A confocal imaging protocol was developed to trace Cy5-inhibitors, combined with Cy3-miR675-5p mimic and Cy5-scrambled control. Further studies will be conducted to assess miRNA675-5p efficacy *in vivo*. These data reveal new insights into tumor resistance to therapy and provide a molecular foundation for proposing miRNA675-5p inhibitor as a novel therapeutic strategy in glioma, particularly in overcoming resistance to existing treatments.

Acknowledgment: PNRR 2022 P2022YK2XR “MiR675 inhibition: from benchtop to potential Glioma therapy”

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A systematic evaluation of Ferroptosis induction as a therapeutic approach in Temozolomide-resistant glioblastoma

NO 26



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Glioblastoma (GBM) is the most aggressive astrocytic tumor in adults, with poor response to conventional therapies, such as Temozolomide (TMZ) and radiotherapy. Resistance to TMZ remains a major clinical challenge, highlighting the urgent need for alternative treatment strategies. Ferroptosis—an iron-dependent cell death mechanism characterized by lipid peroxidation and reactive oxygen species (ROS) accumulation—has emerged as a promising avenue to overcome drug resistance in GBM. In this study, we evaluated the effects of three well-characterized Ferroptosis inducers (Erastin, FIN56, RSL3) on TMZ-resistant human GBM cell lines. The results were robust and multidimensional: all treatments significantly reduced cell viability and induced classical Ferroptosis markers, including iron accumulation, lipid peroxidation, and mitochondrial ROS release. At the molecular level, we observed downregulation of key antioxidant genes, reinforcing ferroptotic pathway activation. Confocal imaging enabled direct visualization of intracellular oxidative damage and activation of Ferroptosis-specific molecular signatures. Notably, Ferroptosis induction also led to suppression of hypoxia-inducible factor-1 α (HIF-1 α), a major player in GBM progression and therapy resistance. All cellular and biochemical results were further supported by an integrated multi-omics approach, combining metabolomic and proteomic analyses. Most compellingly, *in vivo* experiments in mice orthotopically injected with TMZ-resistant U118-luc cells showed a significant reduction in tumor mass after treatment, as assessed by optical imaging. This provides strong translational evidence for the therapeutic relevance of Ferroptosis inducers. In conclusion, our findings demonstrate that Ferroptosis inducers can effectively target TMZ-resistant GBM at the cellular, molecular, and whole-organism level, supporting their potential as innovative therapeutic agents against treatment-refractory GBM.

Expression and roles of Creatine biosynthetic enzymes and transporters in human and mouse oligodendroglia: an *in silico* and *in vivo* study

NP 23



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Creatine (Cr) is a key metabolic hub for high-energy demanding cells, such as interneurons and oligodendrocytes (OLs). Consistently, Creatine Deficiency Syndromes (CDS) – caused by mutations in genes coding for Cr biosynthetic enzymes (i.e. AGAT and GAMT) or transporters (i.e. SLC6A8) – are associated with intellectual disability, autistic-like behaviour and seizures. OLs are essential suppliers of energy metabolites to neurons and are considered the major source of the cerebral Cr, as they express AGAT, GAMT, SLC6A8 and the recently identified Cr transporter SLC22A15. Moreover, OLs *per se* may require high levels of Cr, to sustain the energy burden associated with myelination. Here, we performed an *in silico* and *in vivo* study to assess whether: (i) the expression of Cr biosynthetic enzymes and transporters was a feature of all oligodendroglia subpopulations and maturation stages; (ii) SLC6A8 deficiency influenced myelination. Publicly available single-cell transcriptomic databases were interrogated to assess AGAT, GAMT, SLC6A8 and SLC22A15 (co-)expression in oligodendroglia in the human and mouse cortex at developmental and adult stages. In both species, Cr biosynthetic enzymes and transporters were expressed by specific oligodendroglia subsets. Yet, SLC6A8 was more broadly expressed in mouse cells, while SLC22A15 more frequently found in human oligodendroglia. In the human cortex, the percentages of SLC6A8- and SLC22A15- positive cells increased along the lineage, with a peak in mature OLs. SLC6A8 had the same trend in the mouse cortex, where, in contrast, OL maturation was paralleled by a reduction of SLC22A15-positivity. To address the second aim, we studied the myelination pattern of the SLC6A8-KO mouse brain, where a prominent hypomyelination was detected in the cerebral cortex at juvenile stages. These findings prompt future research on the role of Cr biosynthetic enzymes and transporters in OL biology and on the possible contribution of OL dysfunction to CDS.

Brain-wide analysis of perineuronal nets and parvalbumin-positive interneurons in fragile X syndrome mice across postnatal development highlights region-specific alterations influenced by age and sex

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Fragile X syndrome (FXS), caused by inactivation of the *Fmr1* gene, is the most common monogenetic cause of intellectual disability and autism spectrum disorder. *Fmr1* knockout (*Fmr1KO*) mice display a disorganization of perineuronal nets (PNNs), extracellular matrix structures that preferentially form around parvalbumin-positive interneurons (PVIs) during postnatal development, limiting plasticity. PNN and PVI expression is altered in various neuropsychiatric disorders, and their manipulation in specific brain areas has reversed related deficits; despite that, a systematic study in *Fmr1KO* mice has never been conducted. We performed a brain-wide analysis in adult *Fmr1KO* and control (WT) male mice to study FXS-dependent alterations of PNNs and PVIs. To this aim, we developed a customized method for whole-brain 3D reconstruction of histologically stained sections, followed by high-throughput automated image analysis. We found a general reduction of PNNs and PVIs in FXS mice compared to WT, greatly affecting cortex, amygdala, hippocampus, and striatum. PNNs were dramatically decreased in the isocortex, more markedly in primary areas than in associative ones, whereas PVI density was significantly decreased in the amygdala. The whole-brain analysis allowed us to define a section sampling distance sufficient to robustly identify the PNN reduction in *Fmr1KO* mice. By combining this sampling strategy with our customized method, we were able to extend the analysis to different developmental stages in male and female mice, highlighting peculiar region-specific alterations influenced by sex and age. Thanks to our new customized biocomputational method, we precisely described for the first time the expression of PNNs and PVIs in the FXS model. This comprehensive study on PNN and PVI expression in *Fmr1KO* mice at different developmental stages paves the way to identify specific brain areas and corresponding time windows of intervention that could be therapeutically relevant for FXS.

Sex- and region-specific modulation of perineuronal nets, parvalbumin interneurons, and microglia by ketogenic diet in a Grin2a mouse model of developmental and epileptic encephalopathy

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Developmental and epileptic encephalopathies (DEEs) are early-life onset syndromes in which drug-resistant epilepsy is associated with developmental delay and cognitive impairments. The GluN2A(N615S)-knock-in mice carry a mutation in the Grin2a gene coding for the GluN2A subunit of the glutamate NMDA receptor and display symptoms similar to those described in patients with analogous mutations and affected by GRIN-related DEEs. We investigated the effects of a ketogenic diet (KD) in the GluN2A(N615S) model: after behavioral tests and seizure induction, we performed *ex vivo* analysis on stained brain slices to investigate the expression of neuroplasticity markers – perineuronal nets (PNNs) and parvalbumin inhibitory interneurons (PVIs) – and neuroinflammation (microglia). After epifluorescence acquisition, we employed a customized method for high-throughput automated image analysis. We demonstrated for the first time in this model that KD reduces the susceptibility of mutant mice to audiogenic seizures (AGS) and slightly improves performance in some behavioral tests. We also investigated its impact on PNNs, PVIs and microglia – previously unreported in this model – highlighting interesting region-specific differences according to genotype and sex. Very preliminary data show that the protective role of KD against AGS could be mediated by a KD-promoted increase of PVIs in mutated mice, and KD-induced reduction of PNNs. We found that mutated mice display a general reduction of microglia density, especially in the amygdala, a brain area highly involved in the spreading of the epileptic activity from the brainstem to the forebrain, that increases convulsion severity, possibly suggesting that lower microglia density here can lead to increased seizure intensity. Results of behavioral tests, together with *ex vivo* analysis of the brains, are leading us to identify potential molecular and physiological mechanisms underlying the protective role of KD against refractory epilepsy.

Cystatin B as a mediator in cell-cell communication: its neuronal secretion through extracellular vesicle

NP 26



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Cystatin B (CSTB) is a small protein inhibitor of lysosomal cathepsins, involved in cell proliferation and migration during brain development. Loss-of-function mutations in CSTB gene cause progressive myoclonic epilepsy1A (EPM1A), a neurodegenerative disease characterized by myoclonus, ataxia and seizures. We previously demonstrated that CSTB is synthesized and secreted into the media from synapses, indicating its role in synaptic plasticity. In this study, we investigated the molecular mechanisms underlying synaptic CSTB secretion and its involvement in cell communication. Using synaptosomes isolated from adult rat brain, we demonstrated that the release of CSTB through EVs from synapses is finely regulated. In particular, it depends on actin filaments integrity and on the intracellular homeostasis of Ca^{2+} ions. The next step was to investigate the possible impairment of CSTB secretion in EPM1. We observed that EVs secreted by EPM1 neurons are fewer and bigger in size compared to control neurons. Proteomic analyses indicated that proteins involved in the regulation of actin filament organization and in vesicles maturation and release pathways are missing in EPM1 EVs compared to controls. Thus, we concluded that when CSTB is lacking, the information conveyed by EVs is also altered. Altogether, our findings suggest a novel role of CSTB as a mediator in the cell-cell communication.

Microglia-neuron crosstalk in the remodelling of peritumoral circuits**NP 27****Erika Di Pietro** ⁽¹⁾ - Letizia Mazzearella ⁽¹⁾ - Stefano Garofalo ⁽¹⁾ - Maria Amalia Di Castro ⁽¹⁾ - Cristina Limatola ⁽¹⁾⁽¹⁾ Sapienza University of Rome, Italy, Department of Physiology and Pharmacology "Vittorio Ersamer", Roma, Italia

Glioblastoma (GB) malignancy results from a crosstalk between tumor cells and their microenvironment (TME) including neurons, glia cells and the immune system. Tumor derived factors instruct non-neoplastic cells in the TME toward an immunosuppressive, pro-tumor phenotype. Tumor-associated microglia contribute to tumor proliferation and invasion as a function of their activation state. GB cells are also connected to each other via microtubes enabling intra-intercellular communication via Ca^{2+} -signaling and receive synaptic contacts from surrounding neurons. Here, we investigated the role of microglia in GB-induced neuronal dysfunction and neuron-GB communication. Layer 5 peritumoral neurons (PTNs) in acute slices of mouse motor cortex showed a depolarized resting membrane potential (RMP), lower firing threshold, and increased firing frequency. In GB-bearing mice treated with PLX5622, a CSF1R inhibitor that depletes microglia, the tumor proliferation index decreased and PTNs showed normalized RMP and firing frequency. When slices were exposed to 4-aminopyridine, a K^+ channel blocker, PTNs from glioma-bearing mice developed epileptic discharges, while PLX-treated mice showed increased latency and reduced seizure-like events, suggesting restored excitability. The mAHP, mediated by small-conductance Ca^{2+} activated K^+ channels-2 (SK-2) and inversely related to firing rate, was reduced in PTNs from GB mice and increased upon microglia depletion. Lastly, we investigated the spontaneous Ca^{2+} activity of glioma cells expressing GCaMP6 in acute slices, under perfusion with tetrodotoxin to assess the contribution of neuronal hyperactivity in modulating Ca^{2+} transients in GB network. So far, our findings demonstrate that GB alters intrinsic neuronal excitability in the TME via modulation in mAHP and SK-2 channel expression, and that microglia depletion can counteract these alterations, demonstrating a protective role of microglia depletion in neuronal alterations induced by GBM.

Overexpression of Anosmin-1 alters the myelination pattern and the architecture of axonal components in cerebellar Purkinje cells

NP 28

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Kallmann syndrome (KS) is a rare congenital disorder characterized by hypogonadotropic hypogonadism and anosmia. KS results from genetic mutations affecting ANOS1 gene (former KAL1) which encodes the Anosmin-1 (A1), an extracellular matrix glycoprotein broadly expressed throughout the central nervous system, including the cerebellum. Since the A1-overexpressing mouse model shows ataxia-like motor deficits, we hypothesized that these impairments may be due to cerebellar dysfunction. Alterations in myelin formation and in the structural components of axons are major contributors to both the generation and conduction velocity of action potentials and may represent a key mechanism underlying the motor impairments observed in A1-overexpressing mice. To investigate this, we examined both the maturation dynamics of the axon initial segment and the gross myelination pattern. Immunostaining of Ankyrin-g, a reliable marker of the axon initial segment (AIS), revealed that AIS in cerebellar Purkinje cell axons of A1-overexpressing mice are longer compared to wild-type (WT) controls at adult stages. To assess structural modifications in the nodal and paranodal regions, we analyzed the length of nodes of Ranvier and found that at postnatal day 60, mutant mice display longer nodes compared to WT. Moreover, during development, mutant mice exhibited an altered timeline of myelination with evidence that the deposition occurs earlier compared to WT. Yet, in the mutants myelination reaches normal levels at adult stages. These data suggest that A1 regulates the time of myelin deposition and participates in the interplay between Purkinje cell axons and myelin in the organization of specialized axon domains for signal transmission.

The interplay between importin alpha3 and CBX2 in axonal regeneration and pain

NP 29

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In 2020 Marvaldi *et al.* discovered that Importin $\alpha 3$ (KPNA4) knockout (Impa3KO) or knockdown led to increased pain tolerance in the late phase of chronic pain in mice. However, the role of Impa3 in the early phase remained unexplored. To address this gap, we ran a bioinformatic analysis on DRG tissue naïve vs 1-week post-SNI. The transcription factor CBX2 was found to be enriched in both Impa3KO and WT datasets after SNI. While CBX2 is known for its role in sex determination, recent evidence implicates it in pain and axonal regeneration. To further investigate regeneration capacity and CBX2 cellular localization, we performed both naïve and *in vitro*-injury DRG cultures. Following injury, DRG neurons of both genotypes re-organized their cytoskeletal apparatus in a target-oriented way and displayed changes in soma area and polarity. Both genotypes showed a significant increase in axonal elongation after injury compared to naïve. Notably, Impa3-deficient neurons exhibited significantly longer neurites than WT neurons under both *in vivo* and *in vitro*-injury conditions. In the naïve condition of both sexes, WT neurons displayed higher localization of CBX2 in the nucleus, whereas Impa3KO neurons showed higher levels in the cytoplasm and the axon. Upon injury, however, WT neurons displayed a sex-specific localization of CBX2. Female neurons showed a strong nuclear enrichment, whereas males exhibited a distinct punctate pattern along the axonal shaft. To further investigate the role of CBX2 in axon regeneration, we examined embryonic (E13.5) DRG neurons. RNA-seq analysis revealed a significant enrichment of CBX2 when comparing Impa3KO with WT. Remarkably, whole DRG explants from Impa3-mutant embryos exhibited longer neurites than their WT counterparts under basal conditions and responded more robustly to neurotrophins stimulation. These results support the role of CBX2 and KPNA4 in axonal regeneration, pain and marks them as potential therapeutic targets for new analgesics.

Alcohol mixed with energy drinks increases compulsive operant behavior by altering the mesocorticolimbic dopaminergic system

NP 30

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Combining alcohol with energy drinks (AMED) is becoming increasingly popular among young adults, leading to a higher risk of binge drinking behaviors and a greater vulnerability to developing alcohol use disorders. Nonetheless, the neurochemical effects on the brain's reward pathways caused by AMED consumption have not been widely studied. Therefore, this study aims to explore the different responses of the mesocorticolimbic dopaminergic system during operant self-administration (SA) of Tap Water (TW), alcohol (10% weight/volume in TW), Red Bull (RB), or AMED (10% weight/volume in RB). Adolescence rats were trained to perform 30-minute daily SA sessions in Skinner boxes equipped with nose-poke devices, starting with a Fixed Ratio (FR)-1 schedule and progressively increasing to FR-5. After the training phase, the rats were stereotactically implanted with a guide cannula for subsequent insertion of a microdialysis probe targeting either the nucleus accumbens shell (AcbSh) or the medial prefrontal cortex (mPFCX), in order to measure the responsiveness of dopaminergic neurons in the mesocorticolimbic pathway during SA. Our results confirm that there is a correspondence between compulsive behavior and alterations in the reward system. In particular, behavioral observations revealed that rats that self-administered RB and AMED demonstrated faster acquisition in differentiating between active and inactive nose pokes, with a notable increase in correct responses throughout the training sessions. Additionally, microdialysis measurements showed that both RB and AMED administration resulted in elevated dopamine levels in the AcbSh and mPFCX regions. These findings suggest that the AMED assumption could lead to an increased risk of compulsive drug-seeking behaviors by affecting the brain's reward pathways, specifically the mesocorticolimbic dopaminergic system. This alteration may enhance the likelihood of developing addictive behaviors and alcohol use disorders in adulthood.

Psilocybin (un)locked: intracellular drug retention as a novel strategy to modulate neuroplasticity in depression

NP 31



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Psilocybin, the compound found in “magic mushrooms” and prodrug of psilocin, has emerged as a promising fast-acting antidepressant, producing rapid and long-lasting clinical benefits after a single dose. These effects are thought to rely on the drug’s ability to promote structural and functional neuroplasticity, particularly in the prefrontal cortex, a brain region affected in depressed patients. Psilocin is a serotonin 5-HT_{2A} receptor (5-HT_{2A}R) agonist, but how its activation leads to long-term plastic remodeling remains unclear. Recent evidence suggests that psilocin may diffuse into cells and promote neuroplasticity by activating intracellular 5-HT_{2A}Rs, rather than through conventional signaling at the plasma membrane. An intriguing hypothesis is that, once inside the cell, psilocin could be protonated and retained inside intracellular acidic compartments, leading to its accumulation and explaining its sustained activity. To investigate this possibility, we developed a fluorescent tool to track psilocin’s intracellular localization in real time. Using advanced imaging techniques both *in vitro* and *ex vivo*, we demonstrate that psilocin rapidly diffuses into cells and accumulates in acidic compartments. Disrupting this accumulation does not impair 5-HT_{2A}R signaling but impacts psilocin’s ability to induce dendritic spine remodeling, a key marker of neuroplasticity. These findings reveal a previously unrecognized role for intracellular retention in mediating the neuroplastic effects of psilocin. Our approach, which we are currently extending to *in vivo* studies, offers a novel and powerful strategy to elucidate the role of intracellular acidic compartments in the pharmacology of fast-acting antidepressants. In addition to addressing a key open question regarding the neuroplastic potential of 5-HT_{2A}R agonists, this work lays the foundation for the rational design of next-generation serotonergic agents that exploit intracellular drug retention for enhanced efficacy.

Modelling nervous tissue complexity through biofabrication technologies**NP 32****Cecilia Traldi** ⁽¹⁾ - Vanessa Chiappini ⁽²⁾ - Silvia Chasseur ⁽²⁾ - Federica Aiello ⁽³⁾ - Marina Boido ⁽²⁾ - Chiara Tonda-Turo ⁽¹⁾

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Human central nervous system (CNS) is characterized by unique three-dimensional (3D) complexity, encompassing its multi-scale heterogeneity and structural anisotropy, which are closely related to CNS tissue connectivity. Replicating such complexity *in vitro* is fundamental in the development of reliable experimental models, which can serve as effective platforms for investigating CNS physiology and pathology. In particular, bioengineered *in vitro* systems represent promising tools to model CNS intricacy, as they allow to reproduce the structural features of neural tissues with greater physiological relevance compared to traditional 2D culture models. Here, we propose the development of a bioengineered 3D neural tissue model, by combining the use of neural stem cells (NSCs) and biofabrication technologies to mimic distinctive features of neural microenvironment, including multi-cellularity and 3D anisotropy. Specifically, extrusion-based 3D bioprinting has been employed to fabricate living neural-like constructs, by accurately positioning neural stem cells (NSCs) embedded in a gelatin methacryloyl (GelMA) hydrogel onto an aligned microfibrous scaffold produced by melt electrowriting technique. The obtained results demonstrated that GelMA hydrogel supported NSC bioprinting without affecting cell viability and promoted the growth of encapsulated NSCs, providing a favourable environment for their differentiation towards mature neuronal and astroglial cells. In addition, the presence of anisotropic guidance cues, provided by the microfibrous scaffold, was shown to direct neural cell elongation and arrangement, enabling the formation of a functionally aligned 3D neural network. To conclude, this work highlights the potential of bioengineering-based approaches in creating bioartificial environments that can foster NSC maturation under *in vivo*-like conditions, thus offering new opportunities for the design of physiologically relevant *in vitro* CNS models.

Multisensory Integration of Social Cues in the ventromedial Prefrontal Cortex

NP 33

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The ability to attract and reproduce with a suitable mate is a key driver of evolution. Mate selection depends on social interaction, and animals have evolved diverse behavioural strategies that rely on multimodal communication. Despite the importance of multisensory cues in courtship, the neural circuits responsible for integrating these signals remain poorly understood. In rodents, males use a combination of olfactory (e.g., pheromones) and acoustic (ultrasonic vocalizations, USVs) cues to court females. Recent studies show that female mice prefer USVs from unfamiliar males only when paired with sexual odors, suggesting cross-modal integration. However, where and how this sensory information converges in the brain remains unclear. We first confirmed through behavioural preference tests that female mice favor multimodal cues, but only within a social context. To explore the brain's response to unimodal and multimodal stimuli, we conducted whole-brain analysis of immediate-early gene (IEG) expression, using tissue clearing and light-sheet microscopy. Our results revealed that sexual odors predominantly drive brain activation, with the ventromedial prefrontal cortex (vmPFC) specifically responding to multisensory social cues. To further investigate vmPFC involvement, we used miniscope recording to perform one-photon functional imaging in awake animals. We found that vmPFC neurons respond to both unimodal and multimodal cues, with a distinct subset selectively activated by combined sensory inputs. By performing chemogenetic manipulation of vmPFC neurons, we are now investigating their causal role in shaping female preference for multisensory social stimuli. Together, these findings highlight the vmPFC as a key hub for integrating social cues from different modalities, suggesting its potential role in shaping mate preference.

Metformin Boosts Temozolomide Response in Glioblastoma: Insights from Patient-Derived Models and Multimodal Imaging

NIM 10



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Glioblastoma (GBM) is the most aggressive primary brain tumor, and remains incurable despite surgical intervention, radiation and temozolomide (TMZ) chemotherapy. In a previous study, we demonstrated that Metformin (MET), a metabolic drug, enhanced TMZ efficacy in a classical phenotype of GBM cells. Here, we assess the effectiveness of MET as an add-on therapy to TMZ on patient-derived orthotopic xenograft models (PDOX) from mesenchymal and proneural GBM sphere-forming cells (GSCs) using multimodal imaging.

NOD scid gamma (NSG) mice were intracranially injected with mesenchymal (MES1312) or proneural (PN0605) luciferase-expressing patient-derived GSCs and assigned to four treatment groups: vehicle, TMZ, MET, or both. Tumour growth was monitored weekly via Bioluminescence imaging (BLI). One week after treatment began, [¹⁸F]FLT-PET ([¹⁸F]-fluoro-3'-deoxy-3'-fluorothymidine positron emission tomography) assessed early response and brains were collected post-sacrifice for immunohistochemistry (IHC).

Survival analysis revealed that the MES1312 GBM model was more aggressive than the PN0605 model, with a lower median survival (67 vs. 102.5 days). In the MES1312 GBM subtype, the TMZ+MET combination extended the disease-free interval and reduced recurrence rates. PN0605 tumors exhibited slower proliferation, as reflected in the comparable survival of vehicle- and MET-treated groups (102 and 98 days, respectively). However, PN0605 tumors did not respond to either TMZ alone or the combined treatment, as shown by stable BLI signal emission. MES1312 tumor-bearing mice showed high [¹⁸F]FLT uptake in the control group, which was significantly reduced following TMZ or TMZ+MET treatment. IHC revealed distinct microglial morphologies (IBA1 marker): ameboid in tumor regions and ramified in contralateral areas (MES1312 model). These findings underscore the GBM subtype heterogeneity and highlight the therapeutic potential of TMZ+MET treatment specifically in aggressive MES1312 tumors.

Exploring the phenotypic heterogeneity of Amyotrophic Lateral Sclerosis through blood biomarkers: a prospective study

NIM 11



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Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease characterized by significant phenotypic variability, making its clinical course and prognosis difficult to predict. Our aim is to disentangle this phenotypic heterogeneity exploring a set of blood biomarkers, including serum neurofilament light chain (NfL), phosphorylated tau 181 (p-tau181), and phosphorylated tau 217 (p-tau217) in a prospective cohort of ALS patients. NfL is a widely recognized marker of neuronal damage, while p-tau181 and p-tau217 have been recently linked to lower motor neuron degeneration in ALS. Biomarkers are collected 0, 3, 6, 9, 12 months and analysed with a chemiluminescent enzyme immunoassay at the "Città della Salute e della Scienza" laboratory in Turin. Our preliminary results on 38 ALS patients showed a good correlation between the two isoforms of p-tau. A positive correlation between serum NfL and ALSFRS_r (ALS Functional Rating Scale revised) slope, as well as a negative correlation between both isoforms of p-tau and total ALSFRS_r, were found. We observed significantly higher values of p-tau 181 and creatin kinase (CK) with lower upper motor neuron scores, with NfL showing an opposite trend. Indeed, we found a correlation between p-tau181 and CK levels. Higher levels of p-tau181, but not NfL, were found in spinal patients compared to bulbar patients. Both NfL and p-tau181 correlated with the diagnostic delay. We explored neutrophil-to-lymphocyte ratio (NLR), as a marker of inflammatory status and a negative prognostic factor, finding a significant correlation with NfL and confirming NLR correlation to survival and disease progression. Therefore, our results suggest the complementary utility of blood biomarkers in phenotypic characterization. Further collection of data could provide more robust evidence on the interplay between biomarkers and ALS heterogeneity and their significance in understanding disease underpinnings.

Use of brain 2-^[18F]FDG-PET to discriminate ALS and ALS-mimics

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Some studies showed the capability of brain 2-^[18F]FDG-PET to discriminate ALS patients and healthy controls. Nevertheless, in clinical practice neurologists face the issue of distinguishing ALS from disorders mimicking ALS. A previous study from the ALS Centre of Leuven, Belgium, reported that brain 2-^[18F]FDG-PET showed only an accuracy of 65.4% in discriminating ALS and ALS-mimics. Our study evaluated the capability of brain 2-^[18F]FDG-PET to discriminate ALS and ALS-mimics in the dataset of the ALS Centre of Turin. We considered eligible ALS patients who underwent brain 2-^[18F]FDG-PET at diagnosis at the ALS Centre of Turin between 2009 and 2019, and a group of subjects with ALS-mimics (including cervical spondylogenic myelopathy, multi-focal motor neuropathy, myasthenia gravis). Since further analyses considered each voxel as a single feature, by using Automated Anatomical Labeling Atlas only the whole brain volume has been retained from entire PET scans reducing the amount of candidate voxels to 226954 voxels. Intensity normalization was performed at individual level averaging each voxel for the mean value of the whole brain. The dataset included 663 ALS and 40 ALS-mimics. We randomly collected 40 ALS from the whole group to calculate Laplacian scores. Subsequently, features were extracted from ALS and ALS-mimics. Finally, the dataset (40 ALS and 40 ALS-mimics) was split in a training set (80%) and a test set (20%). A Support Vector Machine (SVM) approach was used as classifier. The procedure was randomly repeated 10 times. SVM showed 85% specificity and 81% sensitivity on the test set, with an error rate of 17%. The classification was based on three cerebellar clusters, situated in left anterior lobe, right uvula and right culmen. Our data support the possible role of brain 2-^[18F]FDG-PET as a single diagnostic marker to discriminate between ALS and ALS-mimics.

NIM 12



Temporal Dynamics of Serum Neurofilament Light Chain in Multiple Sclerosis: A Retrospective Study in a Clinical Routine Setting

NIM 13



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Serum Neurofilament Light Chain (sNfL), a biomarker for neuroaxonal injury, is strongly associated with disease activity in multiple sclerosis (MS). Elevated sNfL levels have been linked to evidence of disease activity (EDA), compared to clinically and radiologically stable patients. However, the temporal dynamics of sNfL in relation to relapses and its clinical implications in routine settings remain understood. We aim to evaluate the temporal changes in sNfL in relation to MS relapsing activity and the potential utility in clinical practice. Retrospective longitudinal data from 162 MS patients (mean±SD age=32.5±7.8 years, median disease duration 2.1[IQR 1-7.1], 64.2% female) were analyzed, with a median of 7[IQR 6-9] samples per patient collected over a median follow-up time of 10.4[IQR 7.8-13.8] years. sNfL levels were quantified using the Simoa HD-X analyzer, adjusting for age and body mass index with Zscores. Radiological activity was assessed through Gadolinium-enhanced or new/enlarging T2 lesions detected using 3T MRI scans. EDA was defined as the occurrence of a clinical relapse, confirmed disability worsening (assessed by Expanded Disability Status Scale), or radiological activity within 6 months of sampling. P-values were corrected for repetitive measurements. The temporal analysis around clinical relapses showed increased sNfL Zscores at relapse onset ($p<0.001$), with elevated levels persisting up to 1 year post-event ($p<0.001$). sNfL Zscores were elevated in patients with future EDA occurring within a year of sample collection, but only when samples were taken at remission ($p<0.001$). Moreover, sNfL levels did not predict EDA beyond this 1-year window. These findings highlight the importance of monitoring sNfL as a dynamic biomarker in MS. Accurate knowledge of the temporal dynamics of sNfL is essential for the correct interpretation of at-risk patients in routine clinical practice. This approach could enhance clinical decision-making and improve MS care.

Sex Differences in Cognitive Reserve Mechanisms in Amyotrophic Lateral Sclerosis: A 2-[18F]FDG-PET Study

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Objectives: We aimed to investigate the influence of sex on cognitive reserve (CR) mechanisms in Amyotrophic Lateral Sclerosis (ALS) patients, focusing on education and its association with brain metabolism.

Materials and Methods: We examined brain 2-[18F]FDG-PET metabolic activity in 95 males (m-ALS) and 95 females (f-ALS), with comparable cognitive performance. Within each group, brain regions showing significant correlations between education and metabolism were identified. These regions were then used as seed areas in an interregional correlation analysis (IRCA) to assess patterns of metabolic connectivity across the brain.

Results: In the f-ALS group, brain metabolism positively correlated with education in the left fusiform gyrus, cerebellum, and pons. IRCA revealed positive correlations with the bilateral cerebellum, pons, right fusiform gyrus and cuneus, and left precuneus, and negative correlations with frontal lobes and caudate nuclei. Conversely, in the m-ALS group, brain metabolism negatively correlated with education in the left frontotemporal and insular cortices. IRCA revealed positive connectivity with bilateral frontotemporal and cingulate cortices and right parietal cortex, and negative correlations with bilateral cerebellum, motor cortex, and left lingual gyrus.

Discussion: The observed frontotemporal hypometabolism in m-ALS suggests a sex-based difference in CR. The negative association between education and metabolism in left frontotemporal-insular areas in m-ALS aligns with the CR hypothesis; the positive correlation in cerebellar regions in f-ALS may reflect sex-specific compensatory processes. Connectivity patterns observed through IRCA further support distinct neural strategies underpinning CR across sexes.

Conclusions: Our findings suggest that cognitive reserve mechanisms in ALS differ by sex, with males and females potentially engaging distinct neural pathways. Recognizing these differences could inform future prevention strategies.

Functional Maturation and Network Integration in Human Cortical Organoids and Assembloids

NDV 38

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Recent advancements in neuroscience have been significantly propelled by the development of three-dimensional (3D) neural organoids derived from human induced pluripotent stem cells (hiPSCs). These organoids faithfully recapitulate key aspects of human brain development and function, including cellular diversity, laminar organization, and mature transcriptional profiles. As such, they represent powerful in vitro platforms for investigating neurodevelopmental processes, elucidating the pathophysiology of neurodegenerative and neuropsychiatric disorders, and evaluating pharmacological responses. In our laboratories, we focus on cerebral organoids with an emphasis on dorsal and ventral forebrain cortical regions. To assess neuronal functionality, we employ whole-cell patch-clamp electrophysiology to characterize intrinsic excitability and synaptic transmission as well as synaptic properties of glutamatergic and GABAergic synapses. In parallel, we investigate neuronal subtype heterogeneity and synaptic protein distribution through immunohistochemistry followed by high-resolution microscopy techniques. Furthermore, to explore more complex inter-regional interactions, we are generating cortical assembloids by fusing distinct brain region-specific organoids. This approach aims to mimic the integration of neural circuits under physiological and pathological conditions. Ultimately, our overarching objective is to delineate the emergence, maturation, and long-term stability of functional neuronal networks within these two models of cerebral organoids. Our work seeks to advance understanding of human neural circuit formation and dysfunction, with applications in modeling neurodevelopmental and neuropsychiatric conditions such as autism spectrum disorders, major depressive disorder, and Alzheimer's disease.

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