NAPLES 2023

27 • 28 • 29 SEPTEMBER

BOOK OF ABSTRACTS

6th BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

CESTEV Aula Magna, 1st floor
Università degli Studi di Napoli Federico II - Facoltà di Biotecnologie
Via Tommaso De Amicis 95, 80145 Napoli

www.braynconference.com

Dear Young Neuroscientists,

The BraYn Association and the BraYn Conference team warmly welcome you to the **6th Brainstorming Research Assembly for Young Neuroscientists**, the BraYn conference.

Inspired and organized by young researchers from different scientific backgrounds, the focus of the BraYn conference is to promote brand-new collaborative connections between the potential future leaders of Neuroscience. The conference philosophy is simple: to **meet**, **connect**, **collaborate**, and **share**. We need to encourage cooperation between different research groups to broaden our horizons and improve the quality of our research.

Nearly **500 delegates** attended the BraYn 2022 conference. They included experienced senior researchers, attending as mentors and contributors and eight invited speakers, including the Nobel Prize winner Prof. Thomas Südhof.

By hosting neuroscientists worldwide, we aim to make the BraYn conference a **flagship event for young European researchers**, where novel national and international research networks will be established to improve future research activities. This goal was fully achieved in the past BraYn conferences, and we want to continue on this path in the future.

In addition to the traditional sessions on neurodegeneration, neuro-oncology, neuroinflammation, and neurophysiology & neural plasticity, **we have expanded the sessions** on neuroimaging and epilepsy, brain development & neurogenetics. Furthermore, to meet the needs and interests of researchers working in the clinical field, we have included a new session on clinical neuroscience in the scientific program. In this session, we will discuss patient-related observations derived from experimental research, clinical research, and clinical trials. We will focus mainly on biomarkers' potential role and use in the clinical setting and on new treatments for neurological diseases.

We are looking forward to welcoming you at the 6th BraYn conference!

The BraYn Staff

MAIN INDEX

You can click/tap on the menu items to go to the section of interest.
You can also click/tap on the names of the Presenting Authors (in the Scientific Program and in the Oral Comm. Index) or on the Abstract Codes (in the Posters Index) to go to the abstract.
Links to return to the main sections of this Book of Abstracts are located at the bottom of each page.

Committees

Sessions

Scientific Program

Oral Communications Index

Oral Communications

Posters Index

Poster Session 1

Poster Session 2

Poster Session 3

Sponsors & Patrons

STEERING COMMITTEE

Giovanni Ferrara PRESIDENT	IRCCS San Martino Hospital, Genoa (Italy)
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Barbara Bettegazzi	San Raffaele Scientific Institute, Milan (Italy)
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Eleonora Vannini	Neuroscience Institute - National Research Council of Italy, Pisa (Italy)

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Rosa C. Paolicelli	University of Lausanne (Switzerland)
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INVITED SPEAKERS

Martina Absinta	Institute of Experimental Neurology, Division of Neuroscience, Vita-Salute San Raffaele University and Hospital, Milan (Italy)
Séverine Boillée	Paris Brain Institute - ICM, INSERM, Pitié-Salpêtrière Hospital, Paris (France)
Michael Khalil	Clinical Department of General Neurology, Medical University of Graz (Austria)
Richard Morris	Department of Neuroscience, The University of Edinburgh (UK)
Claudio Procaccini	Italian National Research Council (CNR), Institute Experimental Endocrinology and Oncology "Gaetano Salvatore" IEOS, Naples (Italy)
Michal Schwartz	Weizmann Institute of Science, Rehovot (Israel)
Sebastian Sulis Sato	IRCCS San Martino Hospital, Genoa (Italy)
Giuseppe Testa	University of Milan and Human Technopole (Italy)
Varun Venkataramani	Universität Heidelberg, Neurological Clinic (Germany)
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Marlene Khin	University of Heidelberg (Germany)
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YOUNG EPILEPSY SECTION-ITALY, YES-ITALY, ILAE

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Luca De Palma	Rare and Complex Epilepsy Unit, Department of Neuroscience, Bambino Gesù Children's Hospital IRCCS, Rome (Italy)
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STARTING GRANT COMMITTEE

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CLINICAL NEUROSCIENCE

Clinical neuroscience is a translational field in which neuroscience data and basic research are coupled with clinical neurology to better understand the neural underpinnings of nervous system disorders, and to improve their diagnosis and treatment. In this session, we encourage the submission of data with a clear translational significance and real-world clinical applications. We will discuss patient-related observations derived from experimental research, clinical research, and clinical trials focusing especially on the potential role and use of biomarkers in the clinical setting and on new treatments for neurological diseases. We also welcome works describing clinical cases (or case-series) that directly discuss the application of new therapies or novel biomarkers in a clinical population.

NEUROINFLAMMATION

Neuroinflammation is 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.

NEUROPHYSIOLOGY & NEURAL PLASTICITY

We will focus on studies addressing the function of the nervous system and of its components, and the capacity of the nervous system to modify itself, functionally and structurally, in response to experience and injury. All levels of function and plastic changes are included, from the membrane and cell to systems and behaviour. Experimental approaches include molecular, cellular and developmental neurobiology, functional neuroanatomy, neurochemistry, neuropharmacology, electrophysiology, and behavioural analysis, in *in vivo*, *ex-vivo* and *in vitro* models in invertebrate or vertebrate species, including humans.

NEURO-ONCOLOGY

Neuro-oncology is an emerging field of investigation that studies nervous system tumors. As many of them 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 would ultimately lead to the development of novel therapeutic approaches for the treatment of life-threatening diseases such as glioma, and medulloblastoma. These topics will be discussed in depth during the NO session.

NEUROIMAGING

Neuroimaging exploits various techniques to image the structure, function, or physiology of the nervous system. Two main neuroimaging approaches exist: i) structural imaging, which evaluates the structure of the nervous system and allows the diagnosis of large-scale intracranial diseases (such as tumors, multiple sclerosis lesions, and stroke) and injuries (like traumatic brain injury); ii) functional imaging, which is used to diagnose metabolic diseases such as Alzheimer's disease, for neurological and cognitive psychology research, as well as for building brain-computer interfaces. The most commonly used techniques for neuroimaging are Computed Tomography (CT), Diffuse Optical Imaging (DOI), Event-Related Optical Signal (EROS), Magnetic Resonance Imaging (MRI), Arterial Spin Labeling (ASL), low to ultra-high frequency ultrasound with photoacoustics, Magnetoencephalography (MEG), Electroencephalography (EEG), Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT), and cranial or functional ultrasound imaging. In this session, we will discuss the use of these techniques, both alone and in combination, to investigate, detect, and understand various aspects of neurological diseases.

NEURODEGENERATION

Neurodegeneration is a key aspect of a large number of diseases characterized by progressive damage of the nervous system that leads to irreversible neuronal death, such as Parkinson's disease (PD) and Alzheimer's disease (AD). PD is 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. Rest tremor is often the first symptom recognized by the patient, but the illness sometimes begins with bradykinesia, and in some patients, tremor may never develop. AD is the most common type of dementia and it is an irreversible, neurodegenerative and progressive central nervous system disorder that slowly destroys memory and thinking skills, and, eventually, other mental abilities. Other examples of neurodegenerative diseases are tauopathies, narcolepsy, depression and psychiatric disorders. During the BraYn conference, we will be updated on the more recent advances in the field.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

Epilepsy, neurodevelopment and neurogenetics are deeply interconnected fields. Human neurodevelopment is a dynamic and extensive process, beginning at the pre-natal stages, driven by genetic information, and influenced by many environmental factors. The alteration of this process at different levels can lead to neurodevelopmental and psychiatric disorders such as autism spectrum disorder, intellectual disability, and epilepsy. Epilepsy is one of the most common neurological diseases globally. Its etiologies cover a wide range, from genetics to trauma, auto-immunity, and tumors. Unfortunately, available therapeutics only treat the symptoms but not the root cause of the disease. This complexity has pushed epilepsy research to embrace many different fields of neuroscience, to discover the pathophysiological processes that cause and sustain seizures, and potential therapeutic targets. In this session we discuss how new insights from the fields of epilepsy research, developmental disorder and neurogenetics can improve our understanding of the human brain and contribute to novel therapeutic perspectives.

SEPTEMBER 27 • Day 1

10:00 Registration

11:00 Opening Ceremony | **Giovanni Ferrara**

BRAYN STARTING GRANT SESSION

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

11:15 **Francesca Fagiani** (Starting Grant 2022 Winner)
The aging brain in Multiple Sclerosis: profiling senescence features in CNS cells.

11:30 Lecture | **Séverine Boillée** (Chairman: G.Nardo)
Microglia and macrophages for the progression of ALS.

12:00 Lunch Box with Poster Session 1

SESSION 1 • CLINICAL NEUROSCIENCE
ORAL COMMUNICATIONS

Chairpersons: F. Di Lorenzo, A. Bombaci, I. Battistella

14:00 Lecture | **Michael Khalil** (Chairman: S. Angiari)
CSF and blood biomarkers in neuroimmunological disorders.

14:30 **Simona Baldassari**
A Blood Brain Barrier (BBB) model to test novel therapeutic strategies for Glut-1 deficiency syndrome.

14:45 **Tommaso Sirito**
The early effect of cladribine versus fingolimod on clinical and MRI measures in relapsing-remitting multiple sclerosis.

15:00 **Elena Ellmeier**
Targeting pyruvate kinase M2 to limit T cell pathogenicity in multiple sclerosis.

15:15 **Luca Scaccini**
Nanostructured materials for the healing of peripheral nervous system (PNS) pathologies.

SESSION 2 • NEUROINFLAMMATION
ORAL COMMUNICATIONS

Chairpersons: V.A. Baldassarro, M. Bottero, G. D'Arrigo, M. Velasco, F. Sciarretta

15:30 Lecture | **Michal Schwartz** (Chairman: V. Chiurchiù)
Transforming understanding of brain immunity and targeting the immune system to defeat Alzheimer's disease.

16:00 **Fionä Caratis**
Immune cell migration towards the blood-brain barrier is mediated by EBI2 under inflammatory conditions.

16:15 **Giada Pessina**
Dendritic cells generated in the presence of specialized pro-resolving mediators display a tolerogenic effect on encephalitogenic T cells.

16:30 **BraYn Educational Symposium • Novartis ▶ Giuseppe Matarese**
The B cell therapy: the impact on bone marrow and lympho-myeloid balance.

16:45 **BraYn Educational Symposium • Beckman Coulter ▶ Valerio Chiurchiù**
Integrated and multi-dimensional approach to dissect the neuro-immune axis in neurodegenerative diseases.

17:00 **Federica Ricciardi**
PEA-OXA ameliorates allodynia, neuropsychiatric and adipose tissue remodeling induced by social isolation.

17:15 **Veronica Ceci**
Characterization of cerebellum alterations in a mouse model of Friedreich's ataxia.

17:30 Closing remarks

SEPTEMBER 28 • Day 2

SESSION 3 • NEUROPHYSIOLOGY & NEURAL PLASTICITY
ORAL COMMUNICATIONS

Chairpersons: P. Lippiello, G. Sansevero, G. Sbrini

9:00 Lecture | **Richard Morris** (Chairman: G. Sansevero)
The neurobiology of memory: Prospective facets and selective retention over time.

9:30 **Sara De Vincentiis**
Response of neural progenitor cells to mechanical stimulation in spinal cord injuries.

9:45 **BraYn Educational Symposium • Voden Medical ▶ Jason Hamlin**
Using human induced pluripotent stem cell-derived model systems to study neurodevelopment, neuronal maturation and neuroinflammation.

10:00 **BraYn Educational Symposium • Revvity Italia ▶ Sergio Braglia**
Innovative technologies for neuroscience application.

10:15 **Stefano Amoretti**
Identification of a novel factor promoting acetylcholine receptor clustering at the neuromuscular junction.

10:30 **Eleonora De Felice**
Physiological role of microglial diversity along the hippocampal longitudinal axis.

10:45 **Camilla Paraciani**
Using the FDA-approved drug vardenafil as a potential treatment to restore access to memories thought to be 'lost' in the sleep-deprived brain.

11:00 **BraYn Educational Symposium • Siemens Healthineers Italia ▶ Domenico Zacà**
Groundbreaking Gemini gradients with a unprecedented strength for whole-body performance on a 3T MRI.

11:15 Poster Session 2 and Lunch Box (from 12:00 pm)

SESSION 4 • NEURO-ONCOLOGY
ORAL COMMUNICATIONS

Chairpersons: G. D'Alessandro, E. Stanzani, E. Vannini, P. Blanco Carlón

13:30 Lecture | **Varun Venkataramani** (Chairwomen: G. D'Alessandro, E. Vannini)
Disconnecting brain tumor networks to tackle gliomas.

14:00 **Irene Appolloni**
Immunoevasive Phenotype of Glioma Cells: Hindering CD8 Lymphocyte Cytotoxicity through CD4 Lymphocyte Modulation.

14:15 **BraYn Educational Symposium • Euroclone ▶ Agnieszka Ciesielska**
Reimagine how you study the brain.

14:30 **Marta Ibáñez Navarro**
NKG2D car T cells target pediatric brain tumor cells in vitro and in a murine model of human glioblastoma in vivo.

14:45 **Veronica Marabitti**
Exploring the role of mitophagy in medulloblastoma stem cells dissemination and therapy resistance.

SESSION 5 • NEUROIMAGING
ORAL COMMUNICATIONS

Chairpersons: G. Ferrara, S. Cocozza, M. Tassan Mazzocco

15:00 Lecture | **Martina Absinta** (Chairwoman: B. Bettegazzi)
Decoding and modelling chronic inflammation in multiple sclerosis.

15:30 **Filomena Grazia Alvino**
Synaptic-dependent developmental dysconnectivity in 22q11.2 deletion syndrome.

15:45 **Sara Bosticardo**
A novel method to estimate Multiple Sclerosis connectomes considering lesional tissue information.

16:00 **BraYn Educational Symposium • Femtonics ▶ Zsolt Iván**
Neuroscience, Illuminated. Femtonics, forming the future of neuroscience.

16:30 **BraYn Educational Symposium • Evident ▶ Luca Cevenini**
"The power to see more": optical strategies to maximize your neuroscience research.

16:45 **Luigi Lorenzini**
Alzheimer's genetic pathways are associated with changes in separate imaging biomarkers in non-demented individuals.

17:00 General assembly for members of the BraYn Association Ets

17:30 Closing remarks

SEPTEMBER 29 • Day 3

SESSION 6 • NEURODEGENERATION ORAL COMMUNICATIONS

Chairpersons: S. Amoretti, S. Negro, N. Piera Palomba

9:15 **Veronica Zatta**
HSV-1 infection in mouse enteric nervous sistem: a trigger for Alzheimer's disease-like neurodegeneration hallmarks.

9:30 **Annamaria Lia**
Recovering stimulation of astrocyte Ca2+ signal to shed light on Alzheimer's Disease.

9:45 **Ambra Del Grosso**
Rapamycin ameliorates the pathological phenotype in the Twitcher mouse by autophagy activation.

10:00 **BraYn Educational Symposium • Merck ▶ Gianmarco Bellucci**
Our evolving understanding of MS pathophysiology.

10:15 **Cristina Somma**
Pharmacological stimulation of autophagy to rescue proteinopathy and cognitive decline in mucopolysaccharidosis-IIIA.

10:30 **Stefania Scala**
Design of an innovative 3D model for blood-brain barrier towards improved translational medicine approaches.

10:45 **BraYn Educational Symposium • Miltenyi Biotec ▶ Vito Antonio Baldassarro**
Blaze light-sheet microscopy: quantifying 3D images.

11:00 Technical Talk | **Sebastian Sulis Sato & Letizia Zullo** (Chairman: G. Ferrara)
Combining molecular biology with in vivo microscopy to study brain networks.

11:50 Lecture | **Claudio Procaccini** (Chairman: G. Ferrara)
Metabolic control of immunological self-tolerance.

12:20 Poster Session 3 and Lunch Box

SESSION 7 • EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS • ORAL COMMUNICATIONS

(curated by Young Epilepsy Section-Italy, YES-Italy, ILAE)
Chairpersons: M. Rasile, P. Scudieri, G. Ferrara, B. Casadei Garofani

14:00 Lecture | **Giuseppe Testa** (Chairman: G. Ferrara)
Towards high resolution maps of neuropsychiatric conditions: translating endophenotypes from cohorts to organoids and back.

14:30 **Greta Volpedo**
Unique metabolic signatures may contribute to the development of post-traumatic epilepsy in mice.

14:45 **Carla Liaci**
The roles of RAC1 regulators ARHGAP15, TRIO, and ARHGEF6 in physiological and pathological forebrain development.

15:00 **Federica Baronchelli**
Mechanisms of synaptic dysfunction in the Angelman syndrome.

15:15 Questions & Answers

15:30 Closing remarks • BraYn Awards (Best Oral & Poster Presentation, BraYn Starting Grant, Creative BraYns)

SESSION 1 • CLINICAL NEUROSCIENCE

Simona Baldassari
A Blood Brain Barrier (BBB) Model to Test Novel Therapeutic Strategies for GLUT-1 Deficiency Syndrome

Tommaso Sirito
The early effect of cladribine versus fingolimod on clinical and MRI measures in relapsing-remitting multiple sclerosis

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Luca Scaccini
Nanostructured materials for the healing of peripheral nervous system (PNS) pathologies

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Veronica Ceci
Characterization of Cerebellum Alterations in a Mouse Model of Friedreich's Ataxia

SESSION 3 • NEUROPHYSIOLOGY & NEURAL PLASTICITY

Sara De Vincentiis
Response of Neural Progenitor Cells to Mechanical Stimulation in Spinal Cord Injuries

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Identification of a novel factor promoting acetylcholine receptor clustering at the neuromuscular junction

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Physiological role of microglial diversity along the hippocampal longitudinal axis

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SESSION 4 • NEURO-ONCOLOGY

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NKG2D CAR T Cells Target Pediatric Brain Tumor Cells In Vitro and in a Murine Model of Human Glioblastoma In Vivo

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SESSION 5 • NEUROIMAGING

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SESSION 7 • EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

Greta Volpedo
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The roles of RAC1 regulators ARHGAP15, TRIO, and ARHGEF6 in physiological and pathological forebrain development

Federica Baronchelli
Mechanisms of synaptic dysfunction in the Angelman syndrome

POSTER SESSION 1 • September 27, 12:00-14:00

CLINICAL NEUROSCIENCE

CN01 | Serum NfL levels and cognitive performance in persons with multiple sclerosis • **Rina Demjaha**

CN02 | Cognitive frailty and oxygen-ozone therapy: differential expressed genes as predictive biological markers of response/improvement to treatment • **Elisabetta Mori**

NEUROINFLAMMATION

NI01 | Investigation of neuronal Ca2+ hyperexcitability and neuroinflammatory state in an Alzheimer's disease mouse model • **Martina Bedetta**

NI02 | Is Circulating AgRP Neuropeptide a Novel Mediator of Neuroimmune Communication in Multiple Sclerosis? • **Eleonora Cornacchia**

NI03 | Spinal and supraspinal characterization of an inflammatory CFA-induced model of Vulvodynia • **Antimo Fusco**

NI04 | Unveiling the role of exosomal miRNAs in the spreading of neuroinflammation • **Francesca Massenzio**

NI05 | Expression and function of TMEM206/PACC1 alternative isoforms • **Serena Tamburro**

NI06 | Systemic inflammation induced in young Tg2576 Alzheimer-mice anticipates the onset of cognitive decline • **Lorenzo Zanella**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP01 | Cardiac functional and structural abnormalities in a mouse model of CDKL5 Deficiency Disorder • **Giulia Candini**

NP02 | Epigenetic effects of exposure to the endocrine disruptor ethinyl estradiol in differentiated SH-SY5Y cells • **Camilla Corrieri**

NP03 | Role of microglia in the GABAergic network plasticity in glioma • **Erika Di Pietro**

NP04 | Recruitment of inhibitory neurons in a murine model of inflammatory pain • **Emma Merlin**

NP05 | Altered neuronal morphology and synaptic protein synthesis in brain cortex of mouse model for Angelman Syndrome: rescuing effect of serotonin receptor 7 stimulation • **Amelia Pizzella**

NP06 | Generation of induced pluripotent stem cells lines to study the cognitive deficits associated with Noonan Syndrome • **Giulia Sbrini**

NP07 | CXCR4 in neurophysiology and neurodegeneration • **Marika Tonellato**

NEURO-ONCOLOGY

NO01 | The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and is involved in medulloblastoma tumorigenesis • **Gennaro Adabbo**

NO02 | Exploring the role of mechanoreceptor Piezo1 in glioblastoma • **Pablo Blanco Carlón**

NO03 | Unmasking Myc's Role in Glioblastoma Clonal Warfare • **Davide Ceresa**

NO04 | Gold nanoparticles (AuNPs) in the radio-sensitization of glioblastoma cells • **Laura Coppola**

NO05 | Autophagy inhibition enhances Natural Killer cell- based therapy in high-risk Medulloblastoma • **Manuela Giansanti**

NEUROIMAGING

NIM01 | Development of a deep-learning tool for the detection and segmentation of contrast-enhanced lesions in multiple sclerosis patients • **Martina Greselin**

NIM02 | Mapping structural disconnection and morphometric similarity in Multiple Sclerosis: a longitudinal study • **Mario Tranfa**

NEURODEGENERATION

ND01 | Bridging the gap from mice to humans: using genetic predictions to investigate the effect of circulating blood factors on the human brain • **Federica Anastasi**

ND02 | A potential therapeutic strategy for CMT2A: combined RNA interference and gene therapy in vitro and in vivo disease models • **Alessia Anastasia**

ND03 | Betaine is a substrate of GAT1 that can modulate extracellular GABA • **Manan Bhatt**

ND04 | Role of GDF15-GFRAL pathway in the regulation of feeding behaviour in hSOD1-G93A mice • **Ludovica Maria Busdraghi**

ND05 | Obstructive sleep apnoea syndrome and effects on central nervous system: from a new in vitro model to peripheral plasma biochemical markers to study cognitive decline insurgence • **Martina De Felice**

ND06 | Generation of hiPSC derived from PD-sporadic patients to dissect the role of rare variants in Parkinson's disease pathogenesis • **Giorgio Fortunato**

ND07 | Visualizing Tau Tangles in AD Retina with a BODIPY-based Fluorescent Ligand • **Ylenia Gigante**

ND08 | Intramuscular IL-10 Administration Enhances the Activity of Myogenic Precursor Cells and Improves Motor Function in ALS Mouse Model • **Cassandra Margotta**

ND10 | Generation and characterization of 3D neuromuscular organoids for the study of Amyotrophic Lateral Sclerosis • **Michela Mochi**

ND11 | Analysis of circulating lipids and metabolites of Parkinson's disease patients undergoing different types of therapy: a pilot study • **Nicole Piera Palomba**

ND12 | An insight into Alzheimer's disease pathogenesis: cell-laden hydrogels and oxidative stress • **Valentina Peluso**

ND13 | In vitro evaluation of the antioxidant capacity of novel benzofuran-2-ones in a cellular model of neurodegeneration • **Sofia Scibetta**

ND14 | Alteration of lipid metabolism in the pathogenesis of Hereditary Spastic Paraplegia: unraveling the mechanisms to recover cell function • **Sonia Sonda**

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

EBN01 | EPM1 3D human model: altered synaptic plasticity and neuronal morphology • **Natalia Abate**

EBN02 | A gene therapy approach for focal epilepsy based on GABAA receptor overexpression • **Martina Bonfanti**

EBN03 | Development of gene therapy as a possible cure for Creatine Transporter Deficiency Syndrome • **Federica Di Vetta**

EBN04 | N-acetyl cysteine rescues cortical glial cell populations and results in functional improvements in a mouse model of primary autosomal recessive microcephaly 17 (MCPH17) • **Maryam Khastkhodaei Ardakani**

EBN05 | Characterization of early communicative deficits and social behaviors in a mouse model of CDKL5 deficiency disorder • **Nicola Mottolese**

EBN06 | The epilepsy-related protein TBC1D24 regulates V-ATPase activity and pH homeostasis in neurons • **Sara Pepe**

EBN07 | Therapeutic Opportunities in Lafora Disease • **Gabriele Trentini**

POSTER SESSION 2 • September 28, 11:15-13:30

CLINICAL NEUROSCIENCE

CN03 | 3D-stem cell spinal cord model to study the therapeutic mechanisms of risdiplam-like compound for Spinal Muscular Atrophy • **Andrea D'Angelo**

CN04 | Human iPSC-based cellular systems to model Autosomal dominant leukodystrophy • **Ingrid Battistella**

NEUROINFLAMMATION

NI07 | Interplay Between Microglial Receptor TREM2 and Maternal Immune Challenges in Schizophrenia • **Matteo Bizzotto**

NI08 | Elucidate the role of microglia in fmr1ko mice model • **Antonella Borreca**

NI09 | dCK intracellular localization is regulated by serine 11 phosphorylation and predicts the response to cladribine treatment in T cells • **Stella Maglio**

NI10 | Pathogenic insights for reducing thalamic hemorrhage-induced pain and depression by regulating microglia and MED1/BDNF/TrkB signaling • **Andrea Maria Morace**

NI11 | Alginate displays anti-inflammatory properties in the ibotenic-lesioned rat brain • **Federica Raimondi**

NI12 | Neuroprotective effect of butyrate in Friedreich's Ataxia models • **Francesca Sciarretta**

NI13 | BTK inhibitors modulate microglial extracellular vesicles release to regulate remyelination in Multiple Sclerosis • **Matteo Tartaglia**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP08 | Sub-toxic glyphosate treatment alters GABAergic synapses in murine hippocampal neurons • **Giuseppe Chiantia**

NP09 | Exposure to interleukin 15 modulates hippocampal synaptic transmission and interfere with episodic memory formation via serotonin receptor activation • **Maria Amalia Di Castro**

NP10 | 5-HT7R Stimulation Modulates Synaptic Protein Synthesis in Autism Spectrum Disorders • **Kardelen Dalım Filiz**

NP11 | Natural Killer cells modulate sleep pressure via Interferon-gamma • **Alessandro Mormino**

NP12 | Voluntary running ameliorates brain development and behavioral performance in a mouse model of CDKL5 deficiency disorder • **Beatrice Uguagliati**

NEURO-ONCOLOGY

NO06 | Astrocytes-derived small extracellular vesicles hinder glioma growth by the regulation of the volume-regulated anion channel (VRAC) • **Mariassunta De Luca**

NO07 | Dissecting the ERAP1/ACP2 interplay in Hedgehog signaling control and Hedgehog-dependent medulloblastoma • **Ludovica Lospinoso Severini**

NO08 | Phospholipases as potential prognostic biomarkers and targets in the development of new therapeutic strategies for glioblastoma • **Maria Vittoria Marvi**

NO09 | Enhancing Natural Killer cell cytotoxicity to counteract Glioblastoma growth • **Letizia Mazzarella**

NO10 | The use of animal proteins in the diet: impact on gut microbiota and glioma growth in a preclinical murine model • **Alice Reccagni**

NEUROIMAGING

NIM03 | Investigating Cerebral Lateralization during Visual Stimulation using Functional Transcranial Doppler: A Preliminary Study • **Rosita Rabbito**

NIM04 | Evaluation of prodromal markers of Parkinson's disease in a progressive neurotoxic mouse model using multi-tracer PET-CT imaging • **Margherita Tassan Mazzocco**

NEURODEGENERATION

ND15 | Nanoparticles used for the targeted delivery of PACAP peptide through an in vitro blood-brain barrier • **Teresa Barra**

ND16 | Brain organoids: a promising approach to investigate neurodegeneration in MSA-C and SCA2 • **Lorenzo Brambilla**

ND17 | Specific alterations of circulating and cellular lipids levels in Parkinson's disease patients carrying TMEM175 mutations • **Federica Carrillo**

ND18 | Identification of a novel class of small molecules for the treatment of TREM2-based diseases • **Mario Dell'Isola**

ND19 | The role of G2019S LRRK2 in excitatory/inhibitory imbalance of Parkinson's disease • **Angela Di Iacovo**

ND20 | Effect of 3D Synthetic Microscaffold Nichoid on the Morphology of Cultured Hippocampal Neurons and Astrocytes • **Arianna Giani**

ND21 | MicroRNA as potential circulating biomarkers for AD diagnosis and novel therapeutic targets • **Agnese Graziosi**

ND22 | What about astrocytes? Elucidating the new role of astrocytes in Autosomal Dominant Leukodystrophy • **Foteini-Dionysia Koufi**

ND23 | Increased apoptotic cell death and autophagy alteration in Riboflavin Transporter Deficiency • **Chiara Marioli**

ND24 | Boosting peripheral nerve regeneration in ALS by the CXCL12-CXCR4 axis • **Samuele Negro**

ND25 | Impaired bioenergetic profile and proliferation in neuron progenitor cells from iPSCs of patients affected by AGC1 deficiency • **Eleonora Poeta**

ND26 | Intensive exercise training counteracts nigrostriatal degeneration and striatal structural changes in an alpha-synuclein based experimental model of Parkinson's disease • **Federica Servillo**

ND27 | Neuroprotective activity of the new metabotropic Glutamate Receptor 3 Positive Allosteric Modulator in Parkinson's Disease in vitro and in vivo models • **Giulia Urone**

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

EBN08 | Metabolic supplementations and epigenetic alterations in in vitro AGC1 deficiency models • **Giorgia Babini**

EBN09 | Auditory-hippocampal alterations and behavioural fluctuation during development in a mouse model of autism and epilepsy • **Lorenzo Ciano**

EBN10 | Dopaminergic alteration triggers autistic-behaviours in lysosomal storage disorders • **Maria De Risi**

EBN11 | Further insights into Allan-Herndon-Dudley syndrome: characterization of two genetic variants in SLC16A2 gene • **Letizia Esposito**

EBN12 | Characterization of the molecular mechanism underlying GABAA-receptor defects in CDKL5 deficiency disorder • **Claudia Lora**

EBN13 | CACNA1A mutations impair neuronal induction and function • **Ilaria Musante**

EBN14 | Dopamine Transporter DNA Methylation modulation evoked by stress in university students • **Alessandro Piccinini**

EBN15 | Investigating the role of rare missense variants in RAB11B in Autism Spectrum Disorder • **Laura Sandoni**

EBN16 | The functional role of CDKL5 at the inhibitory synapse and its interaction with the cytoplasmatic collybistin-gephyrin complex • **Serena Valastro**

POSTER SESSION 3 • September 29, 12:20-14:00

CLINICAL NEUROSCIENCE

CN05 | CRISPR/Cas9 and piggyBac Transposon-Based Conversion of a Pathogenic Biallelic TBCD Variant in a Patient-Derived iPSC Line Allows Correction of PEBAT-Related Endophenotypes • **Federica Benigni**

CN06 | Identification of novel antibodies in patients with small fiber neuropathy • **Luana Morelli**

NEUROINFLAMMATION

NI14 | Nerve growth factor influences microglial activity in vivo via TrkA receptors • **Giulia Borgonovo**

NI15 | A comprehensive molecular imaging study in a mouse model of CMT1B neuropathy • **Mattia Camera**

NI16 | Butyrate decreases the regulatory function of human natural killer cells and promotes their maturation • **Federico Carlini**

NI17 | Transcranial magnetic stimulation restores glial response and microvasculature integrity in experimental Parkinson's disease • **Maria De Carluccio**

NI18 | NLRP3-inflammasome inhibition by Leishmania-derived factors in the neuropathogenesis of Alzheimer's Disease (AD): assessing the molecular and therapeutic role • **Francesca La Rosa**

NI19 | Potential role of the hydroxyl carboxylic acid receptor type 2 (HCAR2) in microglia pathophysiology • **Michela Perrone**

NI20 | Microglia-released extracellular vesicles counteract age-related cognitive impairment and restore microglia homeostasis in the aging brain of male and female mice • **Arianna Rinaldi**

NI21 | Resolution of inflammation is impaired in Parkinson's disease and is rescued by specialized pro-resolving lipid mediator RvD1 through targeting microglia • **Marta Tiberi**

NI22 | Role of Pentraxin3 in neurodevelopmental disorders • **Cecilia Zen**

NEUROPHYSIOLOGY & NEURAL PLASTICITY

NP13 | The effect of daily exercise on improving post-injury symptoms in a mild traumatic brain injury mouse model • **Roozbe Bonsale**

NP14 | Early-life interference with the attachment bond induces depressive-like behaviour and potentiated Ih current in VTA dopaminergic neurons of adult DBA/2J females • **Gilda Chilà**

NP15 | Astrocyte diversity across mammals: a comparative analysis on distribution and single cell morphology • **Caterina Ciani**

NP16 | Homeostatic plasticity in response to short-term monocular deprivation in the visual cortex of adult mice • **Irene Di Marco**

NP17 | Investigating Cerebellar Abnormalities in a mouse model of lysosomal lipid storage disease: Implication for Social Behavior • **Greta Massa**

NP18 | The Brain Anti-Reward Center In Autism Spectrum Disorder • **Manuel Scorrano**

NP19 | Dissecting the mechanism of action and neuroplastic potential of molecules targeting NMDA or 5-HT receptors • **Sonia Sonda**

NEURO-ONCOLOGY

NO11 | Identification of a novel KDM5C-related signature in glioblastoma multiforme • **Denise Drongitis**

NO12 | Developing a Mouse Brain Organoid Model of Glioma Progression • **Chiara Riviera**

NO13 | Cancer-neuronal cross talk in Glioblastoma: how neurons sustain tumor progression • **Chiara Saulle**

NEUROIMAGING

NIM05 | RUBIK: a fluorescent reporter for combinatorial Cre and Flp recombination • **Giada Pessina**

NIM06 | Gradient of dentate-thalamo-cortical tract microstructural disruption: applying diffusion MRI profilometry in Friedreich ataxia • **Mario Tranfa**

NEURODEGENERATION

ND28 | Investigating the role of large microglial extracellular vesicles carrying pathogenic misfolded proteins in Alzheimer's disease and their interaction with neurons • **Elisabetta Battocchio**

ND29 | The interplay between Rab proteins and mitochondrial dysfunction in PD pathology • **Martina Brughera**

ND30 | Developing a localised GDNF gene therapy to treat neurodegenerative diseases • **Lucia Crippa**

ND31 | Analysis of astrocyte calcium activity in alpha7 nicotinic receptor KO Alzheimer's disease mouse model • **Alessandro Di Spiezio**

ND32 | The UPR response and ER stress in a mouse model of Alzheimer's disease obtained by intracerebroventricular injection of β-amyloid oligomers at different ages • **Luca Ghelli**

ND33 | The role of astrocytic Ca2+ dynamics in Alzheimer's disease associated neuroinflammation • **Neha Kachappilly**

ND34 | Modeling Tauopathy-associated neurodegeneration in human iPSC-derived 2D and 3D retinal cultures • **Lorenza Mautone**

ND35 | Identification of Sex-specific autophagy enhancers for dementia • **Mariagrazia Monaco**

ND36 | A novel neural stem cell therapy targeting upper motor neurons provides better outcomes in Amyotrophic Lateral Sclerosis mice models • **Lorenzo Quetti**

ND37 | Corroboration of Stathmin-2 in human and murine models of Spinal Muscular Atrophy as potential therapy target • **Luca Sali**

ND38 | The role of intracellular calcium in GBA2-linked hereditary spastic paraplegia • **Chiara Sinisgalli**

ND39 | An in vivo protocol for screening and readily assessable neurobehavioral investigation in early CRISPR-Cas9 zebrafish mutants of a rare neurodegenerative condition • **Martina Venditti**

ND40 | Emerging value of olfactory neuronal Prokineticin-2 as novel target in Parkinson's disease • **Martina Vincenzi**

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

EBN17 | Human iPSC derived micropatterning models as a new in vitro system for GNAO1 disease modelling and drug testing • **Maria Cristina Benedetti**

EBN18 | Targeting adenosine A2A receptor in FXS-patient derived human cortical organoids and cortical culture for animal free drug discovery and repositioning • **Chiara D'Antoni**

EBN19 | Pol III-related Leukodystrophy -affected patients present a profound transcriptional dysregulation and an impairment in protein synthesis • **Letizia Esposito**

EBN20 | A loss of function zebrafish model of a new disease gene involved in cargo sorting and autophagy recapitulates patients' axonopathy and cerebral atrophy and provides insights into disease mechanism • **Giulia Fasano**

EBN21 | Novel frontiers in Aicardi-Goutières sydrome: characterization of a RNU7-1 mutation • **Erika Maghraby**

EBN22 | Mutations in the stretch-activated ion channel TMEM63B associate with developmental and epileptic encephalopathies and progressive neurodegeneration • **Cristiana Pelorosso**

EBN23 | scRNA transcriptomics reveals a defective corticogenesis in a mouse model of ARX-DEE • **Lucia Verrillo**

EBN24 | Epileptiform activity in a non-epileptic control rat: spontaneous syndrome or lesion-induced epilepsy? • **Beatrice Casadei Garofani**

COMMUNI ORAL CATIONS

A Blood Brain Barrier (BBB) Model to Test Novel Therapeutic Strategies for GLUT-1 Deficiency Syndrome

Simona Baldassari [1] - Valentina Castagnola [2] - Serena Cappato [1] - Ilaria Musante [1] - Renata Bocciardi [1,3] - Paolo Scudieri [1,3] - Fabio Benfenati [2] - Federico Zara [1,3]

Unit of Medical Genetics, IRCCS G. Gaslini, Genova, Italy (1) - Center for Synaptic Neuroscience and Technology, Istituto Italiano di Tecnologia, Genova, Italy (2) - Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DINOGMI), University of Genova, Genova, Italy (3)

Glucose transporter type 1 (GLUT1) is a critical protein allowing glucose efflux to the brain through the Blood-Brain-Barrier (BBB). Monoallelic or bi-allelic mutations in the GLUT1 encoding gene - *SLC2A1* - result in GLUT1-deficiency (GLUT1DS) featuring intractable seizures, intellectual disability, ataxia, and dystonia.
Pathogenic mechanisms are still unclear and specific therapeutic approaches lacking due to the difficulty to obtain appropriate human-derived *in vitro* models.
In this study we aimed to generate a well-established *in vitro* Transwell model of BBB with brain endothelial cells derived from controls and GLUT1DS patients's induced pluripotent stem cells (iPSCs). We selected two patients carrying different mutations and showing a severe (p.Leu124Trpfs*12) and a milder (p.Arg400Cys) GLUT1-deficiency phenotype. We characterized the BBB model with standard tests of BBB functionality, including transendothelial electrical resistance (TEER), GLUT1 expression, immunocytochemistry for endothelial and tight junction markers and paracellular transport across the barrier. The results indicated a different rate of BBB differentiation between the severe and the milder patients' cells with respect to the controls, probably due to the drastic impairment in the nutritional molecules uptake. To improve the BBB model, we are moving from a static to dynamic culture system ensuring optimal microenvironment conditions and mimicking the in vivo physiology.
A standardized BBB model could be used to test novel therapeutical approaches aimed at enhancing BBB glucose permeability. In this regard, we will initiate the search of genetic (*e.g.* by CRISPRa technology) and pharmacological tools able to increase the expression of *SLC2A1* at the transcriptional and translational levels.

The early effect of cladribine versus fingolimod on clinical and MRI measures in relapsing-remitting multiple sclerosis

Tommaso Sirito [1] - Giacomo Boffa [1] - Caterina Lapucci [1] - Daniele Boccia [1] - Kenda Aluan [1] - Lucio Castellan [2] - Antonio Uccelli [1] - Alice Laroni [1] - Elisabetta Capello [1] - Maria Cellerino [1] - Matilde Inglese [1]

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A treatment with cladribine tablets (CLAD) requires two cycles of treatment in Year 1 and Year 2. Data regarding its efficacy during the first 12-moths of treatment in comparison with fingolimod (FINGO) in the real-life setting are still sparse. Our aim was to compare the early impact of CLAD (I course) and FINGO in terms of clinical and MRI outcomes in a cohort of relapsing-remitting multiple sclerosis (RRMS) patients (pts). Patients starting CLAD or FINGO were prospectively enrolled and underwent complete clinical evaluation [including assessment of Expanded-Disability-Status-Scale (EDSS), annualized relapse rate (ARR), nine-hole-peg–test (9HPT) and timed-25-foot-walk (T25 FW)] and 3T-MRI (Siemens MAGNETOM) at baseline and after 12-months follow-up. We used the NIH Toolbox Standing Balance as a measure of balance and calculated theta values at baseline and 1 year. Changes in percentage-brain-volume-change (PBVC) were measured. The probability of disability-/relapse- /MRI activity-free survival, NEDA-3 and NEDA-4 status (defined as NEDA3 + PBVC<0.4% per year) were calculated with Kaplan-Meier estimator.
A total of 63 patients were included in the analysis, [32 CLAD and 31 FINGO; females: 65.1%; mean age, disease duration, ARR in the two previous years: 39.4+14.2, 8.6+9.6 years, 0.47+0.56; median (range) EDSS and number of previous DMTs: 1.5 (0-5.5) and 1 (0-3)]. No differences in terms of age, sex, disease duration, ARR in the two previous years, number of previous treatments, EDSS, total brain volume and lesion load were found between the two groups at baseline. At 1-year FU, MRI-inflammatory-activity-free survival was 81.3% and 74.2% (p=0.55) and relapse-free survival was 93.8% and 100% (p=0.16) in CLAD and FINGO treated pts, respectively. Progression-free survival was 100% in both groups. Mean PBVC was -0.6+1.5% (-0.48+1.6% in CLAD *vs* -0.76+1.4% in FINGO pts; p=0.82). NEDA-3 status was achieved in 81.3% and 74.2% (p=0.57) while NEDA-4 in 35.7% and 27.3% (p=0.53) of pts in CLAD and FINGO groups, respectively. During the first year of treatment 4/32 (12 %) of CLAD treated patients and 2/31 (6%) FINGO treated pts discontinued therapy due to inefficacy (p=0.41). Although not statistically significant, there was a decrease of 0.63+3.0 *vs* an increase of 0.9+3.8 seconds at 9HPT (p=0.13), an increase of 0.46+3.2 *vs* 1.3+3.3 (p=0.51) seconds at T25FW tests and a decrease of 0.19+0.55 *vs* 0.06+0.74 (p=0.57) of theta score at NIH Toolbox Standing Balance in CLAD vs FINGO treated pts, respectively.
In conclusion, although a complete CLAD treatment course requires the 2-dose 2-year protocol, our findings suggest that its efficacy is comparable to FINGO even during the first 12-months, though should be confirmed by larger analysis.

Targeting pyruvate kinase M2 to limit T cell pathogenicity in multiple sclerosis

Elena Ellmeier [1] - Anika Stracke [1] - Katharina Seifried [2] - Cansu Tafrali [2] - Michael Khalil [2] - Stefano Angiari [1]

Medical University of Graz, Division of Immunology, Graz, Austria (1) - Medical University of Graz, Division of General Neurology, Graz, Austria (2)

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system (CNS) in which infiltration of leukocytes into the CNS leads to neuronal death, cognitive impairment, and disability. Among immune cells, T lymphocytes are key players in MS pathogenesis, controlling the development of CNS inflammation. Immunometabolism studies have shown that T cells regulate their activity by modulating their intracellular metabolic profile, and that targeting T cell metabolism represents a novel strategy for the treatment of autoimmunity. In particular, recent works suggested that the glycolytic enzyme pyruvate kinase M2 (PKM2) may be a potential therapeutic target in T cell-mediated autoimmune neuroinflammation. PKM2 can translocate into the nucleus in its monomeric/dimeric form, where it performs moonlighting functions, such as regulation of gene transcription. Several pre-clinical studies have demonstrated that PKM2 moonlighting activity controls T cell pathogenicity in the CNS and modulates neuroinflammatory responses in experimental autoimmune encephalomyelitis, a mouse model of MS. This project aimed to investigate the role of PKM2 in T cell inflammatory potential in MS. We first showed that MS patients express higher PKM2 levels than control individuals in circulating T cells, with differential expression depending on the MS forms. We then evaluated whether limiting PKM2 moonlighting activity with the PKM2 allosteric activator TEPP-46 may impact the inflammatory profile of T cells from MS patients. We found that TEPP-46 inhibits T cell proliferation and decreases the production of pro-inflammatory cytokines by T cells, suggesting that PKM2 may control T cell pathogenicity in MS patients. Overall, our data indicate that PKM2 may represent a novel biomarker for disease activity, and that targeting PKM2 moonlighting functions may be of relevance in MS. PKM2 allosteric activators thus represent promising pharmacological tools for MS treatment.

Nanostructured materials for the healing of peripheral nervous system (PNS) pathologies

Luca Scaccini [1] - Antonella Battisti [2] - Mariacristina Gagliardi [2] - Ambra Del Grosso [1] - Miriam De Sarlo [2] - Laura Colagiorgio [2] - Sara Carpi [2] - Marco Cecchini [2] - Ilaria Tonazzini [2]

Scuola Normale Superiore, NEST - National Enterprise for nanoScience and nanoTechnology, Pisa, Italy (1) - Istituto Nanoscienze (NANO) - CNR, NEST - National Enterprise for nanoScience and nanoTechnology, Pisa, Italy (2)

Peripheral neuropathies are a condition in which peripheral nerves are damaged. This condition affects millions of people every year worldwide and can be caused by external trauma, or by pathologies that impact peripheral nervous system components. Biocompatible scaffolds are emerging as important tools to promote nerve regeneration in case of resection. However, when there is no physical damage of the nerve an effective drug delivery system is still lacking. To address these issues, we are working on two different approaches. We already demonstrated that nano/micro-grooved substrates are capable to direct neuronal and glial cell differentiation, polarization, and migration. We developed microstructured scaffolds, with specific directional topographies and tuneable stiffness, for peripheral nerve regeneration: our scaffolds, made of biodegradable and soft materials (compliant with nerve mechanics), were tested in vitro with neuronal and glial cell models. Moreover, the restricted permeability of nerves, due to the presence of the blood-nerve barrier (BNB), makes difficult to transport drugs into their structure. Polymeric nanoparticles are under investigation for their ability to pass biological barriers (such as the blood-brain barrier). We developed polymeric nanoparticles (NPs) functionalized with peripheral nerve targeting molecules, procaine and a peptide, NP41. We tested their biocompatibility and internalization capability in vitro in neural cell models. These NPs can be further loaded with drugs of interest. These two strategies can be either used as stand-alone systems or can be further combined to create innovative devices (e.g. scaffolds functionalized/enriched with NPs loaded with compounds of interest). Such devices would be able to provide both a physical support for regeneration, and a controlled release of drugs for treating a wide variety of pathological conditions impacting PNS (e.g. nerve trauma, resections, neurodegenerative disorders).

Immune cell migration towards the blood-brain barrier is mediated by EBI2 under inflammatory conditions

Fionä Caratis [1] - Bartosz Karaszewski [2] - Tomomi Furihata [3] - Aleksandra Rutkowska [1]

Medical University of Gdansk, Department of Anatomy and Neurobiology, Gdansk, Poland (1) - Medical University of Gdansk & University Clinical Center, Department of Adult Neurology, Gdansk, Poland (2) - Tokyo University of Pharmacy and Life Sciences, Laboratory of Clinical Pharmacy and Experimental Therapeutics, Tokyo, Japan (3)

Multiple sclerosis (MS) is a chronic, neurodegenerative and neuroinflammatory disease characterised by the entrance of peripheral immune cells into the central nervous system via a disruption of the blood-brain barrier (BBB). The infiltrating immune cells induce inflammatory signalling and attack the myelin sheaths surrounding the neuronal axons, leading to their neurodegeneration and death. The oxysterol 7α,25OHC is a natural ligand for the Epstein-Barr virus-induced receptor 2 (EBI2) which, among other functions, regulates immune cell migration. We specifically showed that brain microvessels express the EBI2 receptor as well as the enzymes necessary for 7α,25OHC synthesis (CH25H and CYP7B1) and that the oxysterol pathway is upregulated in brain's plaques in multiple sclerosis but also in the cerebrospinal fluid of MS patients. Using human in vitro BBB models, comprised of endothelial cells, pericytes and astrocytes, we characterised the expression of EBI2 and EBI2-related enzymes after inflammatory stimuli in the cells forming the BBB and how they impact the BBB permeability. Finally, we investigated immune cells migration patterns with immortalised monocytes and lymphocytes from MS and non-MS patients towards our in vitro spheroid and transwell models of the BBB. The BBB was stimulated with inhibitors of the EBI2/oxysterol pathway and showed that immune cell migration is dependent on the EBI2/oxysterol pathway. Thus, modulating this pathway directly at the BBB represents a therapeutic target in MS to prevent immune cell migration towards the brain parenchyma.

Dendritic cells generated in the presence of specialized pro-resolving mediators display a tolerogenic effect on encephalitogenic T cells

Giada Pessina [1] - Marta Bottero [1] - Fabrizio Loiacono [2] - Nunzio Iraci [3] - Loredana Leggio [3] - Greta Paternò [3] - Silvia Ravera [4] - Nadia Bertola [4] - Santina Bruzzone [4] - Valerio Chiurchiù [5] - Nicole Kerlero de Rosbo [1] - Tiziana Vigo [1] - Antonio Uccelli [1] - Giovanni Ferrara [1]

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Tolerogenic dendritic cells (tDC) are a specialized subset of DC that promote immune tolerance, suppressing excessive immunogenic reaction thereby promoting milder T-cell response. The loss of immunological tolerance is a hallmark of several autoimmune diseases, such as multiple sclerosis (MS) and its animal model, the experimental autoimmune encephalomyelitis (EAE). With the perspective of designing therapeutic approaches, protocols have been developed to generate tDC but they failed in clinical applications. Accordingly, we aimed to use a new protocol to generate tDC by exposure to specialized pro-resolving mediators (SPM), a novel class of bioactive lipids involved in the resolution of inflammation. Our *in vitro* data showed that DC generated in the presence of SPM mixture (RvD1, RvE1 and MCTR1) and activated with LPS/IFNγ (DCSPM) display decreased migratory capacity, upregulation of tolerogenic markers and concomitant downregulation of pro-inflammatory markers. In addition, DCSPM reduce T cells pro-inflammatory response, mainly through a paracrine effect mediated by DC-derived extracellular vesicles (EV). Moreover, since tDC display less oxidative stress in comparison with immunogenic DC, we wondered whether SPM could influence the coupling between ATP synthesis and oxygen consumption (P/O ratio), incrementing the oxidative phosphorylation efficiency. We demonstrated coupled P/O in DCSPM and in T cells cocultured with DCSPM or treated with their secreted EV. We further hypothesized that the injection of DCSPM in EAE-affected mice could modulate the encephalitogenic response and could exert immunomodulatory effects *in vivo*. Our data on preventive and therapeutic treatment of EAE-affected mice revealed a milder disease course and decreased pro-inflammatory profile of lymph nodes. Taken together, these data suggest that DCSPM have a protective effect on EAE and could be promising candidates as a therapeutic approach for possible translation to MS.

PEA-OXA ameliorates allodynia, neuropsychiatric and adipose tissue remodeling induced by social isolation

Federica Ricciardi [1] - Carmela Belardo [1] - Rosmara Infantino [1] - Serena Boccella [1] - Francesca Guida [1] - Nicola Alessio [1] - Michela Perrone [1] - Antimo Fusco [1] - Andrea Maria Morace [1] - Enza Palazzo [1] - Sabatino Maione [1] - Livio Luongo [1]

University of Campania Luigi Vanvitelli, Departement of Experimental Medicine, Naples, Italy (1)

Chronic social isolation (SI) generates a state of stress associated with obesity, neuro-endocrine and central behavioral disorders. Prolonged SI leads to a persistent distress condition which is responsible for several pathological conditions like post-traumatic stress disorder (PTSD). Infact, forced chronic isolation lead to insulin resistance, obesity, altered lipolytic responses, neuro-endocrine alterations, psychiatric and neurological disorders. Chronic stress affect several neuroendocrine systems, causing deep alterations on cognitive and emotional processing. An animal model for studying the PTSD is SI. Chronic SI in mice generates a particular phenotype: exacerbation of aggressive behaviour, increase in anxiety and depressive-like behaviour, associated with the onset of obesity. We evaluated the effect of SI on body weight, on depressive-anxious and aggressive-like behavior, and on phenotypic changes of adipocytes from visceral adipose tissue of group-housed or single-housed male mice and the effect of treatment with pentadecyl-2-oxazoline (PEA-OXA), a natural alpha$_2$ antagonist and histamine H3 protean partial-agonist, on these alterations. Single and group-housed mice treated with vehicle or PEA-OXA underwent body weight, mechanical allodynia, anxious-depressive- and aggressive-like behavior measurements. Mice undergoing SI showed aggressiveness, depression-anxiety-like behavior and developed weight gain and mechanical allodynia. The treatment with PEA-OXA amelyorates physical-metabolic and behavioural alterations in sigle-housed mice. Adipocytes from the adipose tissue of single-housed mice showed an inflamed phenotype and the treatment with PEA-OXA on adipocytes produced a protective/anti-inflammatory phenotype. This study confirms that chronic stress caused by SI predisposes to neuropsychiatric disorders and weight gain. In cocnclusion, PEA-OXA reduces weight gain, systemic pro-inflammatory state, allodynia, and affective disorders associated with SI.

Characterization of Cerebellum Alterations in a Mouse Model of Friedreich's Ataxia

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Friedreich's ataxia (FA) is a neurodegenerative disease characterized by mitochondrial dysfunction due to mutation of the gene encoding the matrix protein frataxin (FXN) which is involved in iron metabolism and in the assembly of iron-sulfur clusters of mitochondrial enzymes. The *Fxn* knock-in/knockout (KIKO) mouse represents a valid model to study FA. Through a bulk mRNAseq, we found that 700 genes were differentially expressed in cerebellum of KIKO with respect to WT mice. Functional enrichment analysis of KEGG pathways revealed 4 clusters of genes pertaining to oxidative phosphorylation, myelin sheath, fatty acid metabolism and synaptic transmission. Taken together, transcriptomics findings revealed a metabolic perturbation in cerebellum of KIKO mice. We also performed qPCR analysis and found that mitochondrial biogenesis, ferroptosis/oxidative stress and inflammatory genes were altered in cerebellum of KIKO mice. Based on the findings that immune cell activation in the cerebellum induces neuronal hyperexcitability and disruption of psychomotor behaviors in animals, we focused our attention on immune cells. We isolated bone marrow-derived macrophages from WT and KIKO mice and found a higher mRNA expression of the inflammatory markers *Nos2* in KIKO than WT mice. We therefore moved at characterizing the immunophenotype of cerebellum of adult non-symptomatic KIKO mice in search for early alterations of resident immune cells. Albeit a similar abundance of microglia cells was found in cerebellum of KIKO and WT mice, KIKO microglia showed increased expression of pro-inflammatory markers. Microglia cells are important in brain development, maintenance of neuronal networks, injury repair and in the elimination of cellular debrides and protein aggregates that may endanger the CNS. Hence, the results highlight the importance to study the phagocytic activity of microglia in FA in order to find new pharmacological targets to counteract neuroinflammation and neurodegeneration.

Response of Neural Progenitor Cells to Mechanical Stimulation in Spinal Cord Injuries

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Spinal cord injury (SCI) is a clinical condition with devastating consequences, but effective therapies are still lacking. Recent studies show that neural precursor cells (NPCs) can be exploited to improve recovery of motor function after SCI. To further enhance their therapeutic potential, nanotechnology can maximize their capability to achieve complete differentiation into a neuronal phenotype and to integrate in lesioned circuits. In this regard, we have already demonstrated that paramagnetic nanoparticles (MNPs) can be uptaken by spinal cord (SC)-derived human neuroepithelial stem (NES) cells, making them responsive to an exogenous magnetic field. The protocol was optimized and validated *in vitro* and *ex vivo*, showing that the mechanical stimulation can influence growth of both isolated and engrafted cells. The collected results led us to begin the in progress pre-clinical trial. After validation of MNPs analogous to human clinical agents, we designed and tested prototypes of magnetic stimulators for *in vivo* treatment, *i.e.* a wearable magnetic device that can be easily scaled up for use in human patients. Then, mice received SCI at the T9-10 level via dorsal hemisection. One week later, SC-NES cells were labeled with MNPs and grafted at two different sites within 500 µm from the lesion site. After two days, the magnetic stimulator was applied. The motor performance was weekly evaluated by behavioral analysis lasting up to 8 weeks post-lesion. Our preliminary findings point to a synergistic effect of mechanical stimulation and SC-NES transplantation, which resulted in enhanced rescue of motor function. In future experiments, these promising data will be combined to neuroanatomical analyses. Moreover, they encourage us to extend the validity of our approach on a rat model of SC contusion, thus recapitulating the most frequent cause of SCI in humans.

Identification of a novel factor promoting acetylcholine receptor clustering at the neuromuscular junction

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The neuromuscular junction (NMJ) is the specialized synapse formed between motor neurons (MNs) and skeletal muscles and it's used as a model to study the ability of the peripheral nervous system (PNS) to remodel and regenerate after damage. An accurate orchestration of signals between the motor axon terminal (MAT), muscle fiber, and perisynaptic Schwann's cells is responsible for NMJ maturation and maintenance. Here, we used Botulinum Neurotoxin type A (BoNT/A), a bacterial exotoxin that blocks acetylcholine (ACh) release and causes prolonged NMJ paralysis, to stimulate PNS remodeling through MAT sprouting and the formation of novel neuromuscular synapses. We found that slow MNs innervating the soleus muscle undergo intense remodeling, while fast MNs innervating the muscle extensor digitorum longus (EDL) undergo little, if any, changes. To identify the molecular determinants of MAT sprouting, we collected NMJs from these two muscles using laser capture microdissection (LCM) and performed RNA-seq. After a series of cutting-edge bioinformatic analysis, we identified a Wnt signaling-related protein specifically expressed in the soleus during MAT sprouting. This protein is a released factor, whose receptors are involved in the formation and maintenance of the NMJ by controlling the clustering of ACh receptors (AChRs). Consistently, we found, in cultured primary myotubes, that this factor promotes AChRs clustering to a similar extent of agrin, a proteoglycan known for its role in NMJ development. Besides, in mice injected in the hindlimb with BoNT/A to trigger sprouting and formation of novel contacts, the neutralization of this factor with a specific antibody reduced the formation of novel nerve-muscle contacts. One receptor for this protein is LRP4, which also binds agrin, a protein involved NMJ development. Since autoantibodies to LRP4 and agrin induce NMJ dysfunction via AChRs disassembly thus causing myasthenia gravis, the identified protein could be exploited to mitigate force loss in these patients.

Physiological role of microglial diversity along the hippocampal longitudinal axis

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The hippocampus is a plastic brain area that shows functional segregation along its longitudinal axis, as reflected by a higher level of long-term potentiation (LTP) in the CA1 region of the dorsal hippocampus (DH) compared to the ventral hippocampus (VH). The mechanisms behind this difference are poorly understood and could be due to different microglial modulations of synaptic transmission and hippocampal plasticity. We characterize the features of microglia in the CA1 region of the VH versus the DH in adult male mice. We observed that microglia regulate LTP amplitude in a region-specific manner. Indeed, their modulation by minocycline and their depletion by PLX5622 reduced the LTP amplitude in the DH while increasing it in the VH. These effects were recapitulated in Cx3cr1 knockout mice, indicating that the CX3CL1-CX3CR1 pathway is a key element in setting the basal level of LTP in the two poles. The observed LTP differences were associated with transcriptional changes in the expression of genes encoding for Il-1, Tnf-α, Il-6, and Bdnf, essential players of neuronal plasticity. These results support proper chemokine signaling between neurons and microglia as crucial for the maintenance of physiological plasticity mechanisms between poles. Furthermore, we identified a higher microglia density in the DH, while in the VH we observed an increase in soma and a more extensive arborization. We also identified an increased microglial prevalence of immature lysosomes and mRNA expression of the phagocytic markers Mertk and Cd68 in the VH compared to the DH, indicating varying microglial phagocytic activity across the hippocampal poles. Overall, we characterized the molecular, morphological, ultrastructural, and functional profiles of microglia among the two poles, suggesting that modifications in hippocampal subregions related to different microglial statuses can contribute to dissect the phenotypical aspects of many diseases in which microglia are known to be involved.

Using the FDA-approved drug vardenafil as a potential treatment to restore access to memories thought to be 'lost' in the sleep-deprived brain

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Sleep deprivation (SD) is a common problem in our current society. SD is known to negatively impact memory processes, particularly those related to the hippocampus. Previous research by us and others has demonstrated that depriving mice of sleep for 6h immediately after training in a hippocampal-dependent learning task leads to retrograde amnesia. However, it has been unclear whether SD results in the loss of information or simply hinders the ability to retrieve this information. We recently showed that object-location memories (OLMs) formed under SD conditions can be effectively recalled several days later using techniques such as optogenetic engram technologies or administration of the PDE4 inhibitor roflumilast. This indicates that these memories are not lost but rather suboptimally stored. Here, we expand on these studies by determining whether the PDE5 inhibitor vardenafil can also be used to reprogram memories thought to be "lost" in such a way that they become and remain accessible over time. We found that OLMs formed under SD condition can be permanently retrieved using a combination of optogenetic engram stimulation and vardenafil treatment. Moreover, we are now investigating whether vardenafil treatment is sufficient to facilitate OLMs retrieval when administered 30 minutes prior to testing, 1 and 5 days after training. By conducting these studies, we aim to enhance our understanding of the molecular mechanisms underlying SD-induced amnesia, and hope to contribute to the development of new therapeutic approaches to treat cognitive deficits including those associated with sleep loss.

Immunoevasive Phenotype of Glioma Cells: Hindering CD8 Lymphocyte Cytotoxicity through CD4 Lymphocyte Modulation

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Gliomas and immune system mutually reshape their phenotypes during malignant progression but, the mechanisms governing it are not fully elucidated. We used a glioma model, based on somatic gene transfer of PDGF-B, that recapitulates glioma progression. PDGF-B overexpression induces tumors that initially show low-grade gliomas (LG) features and do not successfully graft in immunocompetent mouse brains being highly immunostimulatory. Later on, these tumors progress into high-grade gliomas (HG) with a M2 pro-tumorigenic infiltrate and are able to generate secondary tumors when transplanted in immunocompetent mice. Interestingly, we showed that LG cells can successfully graft in immunodeficient NOD/SCID mice. To evaluate the ability of HG to modify the immune system phenotype we co-culture HG cells with freshly isolated splenocytes. Our results show that HG cells reduce the percentage of proliferating $CD8^+$ lymphocytes and drastically reduce the cytolytic activity of both $CD8^+$ and NK cells as shown by the decrease of Granzyme-B expression. Moreover, we noticed that HG cells assist the orthotopic grafting of LG cells, suggesting that the immunosuppressive environment induced by HG cells could tolerate also immunostimulatory LG cells. To dissect which immune subpopulation counteracts the growth of gliomas in the early stages of tumor progression, we orthotopically transplant LG in mice depleted for specific immune population ($CD4^+$, $CD8^+$, NK cells). We show that mice depleted for $CD4^+$ lymphocytes sustain the grafting of LG cells *in vivo* but have a dramatic reduction of $CD8^+$ infiltrating lymphocytes. Cytotoxic cells appear to serve as the ultimate effectors in immunosurveillance, and thus, the reduction of cytotoxic cell activity by glioma during tumor progression emerges as a critical determinant for achieving immunoevasion. However, this evasion mechanism may be indirectly mediated through the action of CD4 lymphocytes.

NKG2D CAR T Cells Target Pediatric Brain Tumor Cells In Vitro and in a Murine Model of Human Glioblastoma In Vivo

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Pediatric malignant Central Nervous System (CNS) tumors are the most common solid tumors and the leading cause of cancer-related death in children, underlying the need for new therapeutic approaches. In this regard, CAR T cells have emerged as a new pillar of treatment for pediatric CNS tumors. The interactions between NKG2D receptor on immune effector cells and NKG2D ligands on tumor cells are essential for tumor immunosurveillance. In the present study, we have explored the ability of NKG2D CAR T cells to target pediatric brain tumors. By using Europium-TDA cytotoxicity assays *in vitro*, we found 6 out of the 6 tested CNS tumor cells lines were sensitive to NKG2D CAR T cells lysis, with a percentage of cytotoxicity ≥30% when effector: target ratios of 20:1 were used. Furthermore, in 3D cultures, NKG2D CAR T cells showed ability to penetrate and eliminate glioblastoma tumor-spheres. In an orthotopic murine model of human glioblastoma, intracranial injections of NKG2D CAR T cells drastically reduced tumor growth, providing their potential to treat glioblastoma *in vivo*. However, NKG2D CAR T cells showed no clinical benefit when they were administered intravenously. Since the entrance of NKG2D CAR T cells to the tumor site could be hampered by the Blood Brain Barrier (BBB), we are currently exploring the permeability of the BBB to NKG2D CAR T cells by using a human BBB model. Additionally, in an attempt to facilitate homing and tumor infiltration, we have isolated Extracellular Vesicles (EVs) from NKG2D CAR T cells. At the moment, we have found EVs derived from NKG2D CAR T cells maintain CAR expression and we are now exploring their anti-tumor potential *in vitro*. In sum, although very preliminary, our results show that NKG2D CAR and Exo-NKG2D CAR could be a promising therapeutic approach to treat these tumors.

Exploring the role of mitophagy in medulloblastoma stem cells dissemination and therapy resistance

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor comprising four main groups characterized by different genetic alterations and rate of mortality. Post-surgery radiotherapy (RT) is actually the standard of care for patients with MB; however, above 40% of patients remains incurable due to treatment failure. Limited treatment efficacy is mainly due to the presence of intrinsically resistant cancer stem cells (CSCs) that survive following completion of standard therapies. Our laboratory recently identified autophagy activation in MB as a strong oncogenic process with translational significance for both patient stratification strategies and for the development of therapies targeting MB CSCs (Nazio et al 2021). Moreover, accumulating evidence indicates that autophagy supports metabolic rewiring of cancer cells and that regulators of mitophagy, selective degradation of mitochondria by autophagy, are frequently altered in CSCs. To date, the role of mitochondrial regulatory mechanisms and their effects on the current therapeutic regimen and tumour dissemination in MB remain unknown as well as mitochondrial properties unique to MBSCs need to be defined. Here, we have identified a novel pro-oncogenic role for the poorly studied mitophagy receptor NDP52 as a regulator of MB CSCs malignant phenotype; NDP52 upregulation supports MB CSCs aggressiveness by regulating: *i*) cell survival, *ii*) invasion capabilities, *iii)* mitophagy execution and *iv*) the response to RT. Moreover, we are setting radiotherapy-adapted-(PDX) models of MB recurrence to study how radiation-induced changes in MBSCs could affect recurrence and metastasis post-radiotherapy by acting on mitochondria-related mechanisms. Therefore, understanding the molecular mechanisms and function of mitophagy in MB during different types of mitochondrial stress and damage may be critical for developing the next generation of MB treatment methods.

Synaptic-dependent developmental dysconnectivity in 22q11.2 deletion syndrome

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22q11.2 Deletion Syndrome (22qDS) is a genetic syndrome associated with increased risk of developmental disorders such as autism and schizophrenia. Brain imaging studies have shown that people with 22qDS exhibit altered large-scale functional connectivity. However, the developmental course and neural underpinnings of these alterations remain undetermined. Here, we investigated the developmental trajectory of functional connectopathy in 22qDS in both a mouse model and in human 22qDS carriers. To this aim, we longitudinally mapped resting-state fMRI connectivity in juvenile and adult LgDel mice, an established mouse model of 22qDS. We found that developmental connectopathy in LgDel mice undergoes a dramatic reconfiguration during the pubertal period, with widespread prepubertal fMRI hyper-connectivity reverting to focal hippocampal hypo-connectivity in adult LgDel mutant mice. We also found that fMRI hyper-connectivity in juvenile Lgdel mice is paralleled by a surplus of cortical dendritic spines, and that both of these phenotypes are normalized by pretreatment with the Gsk3B inhibitor SB216763. To probe the generalizability of these findings to human 22qDS, we examined fMRI connectivity in both pre-pubertal (TD=52, 22qDS n=21) and peri/post-pubertal (TD=204, 22qDS n=118) 22qDS carriers. We found that functional connectopathy in human 22qDS similarly undergoes a reconfiguration from dominant hyperconnectivity in prepubertal carriers, to hippocampal and cortical hypo-connectivity in adulthood. Prompted by our mouse investigation, we next tested the hypothesis that this reconfiguration could be driven by Gsk3B-related synaptic mechanisms. In keeping with this, we found that brain regions exhibiting functional connectivity reversal in 22qDS carriers are spatially enriched for gene transcripts encoding for synaptic proteins that interact with Gsk3B (p=0.001). Collectively, these findings provide evidence of synaptic-dependent, developmental dysconnectivity in 22qDS.

A novel method to estimate Multiple Sclerosis connectomes considering lesional tissue information

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Multiple sclerosis (MS) is a demyelinating disease of the central nervous system that leads to focal lesions that may bias structural connectivity estimates. Here we present a novel method for estimating structural connectivity in the presence of focal white matter alterations. It comprises the computation of connection weights between different gray matter (GM) regions by projecting myelin volume fraction-sensitive scalar map values onto streamlines reconstructed by tractography, modeling lesion-affected voxels, and scaling streamline weights accordingly.
We used data from 82 healthy controls (HC) (44 females, mean age 37.1±12.4) and 139 MS patients (82 females, mean age 45.4±13.8) to evaluate our method's usefulness in studying MS connectome. To assess the method's sensitivity to pathology, we extracted 4 global network metrics: mean strength, global efficiency, modularity, and clustering coefficient. We also evaluated changes in the myelination of bundles, typically affected in MS, connecting the precentral gyrus (PrCG) with the subcortical gray matter nuclei, the corticospinal tract (CS), the pons, and the corpus callosum (CC). We perform a robust linear model considering age, sex, and network density as covariates.
The results show that mean strength, efficiency, and clustering coefficient are notably higher in HCs than in MS patients ($p<0.001$, $R^2>0.40$), while modularity is significantly higher in MS patients than in HCs ($p=0.002$, $R^2=0.52$). We found that all the bundles analyzed are less myelinated in MS than in HC in both hemispheres ($p<0.01$, $R^2>0.10$ for bundles connecting PrCG to subcortical GM nuclei, and $p<0.001$, $R^2>0.10$ for CS, pons, and CC). The method demonstrates significant sensitivity to pathology-induced impairment in the structural connectivity of MS patients. In addition, it allows for precise estimation of damage caused by focal lesions without introducing any bias in the connectivity estimates, which was unreachable using state-of-the-art methods.

Alzheimer's genetic pathways are associated with changes in separate imaging biomarkers in non-demented individuals

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We studied polygenic risk scores (PRS) and pathway-specific PRS in relationship with AD fluid and imaging biomarkers, in non-demented individuals from the European Prevention of Alzheimer's Dementia (EPAD) cohort. Inclusion criteria were age>50 and Clinical Dementia Rating≤0.5 (n=1886,). AD-PRS was determined based on 85 previously identified loci, including and excluding APOE (PRS_{APOE}, PRS_{noAPOE}). Using gene-variant and variant-pathway mapping, six pathway-specific PRS_{noAPOE} were identified, representative of 1) immune-activation, 2) signal-transduction, 3) inflammatory-response, 4) migration, 5) amyloid-production, and 6) clearance. Linear models were used to assess the relationship of the global and pathway PRS with fluid AD biomarkers, including $A\beta_{1-42}$, p-Tau_{181}, and t-tau; and several imaging biomarkers, including hippocampal volume, global and lobar white matter hyperintensities (WMH) volumes, fractional anisotropy (FA) in 9 regions of interest from diffusion tensor imaging, and functional connectivity in 3 default mode network subsystems. PRS_{APOE} was significantly associated with decreased $A\beta_{1-42}$, and increased p-Tau_{181} and t-Tau. PRS_{noAPOE} showed a weaker, but still significant, negative association with $A\beta_{1-42}$ levels, and a significant positive association with p-Tau_{181} and t-Tau. All pathway PRS were also significantly associated with CSF $A\beta_{1-42}$, and CSF p-Tau_{181} (also corrected for CSF $A\beta_{1-42}$,). The clearance pathway was selectively related to WMH volumes in most regions, most strongly in global, frontal, and temporal periventricular, and parietal deep white matter. Only the migration pathway was related to increases in FA in the splenium, body, and genu of the corpus callosum. Lower FC within the dorsal DMN associated with higher PRS for signal transduction. We demonstrate that genetic risk for AD is associated with a broad range of neuropathological features in non-demented individuals, and that distinct AD biomarkers are preferentially associated with specific genetic profiles

HSV-1 Infection in Mouse Enteric Nervous Sistem: a Trigger for Alzheimer's Disease-Like Neurodegeneration Hallmarks

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Alzheimer's disease (AD) is a neurodegenerative disease that induce progressive cognitive impairment in patients. It affects millions of people all over the world with an expected enormous increase in the next years, with a great impact on healthcare systems. In the last decades, a growing body of evidence supports the profound link between AD onset and infectious agents. Among infective agents the neurotropic Herpes simplex virus type 1 (HSV-1) is extremely attractive for a number of experimental and clinical observations. In particular, multiple HSV-1 reactivations from latency are thought to contribute to neuronal dysfunction, thus leading to neurodegeneration. To further investigate this hypothesis, our group established and characterized a mouse model of persistent HSV-1 infection (up to 10 weeks) in the enteric nervous system. By immunohistochemical staining of ileal Longitudinal Muscle/Myenteric Plexus (LMMP) we observe progressive APP (Amyloid Precursor Protein) and β-amyloid accumulation in response to HSV-1 infection, while by qPCR analysis we detect increased expression of β- and γ-secretase genes. A significant decrease in acetylcholine levels in the myenteric plexus was demonstrated via HPLC, suggesting synaptic damage. In addition, HSV-1 persistent infection induces IFNα, IFNβ, IL-1β and IL-6 overexpression in LMMP. Moreover, by immunofluorescence analysis on primary enteric neurons isolated from infected mice we found APP and β-amyloid accumulation and Tau hyperphosphorylation. Furthermore, we analysed myenteric neurons redox homeostasis, revealing peroxidation of membrane lipids and an increased mitochondrial production of free radicals in cells obtained from *in vivo* infected mice. Intriguingly, treatment of HSV-1 infected enteric neurons with a mitochondria-targeting antioxidant (MitoTEMPO) reduced β-amyloid accumulation. These preliminary *in vivo* and *ex vivo* data further support the active role of HSV-1 in AD pathogenesis.

Recovering STIMulation of astrocyte Ca2+ signal to shed light on Alzheimer's Disease

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Alzheimer's disease (AD) is a chronic incurable neurodegenerative disorder characterized by progressive memory loss and cognitive dysfunctions. Brain function is governed by dynamic interactions between neurons and astrocytes. Noteworthy, calcium dynamics in astrocytes represent a fundamental signal that through gliotransmitter release regulates synaptic plasticity and behaviour. Here, by using cutting-edge techniques including 2-photon Ca^{2+} imaging, electrophysiology and behavioural memory tests, we present a longitudinal study in the PS2APP mouse model of AD linking astrocyte Ca^{2+} hypoactivity to memory loss. At the onset of plaque deposition, somatosensory cortical astrocytes of AD female mice swtich to a reactive pro-inflammatory state and exhibit a drastic reduction of Ca^{2+} signaling, closely associated with decreased endoplasmic reticulum Ca^{2+} concentration and reduced expression of the Ca^{2+} sensor STIM1. In parallel, astrocyte-dependent long-term synaptic plasticity declines in the somatosensory circuitry, anticipating specific tactile memory loss. Notably, we show that both astrocyte Ca^{2+} signaling and long-term synaptic plasticity are fully recovered by selective STIM1 overexpression in astrocytes. Our data unveil astrocyte Ca^{2+} hypoactivity in neocortical astrocytes as a functional hallmark of early AD stages and indicate astrocytic STIM1 as a target to rescue memory deficits.

Rapamycin ameliorates the pathological phenotype in the Twitcher mouse by autophagy activation

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Krabbe disease (KD) is a rare condition caused by a deficiency of the lysosomal enzyme galactosylceramidase (GALC). GALC lack leads to the accumulation of the cytotoxic metabolite psychosine (PSY) in the nervous system, with consequent demyelination and neurodegeneration. The KD-related pathogenetic mechanisms are still not completely understood, and no cure is available for KD. Recently, we demonstrated the involvement of autophagy dysfunctions in KD pathogenesis. We found p62-tagged protein aggregates in the brain of KD mice and increased p62 levels in the KD sciatic nerve. Here, we decided to test *in-vitro* and *in-vivo* the autophagy inducer Rapamycin (RAP) to remove unwanted cellular products from KD cells, such as p62 aggregates and PSY. We demonstrated that RAP can partially reinstate the WT phenotype in KD primary cells by decreasing the number of p62 aggregates. Therefore, we tested RAP in the Twitcher (TWI) mouse, a spontaneous KD mouse model. The drug has been administered ad libitum via drinking water (1,5 mg/Kg) starting from PND 21-23. We longitudinally monitored the mouse motor performance through the grip strength and the rotarod tests, and a set of biochemical parameters related to the KD pathogenesis (i.e. PSY accumulation, astrogliosis and autophagy markers expression). We found that RAP is able to improve motor functions at selected time points. Interestingly, we found that the treatment diminishes astrogliosis in the TWI brain, spinal cord, and sciatic nerves. Additionally, by western blot and immunohistochemistry, we found that RAP decreases the amount of p62 in TWI nervous tissues, confirming our *in-vitro* findings. Finally, RAP treatment demonstrated to be able to partially remove PSY in the spinal cord. Overall, these results suggest considering RAP as an option to be tested for KD clinical trials. This is especially encouraged by the fact that RAP is already in clinical practice.

Pharmacological Stimulation of Autophagy to Rescue Proteinopathy and Cognitive Decline in Mucopolysaccharidosis-IIIA

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Mucopolysaccharidosis IIIA (MPS-IIIA) is a lysosomal storage disorder (LSD) characterized by the loss of function of the sulfamidase gene (SGSH), responsible for the degradation of the glycosaminoglycan (GAG) heparan sulfate (HS). Undegraded HS leads to the formation of primary and secondary storages responsible for neurodegeneration and dementia in children (1). Favouring the degradation of secondary storages is one of the most promising therapeutic strategies to prevent neurodegeneration. Genetic overexpression of the transcription factor EB (TFEB), through the control of genes involved in the autophagy/lysosomal degradation process, seems to promote the degradation of protein aggregates in animal models of neurodegeneration (2). However, there are still few synthetic drugs capable to stimulate TFEB and to cross the blood-brain barrier. We are testing a compound that in wild-type/control animal models has been shown to promote TFEB-mediated autophagy and lysosomal biogenesis.
In this project, using validated animal and cellular models of MPS-IIIA, we have tested it in in vitro and in vivo models of MPS-IIIA. The *in vitro* analysis elucidated the mechanism by which the drug activates TFEB in the context of the disease, while the *in vivo* analysis shed light on its ability to improve some of the cognitive deficits associated to the accumulation of undegraded HS in the brain of MPS-IIIA animals, without any major side effect. In addition, *ex-vivo* analysis revealed the ability of the drug to clear secondary storages made of beta-amyloid. These results suggest a new therapeutic approach for the treatment of MPS-IIIA.

Design of an innovative 3D model for blood-brain barrier towards improved translational medicine approaches

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The blood-brain barrier (BBB) is a crucial component of the central nervous system that protects the brain from harmful substances while allowing essential nutrients to pass through. Overcoming the BBB remains a crucial aspect for the delivery of drugs or therapeutics in neurological disease modelling. It is important to underline that *in vivo* models could provide experimental environments that closely mimic the complexity of human physiology, although no animal model can faithfully reproduce all the manifestations of human diseases. In this context, *in vivo* models must be interpreted as an approximation of human biology limited to particular regions or other features. The most important disadvantage of *in vivo* models is the translation of results towards human application. Furthermore, using an *in vitro* model it should be possible to closely reproduce the essential features of the human BBB *in vivo*. In this scenario, an ideal *in vitro* BBB model should have: (i)3D vessel-like structure design; (ii) multiple cell lines in co-culture cells line; (iii) flow-induced shear stress. Accordingly, a novel 3D BBB *in vitro* model has been provided adopting a bipartite vessel-like bioprinted scaffold using cell-laden sodium alginate hydrogels with two different cell lines, namely endothelial and neuronal cells. The optimization of both design and printing parameters has been carried out to achieve high-quality BBB models. The critical parameters that influence print quality and cell viability were also explored and tailored for achieving optimal results. Preliminary results suggested that cell-laden alginate hydrogels represent a valuable bioink for complex biological system modelling, also taking into account the possibility of adopting a bioreactor to study how the shear stress impacts on tight junction protein expression. This innovative *in vitro* BBB model could also be an interesting tool towards high-throughput drug screening.

Unique metabolic signatures may contribute to the development of post-traumatic epilepsy in mice

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Post-traumatic epilepsy (PTE) is a complication of traumatic brain injury (TBI) and it accounts for 5% of all epilepsies and 10-20% of the acquired forms. The mechanisms of TBI-induced epileptogenesis have not yet been fully characterized, leading to a lack of prognostic/diagnostic biomarker(s) of epileptogenesis and therapeutic targets to treat this form of acquired epilepsy. Microbiota-gut-brain (MGB) axis dysfunction is emerging as a new pathogenic mechanism in neurologic disorders, including epilepsy. In particular, patients with epilepsy are known to have intestinal dysbiosis, which can modulate disease outcome. Additionally, special diets such as ketogenic diets or pre-, pro- and post-biotic supplementation can correct the altered microbiota composition andx result in anti-seizure effects in both preclinical models and patients. Different gut microbial populations produce distinct metabolites that may affect brain activity, possibly modulating excitotoxicity and seizures. In this systemic and translational study, we investigated the unique metabolic signatures in PTE vs TBI and naïve mice by using untargeted metabolomics. Adult male CD1 mice underwent left parieto-temporal severe controlled cortical impact (CCI; 2 mm depth). PTE occurrence was determined by 24/7 electrocorticography recordings at 5 months post-TBI. Plasma and feces were collected in all groups longitudinally at 10 days (before PTE onset) and 6 months post-TBI (chronic PTE phase) to investigate any potential differences in the metabolic profile among the experimental groups both locally in the gut and systemically in the blood. Our results show substantial metabolic variation between PTE vs TBI mice and naïve controls that could contribute to PTE onset and progression. Investigating MGB disfunction to uncover the unique metabolic signatures of PTE is crucial to identify biomarkers and potential novel therapeutic targets of the disease, filling a still unmet clinical need.

The roles of RAC1 regulators ARHGAP15, TRIO, and ARHGEF6 in physiological and pathological forebrain development

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Neurodevelopmental disorders affect more than 3% of the world population and are characterized by the inability to reach cognitive, emotional, and motor developmental milestones. A set of gene mutations associated with intellectual disability, microcephaly, and epilepsy causes alterations in the RAC1 pathway. This is the case of the RAC1 regulators ARHGAP15, TRIO, and ARHGEF6 whose roles during neurodevelopment are poorly understood (Liaci et al., 2021). I provided evidence that ARHGAP15 is required for the control of murine cortical interneuron (CIN) migration, morphology, and functionality. Arhgap15 knock-out (KO) CINs show a disoriented leading process during migration and alterations in the cortical lamination. Moreover, Arhgap15-KO mice show increased susceptibility to spontaneous and induced seizures, probably due to reduced CINs intrinsic excitability (Liaci et al., 2022). Regarding ARHGEF6, defects in interneurons migration and number have been observed in KO mice and are currently under investigation. Contextually, I generated human induced pluripotent stem cell-derived models (*i.e.*, radial glia and forebrain neurons) that recapitulate neurodevelopmental processes in the pathological conditions caused by TRIO and ARHGEF6 mutations by using CRISPR/CAS9 technology. In these models, I am currently evaluating the cellular, molecular, and physiological alterations, with a special interest in the potential role of TRIO in radial glia division timing and cytokinesis. To explore this possibility, I'm modeling the TRIO haploinsufficiency in vivo by morpholino-mediated knock-down in zebrafish. Preliminary results show developmental delays and defective neurogenesis.

Mechanisms of synaptic dysfunction in the Angelman syndrome

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The Angelman Syndrome (AS) is a neurodevelopmental disorder caused by the loss of maternally expressed *UBE3A* gene, which encodes an E3 ubiquitin ligase. Although considerable efforts have been put to dissect UBE3A function in the brain, the pathogenic mechanisms remain largely unknown and effective treatments are not available yet. Being an E3 ubiquitin ligase, defective ubiquitination is thought to be a primary mechanism underlying synaptic dysfunction in AS. However, only a few substrates of UBE3A have been shown to be relevant to AS pathogenesis and recent reports suggest that UBE3A might play a global regulatory role instead of acting through a specific neuronal pathway. Increasing evidence indicates a tight functional interplay between ubiquitination and sumoylation, an ubiquitin-related PTM consisting in the covalent conjugation of Small Ubiquitin-like MOdifier (SUMO) proteins to target proteins. In the brain the SUMO machinery finely modulates synaptic and extrasynaptic pathways that are fundamental to neuronal circuit formation and function. In this project, we evaluate the impact of *UBE3A* loss on synaptic development and test the hypothesis that alterations of the functional cross-talk between ubiquitination and sumoylation might contribute to AS pathogenesis. To explore the possibility that an unbalanced sumoylation is at the basis of AS, we are currently evaluating sumoylation of nuclear and cytosolic fractions obtained from cortices of AS and wild type mice throughout neurodevelopment. Strikingly, preliminary data obtained in the laboratory indicate that both SUMO1 and SUMO2/3 conjugation is impaired at different developmental stages in the nucleus and in the cytosol of AS cortices. These data might unveil a novel pathogenic mechanism of AS and provide the rationale to develop new therapeutic strategies to treat AS patients.

POSTER SESSION

1

CN01 | Serum NfL levels and cognitive performance in persons with multiple sclerosis

Rina Demjaha [1,2] - Stefanie Maria Charlotte Hechenberger [1,4§] - Arabella Buchmann [1,2] - Birgit Helmlinger [1,4] - Bettina Heschl [1] - Sebastian Wurth [1,3] - Peter Opriessnig [1] - Stefan Ropele [1] - David Leppert [5,6] - Pascal Benkert [6] - Jens Kuhle [5,6] - Christian Enzinger [1] - Daniela Pinter [1,4*] - Michael Khalil [1,2*§]

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Introduction: Serum neurofilament light (sNfL) is a robust biomarker to indicate neuro-axonal damage in various neurologic conditions including multiple sclerosis (MS). Cognitive impairment (CI) is a frequent feature in MS with a huge impact on quality of life and social functioning. It is still not clear if sNfL correlates with or even predicts CI in MS. This study aims to elucidate the association between sNfL and CI in persons with MS (pwMS).

Methods: 186 pwMS (112 female; mean age=39.6±10.4; mean disease duration=10.6 years; median EDSS=1.5 (IQR=2.75)) and 49 healthy controls (HC) (35 females; mean age=33.4±10.7) underwent clinical examination, neuropsychological (Brief Cognitive Assessment for MS-BICAMS) and 3T brain-MRI assessment, including T2-hyperintense lesion load and normalized brain volumes calculations. sNfL was quantified by single molecule array (Simoa SR-X). We calculated sNfL Z-scores corrected for age and body-mass-index; Symbol Digit Modalities Test (SDMT) Z-scores corrected for age and education; Verbal Learning Memory Test (VLMT) and Brief Visuospatial Memory Test (BVMT) T-scores corrected for age.

Results: In this cross-sectional analysis, 48 pwMS showed CI in at least one BICAMS test and 38 in SDMT (M=-0.2±1.1); 6 in VLMT (M=56.2±9.5) and 20 in BVMT (M=54.3±12.7) in comparison to no CI in HC ($p<0.001$). Baseline sNfL Z-scores (M=0.73±1.27) were unrelated to BICAMS sub-tests, including the SDMT, VLMT and BVMT (all $p>0.05$, n.s.) both in pwMS and HC.

Conclusion: In this cross-sectional analysis, sNfL was unrelated to CI in pwMS. Longitudinal analyses investigating the relation of sNfL dynamics and MRI metrics with cognitive decline are currently ongoing.

Disclosures: M. Khalil has received speaker honoraria from Bayer, Novartis, Merck, Biogen Idec and Teva Pharmaceutical Industries Ltd. and serves on scientific advisory boards for Biogen Idec, Merck Serono, Roche, Novartis, Bristol-Myers Squibb and Gilead. He received research grants from Teva Pharmaceutical Industries, Ltd., Biogen and Novartis. • S. Wurth has participated in meetings sponsored by, received honoraria or travel funding from Allergan, Biogen, Ipsen Pharma, Merck, Novartis, Roche, Sanofi Genzyme, Teva and Bristol Myers Squibb. • D. Leppert is Chief Medical Officer of GeNeuro. MW received speaker honoraria from Novartis Pharma, Chugai Pharmaceutical, Biogen Japan and Alexion. • S. Hechenberger has received speaker honoraria from Roche and Bristol Myers Squibb. • B. Helmlinger has received speaker honoraria from Roche and travel funding from Janssen. • R. Demjaha has received travel funding from Janssen. • All other authors have nothing to disclose.

CN02 | Cognitive frailty and oxygen-ozone therapy: differential expressed genes as predictive biological markers of response/improvement to treatment

Cristian Bonvicini [1] - Catia Scassellati [2] - Antonio Galoforo [3] - Miriam Ciani [1] - Cristina Geroldi [4] - Ivan Arisi [5] - Valeria Manzini [6] - Chiara D'Amelio [7] - Tommaso Gosetti di Sturmeck [5] - Rossella Brandi [8] - Elisabetta Mori [9] - Mario Costa [10] - Mara D'Onofrio [5] - Antonino Cattaneo [9]

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Frailty is a multidimensional geriatric syndrome characterized by increased vulnerability to stressors as a result of the reduced functional capacity of different physiological systems. This heterogeneous clinical syndrome, conceived as an innovative multidimensional concept, includes cognitive frailty (CF). Oxygen-Ozone (O2-O3) therapy is a no-invasive/no-pharmacological low-cost procedure with no side effects based on effects of low O3 concentrations. This therapy induces a mild oxidative stress stimulating antioxidant defenses and prevents the inflammatory response and cell damage. We hypothesized that O2-O3 therapy might promote a significant effect on those oxidative and inflammation processes, strongly altered in CF. Specifically, we aim to identify *in vivo* predictive peripheral biological markers of response/improvement to treatment, integrating clinical and neuropsychological parameters with -omics analysis. We conducted the first pilot double blind randomized controlled trial: seventy-two elderly frail subjects aged between 65 and 80, were treated with free rectal insufflations of air, O2 or O2-O3 mixture for 5 weeks (3 sessions for week). A total amount of 150cc of O2-O3 mixture at the concentration of 30 µg of O3 per cc of O2 over a 5-10 min period was administered. Subjects were monitored at different times: before (T0), 3 months (T1) and 9 months post-treatment (T2) while the RNA profiling was analysed in whole blood by Agilent microarray at T0 and T1. Based on CF evaluation, patients were divided in 3 sub-groups as Low, Moderate and High levels. Following O2-O3 treatment differentially expressed genes (DEGs) have been observed in the 3 sub-groups. Those DEGs involve several molecular pathways highlighting a specific mRNA response to the O2-O3 treatment and representing potential biomarkers associated to O2-O3 therapy. Lastly, these biomarkers integrated with clinical data, might allow to predict and optimize the therapeutic response to O2-O3 therapy.

NI01 | Investigation of neuronal Ca2+ hyperexcitability and neuro-inflammatory state in an Alzheimer's disease mouse model

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Alzheimer's disease (AD), the most common cause of dementia, is an irreversible neurodegenerative disease characterized by progressive memory loss and cognitive deficits. Despite intensive investigations, pathogenic mechanisms of AD are poorly known and effective treatments are still lacking. It is reasonable that some important neurophysiological processes are altered years before the onset of clinical symptoms, highlighting the importance of identifying early dysfunctions and biomarkers useful for both therapeutic and diagnostic purposes. Even though most AD cases are sporadic, a small percentage is due to autosomal dominant mutations in amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2) genes. We used an AD mouse model (B6.152H), double transgenic for PS2 (N141I) and APP (Swedish mutations), which is characterized by neuronal electrical hyperexcitability. In these mice, changes in brain circuitry, relevant to the development of AD, are detectable at both 3 and 6 months of age, earlier than the onset of spatial memory deficits, revealed at 8 months. We first analysed the state of excitatory and inhibitory synapses in the somatosensory cortex (SSCx) of AD and WT mice at 2, 6 and 9 months. Moreover, we evaluated Ca^{2+} activity in neurons from *ex vivo* and *in vivo* SSCx preparations by using advanced imaging techniques, to investigate spontaneous and evoked Ca^{2+} hyperexcitability at different disease stages. Moreover, since glial cells are actively involved in synaptic transmission, and microglia are emerging as critical negative regulators of neuronal Ca^{2+} hyperactivity in mice, we plan to assess and modulate microglia inflammatory state in order to possibly rescue neuronal Ca^{2+} phenotype in AD mice.

NI02 | Is Circulating AgRP Neuropeptide a Novel Mediator of Neuroimmune Communication in Multiple Sclerosis?

Eleonora Cornacchia [1] - Alice Laroni [1,2] - Caterina Bason [1] - Fabrizio Loiacono [1] - Tiziana Altosole [1] - Matilde Inglese [1,2] - Antonio Uccelli [1,2] - Tiziana Vigo [1]

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The interplay between nervous and immune systems is emerging as an important mechanism of immune cell function control. Immune cells express the receptors for neurotransmitters and neuropeptides released by neurons of both peripheral and central nervous system. We have previously demonstrated that activation of hypothalamic neurons producing Agouti-related peptide (AgRP) impairs hematopoiesis and promotes the generation of Treg by reducing the release of norepinephrine in bone marrow and thymus, thus suggesting a possible immune-regulatory function of these neurons. AgRP neurons are activated in the mouse model of Multiple Sclerosis (MS), the experimental autoimmune encephalomyelitis. Activation of AgRP neurons leads to increased expression of AgRP, that is partially released into circulation, as these neurons lye out of the blood brain barrier. Accordingly, we have observed an increase of AgRP level in the serum of people with MS, suggesting that AgRP neurons are activated also in MS. Whether increase of circulating AgRP is specific of MS or also occurs in other neurological diseases of the central nervous system is completely unknown. AgRP is a potent competitive antagonist of the melanocortin receptors 3 and 4 (MCR), and previous studies indicated that immune cells express MC3R at mRNA level. However, a comprehensive expression map of MC3R receptor in immune cells at protein level is still missing, and a possible effect exerted by AgRP on immune cell functions has never been investigated.

Here we have quantified the levels of AgRP peptide in the serum of people with Parkinson disease (PD) and with ischemic stroke (IS), to understand whether increased level of AgRP is a common hallmark or neuronal damage or if it is specific of MS. Then, we have performed an extensive FACS analysis to assess the expression of MC3R in mouse and human immune cells. Our results reveal an increase of circulating AgRP in pwPD and 7 day after IS, when neuronal death is prominent, suggesting that AgRP may be a marker of neurodegeneration. Moreover, we have demonstrated that the AgRP receptor MC3R is expressed by human and mouse dendritic cells (DC) and NK cells. Functional in vitro assays suggest that AgRP may exert a regulatory effect on these cells.

These preliminary results appear promising both in suggesting AgRP as a marker of neuronal damage in neurological diseases and in describing a new mechanism of neuro-immune interplay mediated by AgRP and its receptor MC3R.

NI03 | Spinal and supraspinal characterization of an inflammatory CFA-induced model of Vulvodynia

Antimo Fusco [1] - Michela Perrone [1] - Federica Ricciardi [1] - Serena Boccella [1] - Carmela Belardo [1] - Andrea Maria Morace [1] - Francesca Guida [1] - Sabatino Maione [1] - Livio Luongo [1]

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Vulvodynia is a gynecological disease characterized by chronic pain in the vulva a. The chronic vulvar pain that characterizes the disorder is associated with severe burning, dyspareunia and redness; it has been demonstrated that Amitriptyline and Gabapentin show an efficacy in the treatment of vulvodynia, while a combination of palmitoylethanolamide (PEA) and polydatin (Pol) has important antinflammatory and antioxidant properties. In this study, we have evaluated the development of the tactile allodynia in a mouse model of CFA-induced vulvodynia through behavioural and electrophysiological approaches. The possible depressive-like behaviour has also been investigated. We have used as a pharmacological treatment the Gabapentin, amytriptiline and the PEA/Pol combination. Vulvodynia was induced in female C57BL/6J mice by one injection of Complete Freund Adjuvant for up to four weeks. The chosen dose (5 µl) induced mechanical allodynia in a window between day 1 and 28 post-injection of CFA. During this 28 days Mechanical Allodynia, Tail Suspension and Extracellular Single Unit Recordings were performed. CFA-repeated administration reduced the tactile withdrawal threshold in mice treated with vehicle. The chronic administration with Gabapentin, amytriptiline and PEA/Pol reduced the tactile allodynia as compared to the vehicle treated mice. The same treatments also reduced the immobility time in the tail suspension test highlighting an antidepressive-like effect of the drugs. Electrophysiological data on the nociceptive specific neurons (NS) of the sacral dorsal horn of the spinal cord revealed that the treatment reduced the increased ongoing and evoked firing activity. We have shown for the first time that the CFA-induced vulvodynia is associated to an alteration of the NS neurons' activity in the dorsal horn of the sacral spinal cord segments. We also found that vulvodynia is associated to a depressive-like behaviour appearance that was reduced by the drug treatment.

NI04 | Unveiling the role of exosomal miRNAs in the spreading of neuroinflammation

Francesca De Chirico [1] - Francesca Massenzio [1] - Eleonora Poeta [1] - Giorgia Babini [1] - Sabrina Petralla [1] - Manon Elise Libotte [1] - Giampaolo Zuccheri [1] - Barbara Monti [1]

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Neuroinflammation is a crucial pathogenic mechanism that commonly underlies most neurodegenerative diseases. Microglia, the immune cells of the brain, play a critical role according to the stage of neuropathology. Indeed, at early phases of brain diseases microglia display the neuroprotective, alternatively activated phenotype which is switched to the classically activated pro-inflammatory subtype at later stages, when activated microglia contribute to neurodegeneration. The microglial phenotypic shift is characterized by a change in the release of bioactive molecules (especially proteins and miRNAs), both soluble and through extracellular vesicles. With the progression of neuropathology, microgliosis, i.e. an increase in number and reactivity of microglial cells, is always observed, thus suggesting a spreading of microglia activation. However, the role of extracellular vesicles released by activated microglia in neuroinflammation spreading has never been demonstrated. We took advantage of the in vitro model of murine microglia N9 cells to evaluate the effect of conditioned media or isolated vesicles to spread the neuroinflammation through the release of specific miRNAs. We demonstrated that conditioned media or exosomes obtained from pharmacologically activated microglia were able to promote a pro-inflammatory phenotype to control cells, leading us to prove, in vitro, the existence of a neuroinflammation spreading process mediated by the secretome of microglia with a crucial role of extracellular vesicles in terms of miRNAs content.
In this regard, the downregulation of the inflamma-miR-34a, by using cleaving sequences of anti-miR34a DNAzyme delivered by DNA nanostructures, led to a reduction of microglia polarization towards the neurotoxic phenotype confirming the involvement of miR-34a in the neuroinflammatory process.

NI05 | Expression and function of TMEM206/PACC1 alternative isoforms

Serena Tamburro [1] - Niccolò Callegari [1] - Floriana Guida [1] - Giulia Gorrieri [1] - Francesca Antonini [2] - Ilaria Musante [3] - Lorenzo Muzzi [1] - Simona Baldassari [3] - Paolo Scudieri [1]

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TMEM206, also known as PACC1, is a ubiquitously expressed proton-activated chloride channel mediating outwardly rectifying plasma membrane currents that are elicited only upon a marked extracellular acidification. It is involved in acid-induced neuronal death, in fact, its ablation in mice attenuates the pathogenesis of ischemic brain damage. More recently, it was associated also to housekeeping roles in intracellular compartments, for example in the endosomes, where it functions as a brake preventing excessive acidification.
In this study our aim was to investigate if the different TMEM206 functions could be associated to the existence of alternative isoforms of the channel. Indeed, at least two TMEM206 alternative transcripts can be produced through the inclusion/skipping of exon 2.
Thus, by means of spatial biology approaches we investigated the tissue, cellular and subcellular expression of TMEM206. We found TMEM206 ubiquitously expressed in human tissues, with the highest expression level in the brain. By RT-PCR with primers flanking exon 2, we found the longer isoform (*i.e.* produced by exon 2 inclusion) almost exclusively expressed in the brain. We then overexpressed the two isoforms in heterologous systems and performed a series of biochemical and functional assays, including western blot, immunofluorescence, HS-YFP assay, endosomal pH measurements, and acid-induce cell death quantification. Results from these experiments indicate that the longer isoform functions more as a plasmamembrane channel, whereas the shorter one works more in controlling the endosomal pH. Studies on human iPSC-derived neurons are currently in progress to better investigate the function of the two isoforms in a neuronal model.
Clarifying the roles of TMEM206 will pay the way for future projects aimed at searching pharmacological modulators of this channel, which could be proposed as novel therapeutic options to treat neuroinflammatory diseases characterized by brain tissue acidosis.

NI06 | Systemic inflammation induced in young Tg2576 Alzheimer-mice anticipates the onset of cognitive decline

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It is known that systemic inflammation plays a role on the age of onset and/or worsening of cognitive decline in animal models of Alzheimer's disease. Moreover, emerging epidemiological data indicates a possible correlation between chronic inflammatory bowel diseases and dementia onset in Alzheimer's patients. We investigated whether Dextran Sulphate Sodium (DSS)-induced colitis in young Tg2576 mice (3 months old) anticipates the onset of learning and memory deficits, that normally occurs around 6-7 months of age. To assess cognitive performance, animals were monthly tested by Morris Water Maze (MWM), video-tracking automatic analysis. Results showed an anticipation of cognitive decline, that occurs already at 5 months old in Tg2576+DSS mice, while Tg2576+vehicle perform normally (latency to first entry to the platform zone, Student t test p=0.0141). WT+DSS are not different from WT group. In order to correlate this result with possible peripheral mediators, several inflammatory cytokines were monitored in plasma over the experiment course. Remarkably, TNF-a plasma levels were higher in Tg2576 compared to WT mice before DSS induction (3 months old). We than analyzed neuroinflammation. By tissue cytofluorimetry, we showed that the number of CD11+ cells (activated microglia) was higher in Tg2576 compared to WT during colitis. Finally, we investigated microglia and astroglial phenotypes in 6 months old animals after colitis recovery. While the morphological phenotype of IBA1-positive cells was unchanged, we observed a significant reduction in the GFAP-IR binary area fraction associated with unchanged astrocyte number, thus suggesting a shrinkage of astrocyte morphology. This astrocytic morphological feature has been associated to the very early phase of neurodegenerative diseases. In conclusion, we showed that an experimental model of colitis in very young Tg2576-AD mice anticipates and worsens the cognitive decline usually observed in this Alzheimer mouse model.

NP01 | Cardiac functional and structural abnormalities in a mouse model of CDKL5 Deficiency Disorder

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CDKL5 (cyclin-dependent kinase-like 5) deficiency disorder (CDD) is a rare and severe neurodevelopmental disease that mostly affects girls who are heterozygous for mutations in the X-linked CDKL5 gene. The lack of CDKL5 protein expression or function leads to the appearance of numerous clinical features, including early-onset seizures, marked hypotonia, autistic features, and severe neurodevelopmental impairment. Mouse models of CDD, *Cdkl5* KO mice, exhibit several behavioral phenotypes that mimic CDD features, such as impaired learning and memory, social interaction, and motor coordination. CDD symptomatology, along with the high CDKL5 expression levels in the brain, underscores the critical role that CDKL5 plays in proper brain development and function. Nevertheless, the improvement of the clinical overview of CDD in the past few years has defined a more detailed phenotypic spectrum; this includes very common alterations in peripheral organ and tissue function, such as gastrointestinal problems, irregular breathing, hypotonia, and scoliosis, suggesting that CDKL5 deficiency compromises not only CNS function but also that of other organs/tissues. Here we report, for the first time, that a mouse model of CDD, the heterozygous *Cdkl5* KO (*Cdkl5* +/-) female mouse, exhibits cardiac functional and structural abnormalities. The mice also showed QTc prolongation and increased heart rate. These changes correlate with a marked decrease in parasympathetic activity to the heart and in the expression of the Scn5a and Hcn4 voltage-gated channels. Moreover, the *Cdkl5* +/- heart shows typical signs of heart aging, including increased fibrosis, mitochondrial dysfunctions, and increased ROS production. Overall, our study not only contributes to the understanding of the role of CDKL5 in heart structure/function but also documents a novel preclinical phenotype for future therapeutic investigation.

NP02 | Epigenetic effects of exposure to the endocrine disruptor ethinyl estradiol in differentiated SH-SY5Y cells

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Endocrine disruptors (EDs) are a heterogeneous group of chemicals that may interfere with several mechanisms of the endocrine system. Some of them derived from natural sources, but most are synthetic chemicals released by human activity into the environment, such as ethinyl estradiol (EE), an estrogen medication. Their danger lies in the constant exposure of the global population and in the possibility of an induction of toxic effects not only on the hormones, but also on various physiological systems, such as the central nervous system. Among the mechanisms by which EDs could impact human health, of interest is the modulation of microRNA (miRNA). Therefore, the aim of the study was to identify the cellular pathways deregulated following exposure to subtoxic concentrations of EE and the effects on the expression of miRNAs implicated in pathways involved in neurotoxicity mechanisms. A human neuroblastoma cell line, SH-SY5Y, was exposed to different concentrations of EE for 48h to identify the experimental conditions to which cytotoxicity was not induced and which did not affect the cellular redox state. Afterwards, a miRNA profiling unveiled an important modulation in the expression of some miRNAs implicated in neurotoxicity. The genes target of these miRNAs were identified thanks to a computational analysis and the results showed that several of them were members of the PI3K/Akt/mTOR pathway. The modulation of the genes and the proteins included in this pathway has been validated by Real-Time PCR and Western Blotting assays. The study identified upregulation in the phosphorylation of Akt and mTOR, while p53 underwent downregulation following exposure to EE. Although these analyses are preliminary, they allow to investigate the role of EE as ED able to modulate pathways involved in neurodegeneration and tumor development.

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NP03 | Role of microglia in the GABAergic network plasticity in glioma

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Glioblastoma (GB) is an aggressive and immunosuppressed brain tumor with few therapeutic options. The heterogeneity, the invasiveness and the malignity of GB results from complex interactions between tumor cells and their microenvironment, including the immune system. Tumor-associated microglia play a key role in dampening immune responses, contributing to a supportive microenvironment that facilitates tumor proliferation, survival, and migration as a function of their activation state. In physiological conditions, microglia (MG) interact with inhibitory cortical synapses during a critical window of mouse postnatal development. Here, we investigated plastic changes that occur in peritumoral tissue during glioma growth, focusing on GABAergic neurotransmission, strongly affected during GB progression with implications for the development of clinical symptoms such as seizures. We used a syngeneic mouse model of GB obtained injecting the GL261 cell line into the primary motor cortex. By recording spontaneous and evoked currents with patch clamp technique in acute slices, we found that the presence of glioma drastically reduced the GABAergic inhibitory transmission in peritumoral area, while the presynaptic GABA release probability was not affected. Consistently, immunohistochemical analyses showed a significant reduction in density of VGAT+ inhibitory boutons, indicating synaptic loss. Then, we tested whether MG is involved in the GB-induced alterations of the GABAergic circuit by using PLX5622, a CSF1R inhibitor administered with the diet. One week of MG depletion in CTR mice caused a reduction in the frequency of spontaneous and miniatures GABAergic post-synaptic currents, confirming that MG participate in the modulation of GABAergic circuit. On going experiments on inhibitory synaptic transmission in microglia-depleted GB mice will help to understand whether tuning down the anti-inflammatory and pro-tumor role of MG could ameliorate synaptic impairments in GB mice.

NP04 | Recruitment of inhibitory neurons in a murine model of inflammatory pain

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Perception of pain derives from the integration and elaboration of sensory stimuli conveyed to different areas of the central nervous system (CNS) through nociceptive pathways. At the spinal cord level, the superficial dorsal horn (SDH) is the nociceptive-specific termination site of primary afferent fibers, where modality-specific circuits are activated for the elaboration of pain. A complex network of excitatory and inhibitory interneurons exists, and their balance shapes the way nociceptive signals reach the brain. At the cortical level, multiple areas participate in pain processing. To assess the hypothesis that inflammatory pain activates specific inhibitory circuits in the CNS, adult male and female CD1 mice were used as a model of inflammation induced by hindpaw intraplantar injection of zymosan. Behavioral tests to verify the presence of mechanical allodynia were performed. Through immunofluorescence, activation of inhibitory neurons was detected by the expression of a common marker of neuronal activation (Fos) with phenotypic markers of inhibitory neurons (Pax2 in SDH, calbindin/parvalbumin/calretinin in the primary somatosensory cortex). The colocalization between markers was ascertained by confocal microscopy. The von Frey test showed mechanical allodynia both in male and female. Male mice showed a significant ipsilateral increase in Fos expression at SDH and contralateral at the cortical level. However, a significant increase in the activation of inhibitory neurons was only observed in SDH nociceptive-specific areas, while no changes were detected in the somatosensory cortex. Preliminary data on female mice showed related results in the SDH, while a different activation of inhibitory neurons occurred in the somatosensory cortex. These results confirm the pivotal role of inhibition in the SDH in constraining the spread of nociceptive information in inflammatory pain, with differences related to sex, representing a potential target for controlling chronic pain.

NP05 | Altered neuronal morphology and synaptic protein synthesis in brain cortex of mouse model for Angelman Syndrome: rescuing effect of serotonin receptor 7 stimulation

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The serotonin receptor 7 (5-HT7R) is a G-protein coupled receptor, which is involved in various forms of synaptic plasticity in the brain. This receptor has been associated with several neurological and neurodevelopmental disorders including schizophrenia, depression and Autism Spectrum Disorders (ASD), which are characterized by abnormal neuronal connectivity and intellectual disabilities. Angelman Syndrome (AS) is a rare neurodevelopmental disorder exhibiting a high comorbidity with ASD. Interestingly, our research has revealed that several signalling pathways affected in AS are positively regulated by 5-HT7R. We first demonstrated that 5-HT7R activation by acute systemic injection of LP-211, a potent and selective agonist, could rescue behavioral impairment (fear conditioning test) in AS mice, suggesting a potential involvement of 5-HT7Rs in AS pathogenesis. We next investigated synaptic protein synthesis using synaptosomes isolated from the cortex of AS and wild type (WT) mice. Our results showed a significant impairment in synaptosomal protein synthesis in AS as compared to WT mice and stimulation of synaptosomes with LP-211 rescued this impairment. Additionally, we examined neuronal morphology of hippocampal primary neurons from AS and WT mice brains. Our results revealed a decrease in the density of dendritic spines in neurons from AS mice as compared to WT. Interestingly, chronic stimulation for 3 days with LP-211 restored spine density to the value found in neurons from WT mice. Overall, our study demonstrates that activation of 5-HT7R can rescue multiple synaptic plasticity mechanisms that are disrupted in AS mice. These results thus provide a new perspective for therapeutical approaches of the disease, using 5-HT7R receptor as a potential target.

NP06 | Generation of induced pluripotent stem cells lines to study the cognitive deficits associated with Noonan Syndrome

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Noonan syndrome (NS) is a rare, genetically inherited disease due to mutations on the PTPN11 gene, leading to the hyperactivity of the encoded Shp-2 tyrosine-kinase. Around 50% of NS patients show cognitive disabilities, such as learning and memory impairments. Unfortunately, drugs for the treatment of these symptoms are not available and how Shp-2 hyperactivation affects neuronal functions is still elusive. The comprehension of the molecular mechanisms underlying NS-associated cognitive deficits is needed for understanding their pathogenesis and for the identification of new molecular targets. Hence, we used the induced pluripotent stem cells (iPSCs) technology as an “in vitro” tool to model NS and study the molecular mechanisms underlying the cognitive deficits. Peripheral blood mononuclear cells were isolated from three different NS patients with cognitive deficits and the 188A>G mutation in the PTPN11 gene and treated with the Yamanaka factors (Oct3/4, c-Myc, Klf4, Sox2). We then selected two iPSCs lines for each patient and we confirmed their stemness and their pluripotency both by gene expression and immunofluorescence analyses. Moreover, iPSCs-derived embryoid bodies were analyzed for the expression of typical ectodermic endodermic and mesodermic genes, and therefore for their ability to generate all the body tissues. Each iPSCs line was also karyotyped and analyzed for the presence of the 188A>G mutation in the PTPN11 gene. Since it has been shown how Shp-2 hyperactivity impacts glutamatergic neuron function, we also developed a protocol to differentiate iPSCs into neuronal cells with a high percent of glutamatergic neurons.
Thus, iPSCs lines generated from NS-patients and cognitive deficits, and their corresponding genetic-manipulated lines with the corrected mutation used as control, represent a powerful tool to identify the molecular mechanisms underlying the neurological feature of the disease.

NP07 | CXCR4 in neurophysiology and neurodegeneration

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The ability of the peripheral nervous system to regenerate after injury relies on intrinsic qualities of peripheral neurons and a permissive environment. An array of mediators exchanged by motor axons and nearby cells drives nerve regeneration. In this context, the molecular axis composed of the chemokine CXCL12α and its receptor CXCR4 plays a pivotal role: CXCR4, a G protein-coupled receptor expressed only during development, is re-expressed by adult motor axons upon either crush of entire nerves or nerve terminals neurotoxic damage, while its natural ligand CXCL12α is released by Schwann cells. CXCR4 activation by the chemokine and the novel agonist NUCC-390 drives re-innervation and neuromuscular function recovery. Understanding CXCR4 dynamics upon injury and the intracellular signaling downstream its reactivation is of crucial importance, given the pro-regenerative potential of CXCR4 agonists. We monitored the expression and trafficking of CXCR4 mRNA and protein in control conditions and upon crush of the sciatic nerve by RNAScope Technology and immunostaining, respectively. CXCR4 transcripts are detectable in controls both in the spinal cord and along the sciatic nerve, and accumulate locally after axonal damage. This suggests that injury triggers CXCR4 transcription in the neuronal soma, and the transport of its mRNA to the periphery when axon re-growth is required, and where its local translation occurs. We also found an increase in CXCR4 protein expression upon peripheral damage both in the spinal cord and in the sciatic nerve, with the protein accumulating in discrete structures along the nerve, whose nature is under investigation. We plan to visualize the protein synthesis machinery in the axon and CXCR4 translation by puromycilation assays. *In vitro* assays in primary spinal cord motor neurons (SCMNs) expressing a FRET-based probe reveal a decrease of cAMP levels upon CXCR4 stimulation by CXCL12α and NUCC-390, pointing to the engagement of a Gi protein.

NO01 | The cytoskeleton regulator inverted formin INF2 regulates the SHH pathway and is involved in medulloblastoma tumorigenesis

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Medulloblastoma (MB) is a lethal pediatric malignancy of cerebellum. Identifying a successful therapy for MB is extremely difficult, mainly due to its high molecular and genetic heterogeneity. Among MBs, Sonic Hedgehog subgroup (SHH-MB) is the most abundant and genetically understood. SHH-MB is characterized by genetic alterations in the key players of SHH signaling, a developmental pathway emerged as an attractive therapeutic target for MB treatment. However, the molecular mechanisms governing SHH-MB remain unclear. We recently identified INF2, a formin involved in the regulation of actin cytoskeleton dynamics, as a negative regulator of SHH signaling, with a putative opposite effect to the one previously described for mDia formins. We found that the overexpression of INF2 counteracts the positive effects of mDia on GLI1 (the final and most powerful effector of SHH signaling), by reducing both its transcriptional activity and SAG-induced expression. Accordingly, the INF2 genetic silencing increases mRNA and protein levels of GLI1 as well as the proliferation of granule cell progenitors (GCPs), the cells of origin of MB. Moreover, a correlation between increased INF2 protein expression and time dependent switching off of SHH signaling was observed during normal cerebellum development in mice, showing an opposite trend to mDia. Interestingly, INF2 protein levels were strongly reduced in murine and human SHH MB samples, contrary to what observed for mDIA. Notably, the overexpression of INF2 in SHH-MB primary cells significantly inhibits the cell proliferation as consequence of the reduction of GLI1 expression levels, and increases the stiffness of primary MB cells, thus suggesting that the absence of INF2 could affect tumor cell motility and invasiveness. Overall, these findings strongly support a negative role of INF2 in the regulation of SHH signaling paving the way to study cytoskeletal remodelling proteins as a novel area of investigation in SHH-MB.

NO02 | Exploring the role of mechanoreceptor Piezo1 in glioblastoma

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Glioblastoma multiforme (GBM) is one of the most aggressive types of brain tumour. It affects 2-3 out of every 100,000 adults each year, and has a median survival rate of 14-18 months. This is, in part, due to the existence of cancer stem cells (CSCs), a cellular subtype with stem-like properties and resistant to conventional treatments, being the cause of most relapses. Because of all this, a deeper understanding of this type of tumour is needed, so that new therapeutic strategies can be designed. The study of mechanical properties of tissues has an emergent role in current research. It is known that the tumour microenvironment is harder than healthy tissue and that tumour cells overexpress mechanoreceptor proteins to respond to these changes, which have a key role in cancer. Here, we explore the role of mechanoreceptor Piezo1 in GBM. Previously, our group observed that Piezo1 overexpression in U251 modified cell line increases cell migration, clonogenicity and chemoresistance. To continue this research, we have analysed the transcriptomic profile of Piezo1-KO and Piezo1-overexpressing cells by RNA-Seq, observing that key cancer-related processes such as epithelial-mesenchymal transition, G2/M checkpoint and inflammatory pathways are altered. Thus, we have validated these findings molecularly, explaining how Piezo1 can increase the aggressiveness of glioma cells. We also have created a unique mouse model of primary glioma (GFAP-Cre/Tg.Piezo1 and GFAP-Cre/Tg.Piezo1/p53$^{lox/lox}$) that overexpress Piezo1 in glial cells. We have observed that a high percentage of mice developed brain tumours, which postulates this mechanoreceptor as an oncogene of GBM, and with it, in a potential therapeutic target for this disease. Finally, we have developed a new pharmacological approach to modify Piezo1 activator Yoda-1 and Piezo1 inhibitor GsMTx4 for nasal-to-brain delivery, in collaboration with the ADDRes Lab from Universita' di Parma, and we have tested this formulations *in vitro*.

NO03 | Unmasking Myc's Role in Glioblastoma Clonal Warfare

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Glioblastoma has a complex and poorly understood progression. Our study aimed to unravel the underlying dynamics of glioblastoma evolution, particularly focusing on clonal competition. Using a groundbreaking approach of simultaneous transfer of Platelet Derived Growth Factor B (PDGFB) and a unique genetic barcode in E14 mouse embryos, we have been able to trace the inception and progression of glioblastoma. We uncovered an unexpectedly high incidence of clonal extinction events and progressive clonal size divergence, suggestive of fierce cell-cell competition at the clonal level. Our analysis highlighted the role of Myc transcriptional targets as a major driver of these dynamics. To validate our findings, we conducted additional experiments using Epidermal Growth Factor Receptor variant III (EGFRvIII), another potent oncogenic driver, and found similar clonal evolution patterns. The robustness of these results, regardless of the oncogenic driver, underscores their relevance in glioblastoma progression. Further analysis of human Glioblastoma datasets echoed our findings. Specifically, we noticed the replacement of high frequency genetic variants in primary gliomas by low frequency variants in recurrences. To provide a functional perspective, we assessed the role of Myc expression levels in clonal competition. Despite Myc knockdown did not affect the in-vitro growth rate of cells, it dramatically altered their in-vivo behaviour. Myc downregulated cells (Myc-KD) and Myc wild-type cells (Myc-wt) were introduced into mice brains in isolation or mixed. When transplanted alone, Myc-KD cells developed gliomas similar to Myc-wt cells. However, in a mixed population, Myc-KD cells were nearly completely outcompeted by Myc-wt cells, underscoring Myc role in cell competition during glioma progression. In conclusion, our findings unveil an intricate interplay of clonal competition in glioblastoma progression, with a key role played by Myc pathway.

NO04 | Gold nanoparticles (AuNPs) in the radio-sensitization of glioblastoma cells

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Glioblastoma multiforme (GBM) is the most malignant form of primary brain tumour, with extremely poor prognosis due to bad response to therapeutic regimens. Ionizing radiation (IR) has been identified as a crucial treatment for GBM following surgical resection to improve overall survival. Unfortunately, radiotherapy resistance is a frequently observed phenomenon in affected patients. The mechanisms underlying the intrinsic radio-resistance in GBM are multifactorial, although altered DNA damage response seems to be the most crucial operator in the outcome to IR exposure. In the present work we are investigating the effectiveness of a novel approach to radio-sensitize GBM cells through the use of gold nanoparticles (AuNPs). AuNPs are promising radio-sensitizing agents due to their high biocompatibility and ability to be synthesized with various shapes and structures. AuNPs act by photothermal therapy (PTT), an efficient method of inducing localized hyperthermia aiming to selectively kill tumor cells. In this work, AuNPs, specifically nanoprisms (NPrs), have been tested in two GBM cell lines: U87MG stabilized cell line and a primary cell line named GBM3. Preliminary data show that AuNPrs alone at low concentrations have no toxic effects in both GBM cell lines used, where AuNPrs demonstrated an efficient cytosolic internalization. More importantly, the combination of AuNPrs with increasing IR doses (2Gy-8Gy) showed a greater reduction in cellular viability and colony formation when compared with samples treated with IR alone. This suggests that throughout thermoablation, AuNPrs are able to weaken cells thus making them more susceptible to lower doses of IR. Combination therapy based on AuNPrs and subsequent low-dose IR could be considered a promising alternative to standard GBM treatment involving much higher IR doses (60Gy).

NO05 | Autophagy inhibition enhances Natural Killer cell- based therapy in high-risk Medulloblastoma

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Medulloblastoma (MB) is the most common pediatric brain tumor with Group3 (G3) subtype characterized by poor prognosis and therapy relapse. Autophagy is a self-degradative process increased in G3 MB stem cells (MBSCs) to contribute stemness and survival (Nazio et al., 2021). A promising therapeutic value in the treatment of high-risk MB is represented by Natural Killer cells (NKs)-based immunotherapy but it requires complementary approaches that break immune tolerance. To date, the role of autophagy-mediated mechanisms in regulating tumour heterogeneity and immune cells infiltration capability in the context of brain TME remains unknown as well as the role of autophagy in NK/CAR-NK mediated therapy is completely unexplored. Herein, we are investigating the role of autophagy inhibition as a druggable mechanism to increase NK recognition and killing against MB G3. By flow cytometry analysis, we found low levels of NK-related activating ligands (ULBPs, CD155, CD112, MICA/B) in G3 cell lines and MBSCs derived by MB G3 patients compared to SHH subgroup. Intriguingly, genetic and pharmacologic inhibition of autophagy is able to increase NK-related ligand expression on cell surface of MB G3 cells. Additionally, by means of 2D and 3D models of MB G3 cells, we found that autophagy inhibition increases NK degranulation and MB G3 sensitiveness to NK-mediated cytotoxicity. Immunotherapeutic strategies aimed at restoring and increasing the cytotoxic activity of NK cells in solid tumors, including the adoptive transfer of NK cells, are currently employed in preclinical and clinical studies. New strategies are necessary to make NK cells more resistant to the metabolically restrictive TME as well as to immunosuppressive molecules generated by the tumor. The completion of our project would be helpful to design a novel therapeutic approach for children with high-risk MB, aiming to a higher clinical response rate coupled with less toxicity as compared to conventional therapies.

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NIM01 | Development of a deep-learning tool for the detection and segmentation of contrast-enhanced lesions in multiple sclerosis patients

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Background: The detection of contrast-enhanced lesions (CELs) is fundamental for the diagnosis of multiple sclerosis (MS) and for the monitoring of MS patients. CELs detection in clinical practice is time-consuming and suffers from high inter- and intra-rater variability, especially for small CELs. **Objective**: To develop a deep-learning tool to automatically detect and segment CELs, which can support clinical radiological practice. **Methods**: We studied 157 MS patients with CELs and 129 patients without CELs who underwent a clinical MRI scan including a T1-weighted image acquired with pre- and post-injection of a gadolinium-based contrast agent, as well as FLAIR images. White matter lesion (WML) masks were obtained with an automated method and then manually corrected. 557 CELs were segmented by experienced clinicians and the masks are utilized as ground truth. For the purpose of this study, we adapted a UNet-based convolutional neural network that had been previously tested for the detection of cortical lesions, which are notoriously small. To overcome the problem of the low frequency of CELs smaller patches are cropped based on WML mask regions. Moreover, a new loss function is introduced, which takes into account the class imbalance and partly also the heterogeneous shape of CELs. Finally, an ablation study was performed to fine-tune the neural network architecture. **Results**: In the test dataset (n= 63 patients, 125 CELs) we obtained a DSC of 0.74, a True Positive (TP) rate of 0.94 and a False Positive (FP) rate of 0.0085. These values for small lesion sizes (3-10 mm^3) are 0.76/0.769/0.090, for medium lesion sizes are 0.67/0.92/0 and for lesions with volume larger than 50 mm^3 are 0.79/1/0. **Conclusions**: Our results were comparable with those obtained in a few previous studies performed using larger datasets, more contrast images and considering larger lesion size. Future work will aim for improving its performance and integration into clinical practice.

NIM02 | Mapping structural disconnection and morphometric similarity in Multiple Sclerosis: a longitudinal study

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Multiple sclerosis (MS) can be conceptualized as a network disorder. The accumulation of white matter (WM) lesions leads to progressive disconnection, while the development of atrophy disrupts the morphological similarity between brain regions. We used conventional MRI to assess cross-sectional and longitudinal modifications of structural disconnection and morphometric similarity networks in MS.

We retrospectively collected 3T structural brain MRIs of 461 MS patients (age=37.2±10.6y; F:M=324:137), corresponding to 1235 visits (follow-up time=1.9±2.0y; range=0.1-13.3y), and 55 healthy controls (age=42.4±15.7y;F:M=25:30). From 3D-T1w and FLAIR-T2w scans, WM lesions were automatically segmented and the brain was parcellated into 100 cortical (Schaefer atlas) and 14 subcortical (Aseg atlas) regions. For MS patients, subject-level WM masks were registered to MNI space and, using the Lesion Quantification Toolkit, disconnection between pairs of regions was estimated as the proportion of connecting streamlines passing through WM lesions. Likewise, with the Morphometric Inverse Divergence (MIND) method, we computed networks of morphometric similarity between cortical regions from 3D-T1w derived FreeSurfer outputs for both groups. Using network-based statistics, effect of time (and group, for MIND networks) was tested with linear mixed-effects models.

We identified a small subnetwork (27 edges) of significant progressive structural disconnection mainly encompassing cortico-subcortical tracts ($p \leq 0.001$). MIND networks were sensitive to disease status and time, with distributed effects of decreased morphological similarity in large subnetworks of 125 and 174 edges, respectively ($p \leq 0.001$).

We demonstrated that structural disconnection and morphometric similarity networks, as assessed through conventional MRI, are sensitive to MS-related brain damage and its evolution over time, potentially adding to established MRI-derived measures as biomarkers of disease severity and progression.

ND01 | Bridging the gap from mice to humans: using genetic predictions to investigate the effect of circulating blood factors on the human brain

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Mice studies have identified blood proteins that influence brain aging, but translating these findings to humans remains a challenge. Here, we report an innovative approach to investigate the potential effect of these proteins on the human brain by genetically predicting their plasma levels and assessing their association with cognitive performance. Through a systematic review, we identified 13 blood proteins with an aging/rejuvenating effect on the mouse brain. Leveraging data from a previous genome-wide association study, we retrieved DNA variants associated with the level of the targeted proteins in human plasma. We used these DNA variants to compute protein-based genetic scores (PRS) for 410 cognitively unimpaired individuals at risk of Alzheimer's disease (age: 53-61; 61% women; 54% *APOE*-ε4 carriers). Computed PRS estimate the individual predisposition to high protein blood levels. Our results revealed that genetic predisposition to elevated plasma levels of TIMP2 (Metalloproteinase inhibitor 2) is significantly associated with better cognitive performance as measured by the Preclinical Alzheimer Cognitive Composite (PACC) scores. Results were also significant when stratifying by sex and *APOE*-ε4 carriership (FDR corrected p-value <0.05). Our findings align with a previous mouse study that demonstrated the effects of plasma TIMP2 on improving brain performance (synaptic plasticity and cognition). To validate the PRS of TIMP2 as a proxy of TIMP2 levels, we measured TIMP2 in the plasma of the study participants using an ELISA assay and found a significant association with the TIMP2 PRS. This innovative use of protein based PRS computation overcomes translational challenges encountered in animal studies, providing a complementary mean to screen the association of various proteins with brain performance in humans. This approach was successful in highlighting TIMP2 as a potential therapeutic target in brain aging and neurodegeneration.

ND02 | A potential therapeutic strategy for CMT2A: combined RNA interference and gene therapy in vitro and in vivo disease models

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CMT2A is an autosomal dominantly inherited disease characterized by progressive muscle weakness, and atrophy, loss of sensation and motor difficulties mainly in the distal limbs. It is caused by missense mutations in the *Mitofusin2 (MFN2)* gene which induce the disease with a dominant negative mechanism, negatively regulating the wild-type MFN2 allele expression. Gene therapy for dominant inherited diseases uses RNA interference (RNAi) to selectively inhibit expression of the mutant allele, which results in a toxic protein. Since this approach can also reduce the expression of the wild-type functional allele, wild-type allele restoration, in combination with mutant allele silencing, could improve the therapeutic effects. Here, we propose this novel potential therapeutic approach combining RNAi and gene therapy, whereby mutant and wild-type *MFN2* mRNA are inhibited by RNAi, while the wild-type protein is restored by overexpressing cDNA encoding functional MFN2 modified to be resistant to RNAi. First, we tested the effective silence of the endogenous MFN2 (both mutant and wild-type *MFN2* alleles) and its replacement with an exogenous copy of the wild-type *MFN2* gene in CMT2A human induced pluripotent stem cells (iPSCs)-differentiated motor neurons, confirming the molecular efficacy of our strategy. To evaluate the amelioration of the disease phenotype, we analysed key motoneuronal features relevant to CMT2A, observing an enhancement in mitochondrial distribution and function, beyond in apoptotic and autophagic parameters in CMT2A MNs. We, also, assessed the molecular efficacy in Mitocharc1 CMT2A mouse model after cerebrospinal fluid (CSF) delivery of the constructs into newborn mice using adeno-associated virus 9 (AAV9). Our data confirm the feasibility of combined RNAi and gene therapy approach as potential therapeutic strategy for treating CMT2A and other similar genetic neurological disorders even if the therapeutical efficacy need to be validated in vivo.

ND03 | Betaine is a substrate of GAT1 that can modulate extracellular GABA

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Betaine, N,N,N-trimethyl glycine, is an osmolyte that shows ameliorating effects in neurological and neurodegenerative diseases like Alzheimer's, Parkinson's, schizophrenia. While there has been an ongoing surge of the studies demonstrating positive effects of betaine, the molecular mechanism of action and the translocation process is still not clear. The betaine/ γ-aminobutyric acid (GABA) transporter 1 (BGT-1, slc6a12) and the sodium-coupled neutral amino acid transporter 2 (SNAT2, slc38a2) can transport betaine in the brain, however due to their little expression, it has been suggested that betaine should be interacting with proteins other than these, especially across the GABAergic pathways.

In this work, we show that GAT1 (slc6a1), the most expressed GABA transporter in the central nervous system (CNS), can translocate betaine across the neuronal membrane, though with lower affinity ($K_{0.5}$≈11mM at -60mV) than GABA. Using electrophysiological experiments on *Xenopus laevis* oocytes heterologously expressing rGAT1, we demonstrate that betaine induces inward transport currents, which are dose-, voltage-, and Na^+ dependent. The betaine transport can be blocked by GAT1 specific inhibitor like SKF89976a. We also confirmed that betaine is a substrate of GAT1 using molecular docking, radiolabelled release assay in HEK293 cells, and LCMS-MS technique. More interestingly, our results on GABA-betaine relationship for GAT1 show that betaine at µM concentration can effectively modulate the GABA transport. It inhibits the transport when the extracellular GABA concentration is less than its $K_{0.5}$ (≈16 µM at -60mV), while at higher extracellular GABA concentration, betaine behaves like a secondary substrate. Uptake data obtained by LCMS-MS detection of GABA and betaine inside the oocytes expressing rGAT1, provide direct supporting evidence for the same phenomena. This modulatory behaviour of betaine on GABA transport suggests a role of neuromodulator in maintaining GABA homeostasis and excitatory/inhibitory balance in the CNS.

ND04 | Role of GDF15-GFRAL pathway in the regulation of feeding behaviour in hSOD1-G93A mice

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that affects the motor system. Pro-inflammatory microglial cells are able to impact the disease progression in ALS, participating to impaired mechanisms that regulate feeding behaviour. In the last years, Growth Differentiation Factor 15 (GDF15) has become an attractive target for its metabolic role in non-homeostatic conditions, including cancer cachexia, metabolic diseases and neuroinflammation, exerting an anorectic effect through a GDNF family receptor alpha-like (GFRAL), which expression is restricted to the brainstem. In the present study we used hSOD1^{G93A} mouse model of familial ALS to investigate whether GDF15-GFRAL signaling could be involved in the regulation of body weight and feeding behaviour in ALS. To this aim, we first quantified serum levels of GDF15 reporting a significant increase in our model. In addition, we observed that pro-inflammatory microglial cells show higher expression of GDF15, both in vitro and in vivo. Consistently, pharmacological depletion of microglia dampened body weight loss in hSOD1^{G93A} and led to a reduction of GDF15 levels in the brainstem, suggesting the involvement of microglial cells in GDF15 signaling pathway. Then, to investigate whether inhibition of the GDF15–GFRAL pathway influences mouse feeding behaviour, we knocked down GFRAL expression in the hindbrain in hSOD1^{G93A} mice. Under these conditions, we observed a positive effect on food intake and how body weight loss was successfully prevented. Our results provide a novel mechanism involved in the regulation feeding behaviour and body weight in amyotrophic lateral sclerosis, shedding light on the essential role played by GDF15.

ND05 | Obstructive sleep apnoea syndrome and effects on central nervous system: from a new in vitro model to peripheral plasma biochemical markers to study cognitive decline insurgence

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Obstructive sleep apnea syndrome (OSAS) is a breathing disorder characterised by nocturnal fluctuations in blood oxygen levels causing intermittent hypoxia (IH) at tissues level. Since OSAS is often associated with the onset of mild cognitive impairment (MCI),this work has two main aims: (1) the development of a new in vitro model to mimic IH condition on human microglial cell line to study O_2 oscillations consequences on central nervous system (CNS); (2) the investigation of new possible peripheral biomarkers in plasma of OSAS patients to discriminate patients with MCI (OSAS+MCI) from patients without clinical signs of cognitive decline (OSAS-MCI). Our in vitro model was firstly validated by assessing cells normoxic/hypoxic condition; then inflammatory markers (NF-kB and IL-6) mRNA expression and proteins levels analysis were performed too. In addition, to verify if IH can modulate the expression of membrane markers related to microglial priming (CX3CR1, HLA-DRα and CD86), a transcriptional analysis was performed. Lastly, to confirm that IH can lead to microglial priming, an analysis of NF-kB and IL-6 mRNA expression and protein levels after IH treatment followed by a mild inflammatory stimulus (IL-1b treatment) were performed too. Results showed as IH condition causes an increase in microglial priming markers' mRNA expression and an exaggerated cellular response after IH followed by a secondary inflammatory stimulus.Parallely, 30 OSAS patients and 15 controls were enrolled and subjected to clinical evaluation and blood sampling. A positive correlation between HIF-1a and p-Tau plasma levels and polygraphic parameter T90 (the cumulative time with oxygen saturation below 90% during sleep) was obtained. In addition, T90, HIF-1a and p-Tau, showed increased levels in OSAS+MCI group compared to OSAS-MCI. ROC curve analysis confirmed the discriminatory ability of HIF-1a and p-Tau between OSAS+MCI and OSAS-MCI, suggesting these proteins new peripheral biomarkers useful to predict MCI insurgence.

ND06 | Generation of hiPSC derived from PD-sporadic patients to dissect the role of rare variants in Parkinson's disease pathogenesis.

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Parkinson's disease (PD) is a neurodegenerative movement disorder caused by the inexorable and progressive loss of dopaminergic neurons in the substantia nigra and associated with diffuse accumulation of Lewy bodies. The genetic basis of sporadic PD remains poorly understood preventing the development of effective tools for disease stratification and treatment. We have recently reported the identification of new rare gene variants in PD patients that support polygenic contribution to the disease, even in sporadic forms. Given this genetic complexity, patient-derived induced pluripotent stem cells (iPSCs) provide an invaluable system where the cellular alterations associated with the patient genetic background can be directly assessed.
In this study, we generated a set of episomal integration-free hiPSCs (6 of PD-patients and 3 of healthy subjects) reprogrammed from peripheral mononuclear cells (PBMc) of patients carrying highly penetrant genetic combinations of variants in novel PD genes (AIMP2, HMOX2, IMMT, KIF21B, LRRK2, RHOT2, TMEM175, TOMM22, TVP23A, ZSCAN21).
A well-defined characterization protocol was performed to ensure high standard quality of collected iPSCs including: i) human embryonic stem cell-like colonies morphology; ii) episomal elimination, through PCR analysis; iii) pluripotency assessment, through the analysis of pluripotency-associated markers including, OCT3/4, SOX2 and NANOG, both at RNA and protein level; iv) karyotype analysis to reveal chromosomal abnormalities. Finally, generation of BIII-tubulin-, GFAP- and TH-positive cells demonstrated the differentiation ability of the reprogrammed iPSCs.
In conclusion, the characterization of a large collection of iPSCs carrying novel PD-associated predictive genetic combinations of mutations in novel candidate genes will represent unique tools to decipher the molecular basis underlying neurodegeneration in PD and will contribute to the design of new therapeutic strategies.

ND07 | Visualizing Tau Tangles in AD Retina with a BODIPY-based Fluorescent Ligand

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Background: Alzheimer's disease (AD) is a neurodegenerative disease responsible for most cases of senile dementia. Despite numerous studies, there are no effective therapies or significant progress in symptom progression. To develop a therapeutic strategy, research has focused on accurate diagnostic methods. Coupled units of hyperphosphorylated protein (P-Tau) and total-Tau monomers (t-Tau) are reliable biomarkers for AD. Early identification of neurofibrillary tangles (NFTs) in retinal tissue is a promising diagnostic tool. The development of highly specific Tau fluorophores has been achieved through the construction of a computational model of TAU oligomers.

Methods: The construction of the highly specific Tau fluorophore BT-1 (Soloperto et al., 2021) consisting of a BODIPY-based probe showed excellent photophysical properties and high selectivity allowing in vitro imaging of hyperphosphorylated tau protein filaments with minimal background noise. Delivery to living iPSC derived retinal cells was achieved by identifying a class of delivery cargoes among cell permeant peptides (CPP), natural nanocages that can embed small molecules and enter human cells.

Results: BT-1 colocalizes with phosphorylated and oligomeric tau in AD retinal slices and iPSC derived retinal cells. Loading the CPP nanocage with the fluorescent probe BT-1 generated a unique formulation for the delivery of the fluorescent probe to retinal tissue.

Conclusion: The use of CPP nanocages loaded with the fluorescent probe BT-1 provides a promising method for the specific identification of NFTs in retinal tissue for the clinical diagnosis of AD. The encapsulation of other substrates in CPP has potential applications in nanomedicine.

ND08 | Intramuscular IL-10 Administration Enhances the Activity of Myogenic Precursor Cells and Improves Motor Function in ALS Mouse Model

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Amyotrophic Lateral Sclerosis (ALS) is the most common adult motor neuron disease, with a poor prognosis, a highly unmet therapeutic need, and a burden on health care costs. Hitherto, strategies aimed at protecting motor neurons have missed or modestly delayed ALS due to a failure in countering the irreversible muscular atrophy. We recently provided direct evidence underlying the pivotal role of macrophages in preserving skeletal muscle mass.
Based on these results, we explored whether the modulation of macrophage muscle response and the enhancement of satellite cell differentiation could effectively promote the generation of new myofibers and counteract muscle dysfunction in ALS mice. For this purpose, disease progression and the survival of SOD1G93A mice were evaluated following IL-10 injections in the hindlimb skeletal muscles. Thereafter, we used ex vivo methodologies and in vitro approaches on primary cells to assess the effect of the treatment on the main pathological signatures.
We found that IL-10 improved the motor performance of ALS mice by enhancing satellite cells and the muscle pro-regenerative activity of macrophages. This resulted in delayed muscle atrophy and motor neuron loss. Our findings provide the basis for a suitable adjunct multisystem therapeutic approach that pinpoints a primary role of muscle pathology in ALS.

ND10 | Generation and characterization of 3D neuromuscular organoids for the study of Amyotrophic Lateral Sclerosis

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Neuromuscular organoids (NMOs) are a complex 3D model system in which different tissues, including spinal cord neurons and skeletal muscle, develop in parallel, self-organize and interact to form functional networks. Since that, they represent a useful system to study neuromuscular disorders, as the development of 3D NMOs also allows the study of the possible several cell types contribution to the disease, investigating the behaviour of neuromuscular junction (NMJ) different cellular components. Further, this model system enables the analysis of different NMJ maturation stages. These properties make NMOs an appropriate model system for studying Amyotrophic Lateral Sclerosis (ALS), a neurodegenerative disease characterized by the loss of motor neurons, in which functionality of NMJ is affected. In particular, the mutation P525L of the FUS gene has been associated with a severe familiar form of the pathology, corresponding to a juvenile onset. For these reasons, the aim of this project is the study of possible alterations in the development of NMOs generated by iPSCs carrying FUS P525L mutation. By using gene edited FUS P525L iPS cell line and an isogenic wild type control, I will analyse, at different development stages, neuronal and muscular genes expression, contractile activity and NMOs morphology; further, we will investigate mechanisms underlying eventual alterations, and, by taking advantage of this more complex model system, we would try to find new therapeutic strategies for the FUS-ALS.

ND11 | Analysis of circulating lipids and metabolites of Parkinson's disease patients undergoing different types of therapy: a pilot study

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Parkinson's disease (PD) is a complex neurodegenerative disorder characterized by the impairment of several cellular pathways including autophagy, mitochondrial metabolism, oxidative stress, and vesicular trafficking, leading to the formation of alpha-synuclein aggregates. Currently, only symptomatic therapies are available and long-term medical treatments are associated with a range of adverse effects. It is well demonstrated that circulating lipids and metabolites may mirror the alteration of metabolic pathways at cellular level, even at neuronal one.
In this study, we explored whether long-term treatment of PD patients induced alteration on metabolism. To this aim we performed a multi-omics approach to profile lipidomic and metabolomics in plasma as well as we used a multiplex ELISA assay to measure circulating marker associated to lipids mobilization and catabolism (leptin, ghrelin, FABP3 and FABP7), inflammation (TNF-alpha, Gro-alpha), neurotrophy (BDNF) and neurodegeneration (GFAP). The study cohort included three groups of PD patients, undergoing different therapies, and healthy subjects matched for age, sex, and BMI. Specifically, the PD cohort included patients treated for at least 10 years with L-Dopa standard therapy (n=15), or with deep brain stimulation (DBS) and L-Dopa maintenance therapy (n=16), and patients taking only dopamine agonists (n=15) and controls (n=15). We found an extensive dysregulation of lipids and metabolites in PD patients compared to healthy subjects. This effect was more evident when comparing patients treated with DBS *vs* L-Dopa. The DBS group also showed an increase in peripheral inflammation and lipid mobilization, as well as a significant reduction of the neurodegenerative marker GFAP. These preliminary data are encouraging to extent this analysis to a larger cohort of patients to discover novel biomarkers for the monitoring of the therapy and to identify drug targets that will improve long-term treatments for PD.

ND12 | An insight into Alzheimer's disease pathogenesis: cell-laden hydrogels and oxidative stress

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder leading to the most common form of dementia in elderly people. Over time, a person with Alzheimer's gradually loses his/her ability to live independently. Research on the pathogenesis of AD is mainly focused on the amyloid cascade, tau protein, neuroinflammation, metal ions, and oxidative stress hypotheses. Oxidative stress is a process that causes neuronal damage and occurs in various pathways, acting as a bridge between the different hypotheses and mechanisms of AD. Meanwhile, nanocomposite and nanostructured hydrogels, functionalized for drug and cell delivery, offer a variety of possible cutting-edge scenarios such as tissue repairing and bioinspired scaffolds, extracellular matrix analogues, exploiting their good biocompatibility. The aim of this study was to develop a novel *in vitro* model based on co-culture of MG63/SHSY5Y cell lines embedded in different formulations of collagen and hyaluronic acid-based semi-IPNs. Alamar Blue assay and western blot analysis were performed to investigate the differentiative and proliferative capacities of the proposed model after oxidative stress. Moreover, Confocal Laser Scanning Microscopy has allowed to evaluate the capability to improve the expression of neurological differentiation markers for the co-culture system, if compared to single culture system. Results showed an improved expression of Neuron Specific Enolase(NSE) in co-culture systems in comparison to single culture system. The obtained results support a biomaterials-based approach for controlled delivery of cell-produced neuroprotective factors in AD experimental context. The authors thank PRIN2017 2017XKJTLW_003 - "EXPLOITATION OF CIRCULATING MIRNAS FOR DIAGNOSIS AND NEUROPROTECTION IN TRANSLATIONAL STROKE STUDIES" and PRIN 2020WREYF2 "3D Customized HYbrid MedicAl Devices for Alzheimer's disease-related Periodontitis Treatment - 3D CHYM ADAPT" for providing the financial support to this work.

ND13 | In vitro evaluation of the antioxidant capacity of novel benzofuran-2-ones in a cellular model of neurodegeneration

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Neurodegenerative diseases (NDs) are characterized by the progressive loss of neurons in the Central Nervous System (CNS). In the healthy brain, oxidative stress (OS) is well counterbalanced by antioxidative defences. The augmented OS is generally associated with neuronal loss, a typical hallmark of several NDs. A pivotal role in the pathogenesis and progression of NDs is played by heme oxygenase-1 (HO-1), a 32 kDa heat-shock protein which may be protective or toxic, depending on its levels of expression. Therefore, it becomes of fundamental importance to consider antioxidants (AOs) as an adjuvant strategy to control cellular OS within the CNS.
The aim of this study was to investigate the antioxidant activity of four newly synthesized benzofuran-2-one derivatives in differentiated SH-SY5Y cells exposed to catechol-induced OS.
Our results show that, upon phorbol 12-myristate 13-acetate (PMA) differentiation, SH-SY5Y cells were more sensitive to OS than the undifferentiated counterpart. Among the tested AOs, compounds 1 and 4 significantly reduced catechol-induced cell death, being the AO 4 the most effective. Moreover, dichlorofluorescin diacetate (DCFH-DA) assay confirmed that both molecules can reduce intracellular ROS concentration in our cellular model in a greater extent than Trolox, a reference AO. Further, western blot analysis showed that catechol induced high levels of HO-1, thus confirming toxic effects on cells, and that AOs 1 and 4 can limit this induction. Additionally, we have evaluated the efficacy of the AOs in an *in vitro* model of microglia/neuron interplay by treating SH-SY5Y cells with supernatants collected from LPS/IFN-γ activated THP-1 monocytes. In this condition, only AO 4 showed an appreciable antioxidant capacity.
In conclusion, the newly synthesized benzofuran-2-ones tested in this work could represent promising molecules to contrast OS, opening the way for new adjuvant strategies that might improve the life quality of patients with NDs.

ND14 | Alteration of lipid metabolism in the pathogenesis of Hereditary Spastic Paraplegia: unraveling the mechanisms to recover cell function

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Hereditary Spastic Paraplegias (HSPs) are a group of inherited neurologic disorders in which lower extremity weakness and spasticity are the predominant symptoms. HSPs are characterized by high genetic heterogeneity. Nevertheless, alterations in morphology or distribution of the Endoplasmic Reticulum (ER) appear to be a critical pathogenic factor. Mutations in two genes encoding crucial enzymes to the plasmalogens (PLs) biosynthetic pathway, *i.e.*, SPG81 and SPG82, have been recently identified in HSP patients. PLs are ether phospholipids abundant in ER membranes. Ethanolamine-based PLs (PE-PLs) are enriched in nervous system membranes, constituting up to 85 mol% of total phosphatidylethanolamine (PE) species and up to 30 mol% of total phospholipids in mammalian brains. Notably, PLs amount was found decreased in several neurological diseases, suggesting that PLs could play a role in neuronal membranes welfare. PE-PLs are suggested to promote the formation of inverted hexagonal phases, thus facilitating membrane fusion events. However, the sub-molecular details behind the above properties are not fully understood. We aim at identifying a potential role for PLs in the remodeling of ER membranes. Our hypothesis is that manipulating ER membrane lipid composition in a way that favors membrane dynamics, we could rescue HSP-related ER morphology defects. Our goal is to test if administration of bioavailable PLs precursors to validated HSP fly models is able to increase the amount of membrane PLs, and to improve HSP-related phenotypes (birth rate, survival rate, and locomotor ability). The validated approach could prove a new therapeutic option for HSPs and potentially for other neurodegenerative diseases involving phospholipid-related membrane impairment.

EBN01 | EPM1 3D human model: altered synaptic plasticity and neuronal morphology

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Progressive myoclonic epilepsy 1 (EPM1) is a neurodegenerative disease characterized by loss-of-function mutations in Cystatin B (CSTB) gene. CSTB is an inhibitor of lysosomal cathepsins and it is involved in the development of human brain cortex: low levels of functional CSTB impact cell proliferation, differentiation and interneuron recruitment during neurogenesis. We previously demonstrated that CSTB is locally synthesized in rat brain synaptosomes and secreted into the media, suggesting its role in synaptic plasticity. In this work we investigate if synaptic physiology is impaired by pathological low levels of CSTB in human Cerebral Organoids (hCOs) from EPM1 patients. We showed that the synaptosomal fraction isolated from control hCOs at different developmental stages is enriched in pre-synaptic proteins and contains CSTB. CSTB presence in the synaptic territories was confirmed also by immunostaining on human neurons *in vitro*. Interestingly, CSTB is released by synaptosomes as a soluble protein and in extracellular vesicles-mediated manner. In synaptosomes from EPM1 hCOs, the levels of pre-synaptic proteins were significantly reduced and the extracellular vesicles trafficking was impaired. Furthermore, the expression levels of initiation factor linked to local protein synthesis was reduced in synaptosomes from EPM1 hCOs, suggesting an impairment in the synaptic translation system in the pathology. In addition, neurons differentiated from EPM1 patients NPCs showed longer, thinner and more branched neurites compared to controls, suggesting that altered neuronal morphology and connectivity are associated with the pathology. Altogether, these data indicate alteration of synaptic plasticity and neuronal morphology in EPM1, opening new venues toward the understanding of molecular mechanisms underlying the disease.

EBN02 | A gene therapy approach for focal epilepsy based on GABAA receptor overexpression

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[Abstract no longer available]

EBN03 | Development of gene therapy as a possible cure for Creatine Transporter Deficiency Syndrome

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Creatine Transporter Deficiency (CTD) is an X-linked inherited metabolic disorder presenting with cerebral creatine (Cr) deficiency, early intellectual disability, epilepsy and autistic-like behavior. Although rare, CTD represents a major issue in health care, leading to a significant decrease of life expectancy and causing chronic illnesses with a large impact on patient quality of life and health-care system. There is currently no cure for this disorder. To understand whether gene therapy might be a potential disease-modifying treatment for CTD, we developed an adeno-associated viral vector (AAV9) carrying a functional copy of human *SLC6A8* gene (encoding for creatine transporter, CrT) driven by the JeT promoter. We administered this AAV into male newborn wild-type (WT) and CrT knockout (KO) mice by intracerebroventricular injection. After three weeks, we found a high expression and widespread distribution of the exogenous transgene in the brain, and a significant increase in cerebral Cr levels, demonstrating that the exogenous CrT was fully functional. However, this treatment was not sufficient to improve cognitive function and brain CrT overexpression resulted in neurotoxic effects, including neurodegeneration and neuroinflammation. Our results suggest that this adverse outcome might derive from a set of different processes, including the stress of endoplasmic reticulum and the osmolitic action of Cr. As an alternative to using non-native promoters to express *SLC6A8*, we are now testing a characterized portion of its endogenous regulatory sequence. Preliminary results in HEK293 cells and primary astrocytes suggest that this strategy allow yielding lower levels of *SLC6A8* expression, possibly overcoming toxicity. Our results provide proof-of-concept evidence that gene therapy has potential applications for treating CTD and suggest that further steps of vector engineering to finely tune CrT expression are crucial for maximizing safety and efficacy.

EBN04 | N-acetyl cysteine rescues cortical glial cell populations and results in functional improvements in a mouse model of primary autosomal recessive microcephaly 17 (MCPH17)

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Primary autosomal recessive microcephaly 17 (MCPH17) is a rare neurodevelopmental disorder caused by mutations in the CIT gene, which encodes for the Citron Kinase (CIT-K), a kinase involved in DNA repair and cytoskeletal dynamics. Patients show reduced brain volume, intellectual disability, motor deficits, epilepsy, and early mortality. Cit-k KO mice recapitulate MCPH17 phenotype. In the Cit-k KO mouse brain, DNA damage and reactive oxygen species (ROS) accumulation is accompanied by neural progenitor apoptosis and glial cell alterations, including oligodendroglia and astroglia reduction, hypomyelination, and increased numbers of microglia presenting dysmorphic features and engulfed synaptic material. To identify pharmacological treatments that can reduce cellular damage accumulation and improve the pathological features in Cit-k KO mice, we chronically treated Cit-k KO mice during the first 2 postnatal weeks with the FDA-/EMA-approved antioxidant drug N-acetylcysteine (NAC), which can pass the blood-brain-barrier. Treated mice showed motor improvement, reduction of epileptic myoclonus and decreased susceptibility to epileptogenic drugs, in association with a slight increase in the Cit-k KO mouse life span. These changes were accompanied by reduced brain ROS levels and by a prominent increase in the number of cortical oligodendroglia and astrocytes, though in the absence of a rescue of myelination. Moreover, microglia density and dysmorphic features decreased. Notably, deposition of perineuronal nets around cortical parvalbumin-positive interneurons was also significantly rescued by NAC treatment, suggesting a positive effect on the maturation/function of inhibitory neurons. Our data show that NAC treatment pervasively improves brain pathology and mitigates MCPH17 mouse phenotype. They also suggest that NAC-induced functional improvements may be at least in part mediated by the correction of Cit-k KO glial cell dysfunctions. Ongoing analyses will define the cellular and molecular bases of NAC effects.

EBN05 | Characterization of Early Communicative Deficits and Social Behaviors in a Mouse Model of Cdkl5 Deficiency Disorder

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Mutations in the X-linked *CDKL5* gene cause CDKL5 deficiency disorder (CDD), a rare neurodevelopmental disease characterized by early onset epilepsy, severe intellectual disability, and autistic features. To date, no therapies are available for CDD. Mouse models that mimic the genetic disease, the *Cdkl5* knockout (KO) mice, were recently generated to study the pathophysiological mechanisms of CDD. *Cdkl5* KO mice recapitulate different features of CDD, showing impairments in hippocampus-dependent learning and memory, visual deficits, and autistic features. However, although CDD is an early onset neurodevelopmental disorder, most of the studies published so far have been conducted on adult *Cdkl5* KO mice, and the role of CDKL5 in the first weeks of life has not been thoroughly investigated. Importantly, none of the existing *Cdkl5* mouse models have been assessed for a thorough characterization of early communicative deficits through ultrasonic vocalizations (USVs), which could provide insights into brain circuit disruptions relevant to autism spectrum disorder (ASD) and language impairments. The aim of this study was to characterize communication in newborn/juvenile *Cdkl5* KO mice and to further investigate social behaviors during adulthood. We recorded USVs and conducted open field and auditory startle tests in newborn/juvenile mice. In addition, to evaluate social behaviors in adulthood, mice were subjected to the three-chamber test and direct social investigation towards a conspecific. We found that *Cdkl5* KO mice exhibited early quantitative and qualitative alterations in USVs and showed impairments in social behaviors, together with hyperacusis and higher levels of anxiety compared to wild-type mice. Our findings provide, for the first time, a comprehensive characterization of communication and social deficits in *Cdkl5* KO mice during early life phases, which could be useful to test therapeutic interventions for ASD in CDD patients.

EBN06 | The epilepsy-related protein TBC1D24 regulates V-ATPase activity and pH homeostasis in neurons

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TBC1D24 is a gene mutated in a broad spectrum of neurodevelopmental disorders, from mild epilepsy to severe epileptic encephalopathy. TBC1D24 protein regulates neuronal development, synaptic vesicle cycling and synaptic function; yet the molecular mechanisms mediating these complex roles and their relation to brain dysfunction are largely unknown. TBC1D24 is unique in containing the conserved TBC and TLDc functional domains. Recently, TLDc-containing proteins have been proposed to interact with the proton pump V-ATPase. By immunoprecipitation and cell staining experiments, we revealed the interaction between Tbc1d24 and the cytosolic domain of V-ATPase in the brain. Using *Tbc1d24* knockout neurons, we found that loss of Tbc1d24 leads to a decrease in the assembled and active V-ATPase complex with parallel impairment of intracellular organelles acidification. This phenotype was accompanied by defects in the autophagic clearance. At synaptic level, Tbc1d24 loss resulted in a reduction in the number of synaptic vesicles with the accumulation of endosomal-like structures, dysregulated synaptic autophagy and altered synaptic vesicle acidification. We propose a novel function for TBC1D24 as a regulator of V-ATPase activity in neurons and suggest pH homeostasis dysregulation as a key cellular mechanism that underpins the synaptic defects and pathogenesis in *TBC1D24*-related disorders.

EBN07 | Therapeutic Opportunities in Lafora Disease

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Lafora disease (LD) is a lethal autosomal recessive epilepsy, caused by loss-of-function mutations in EPM2A (encoding laforin) or NHLRC1 (expressing malin). In healthy condition, malin ubiquitinates its targets, among which protein targeting to glycogen (PTG), in a laforin dependent manner. PTG is a master regulator of brain glycogen synthesis, which is mainly metabolized and stored in astrocytes as glucose source for neurons. In LD, PTG escapes from degradation due to dysfunctional laforin-malin complex, leading to the accumulation of abnormal glycogen and neurodegeneration. Nowadays, no treatments are available as well as cellular models and clinically relevant targets for screening and drug development campaigns. In this window of opportunities, two parallel strategies are being investigated.

On one hand, a reverse chemogenomic approach has been employed with PTG as therapeutic target. The crystal structure of the PTG carbohydrate binding motif 21 (CBM21) has been determined for the first time. Hence, the molecular docking on PTG-CBM21 uncovered two potential druggable pockets. In the next future, the screening of compound libraries will be run to identify PTG-interacting molecules in order to design specific inhibitors.

On the other hand, a forward chemogenomic approach is being explored to screen FDA-approved drugs. To set up HTS experiments, different phenotypic assays to detect glycogen have been tested and validated. Moreover, genetically modified cell lines and LD patient-derived iPSCs have been developed and characterized as LD cellular models.

In this work, a druggable target involved in glycogen synthesis has been identified, aiming to reduce brain glycogen accumulation in LD. Meanwhile, the first LD-patient derived iPSCs have been reprogrammed and characterized to generate an *in vitro* model of LD.

Taken together, these findings will allow a multimethodological approach for drug screenings looking for candidates to be advanced to pre-clinical testing.

POSTER SESSION

2

POSTER SESSION

CN03 | 3D-stem cell spinal cord model to study the therapeutic mechanisms of risdiplam-like compound for Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a severe neurological disorder characterized by early onset and degeneration of lower motor neurons due to mutations in the *SMN1* gene. To reproduce reliable human models, we generated and phenotypically characterized human spinal cord organoids from induced pluripotent stem cells (iPSCs) of SMA type 1 subjects (n=3) and healthy controls (n=2). Our study aimed at improving the treatment of SMA by investigating the efficacy of a Risdiplam-like compound on 3-dimensional (3D) spinal cord model. Treatment, whose main action is restoring SMN protein level, was started at different time points during the first 80 days of organoid development which parallels the first trimester post conception and was provided as daily therapy every two days. We observed that SMA samples present a pervasive cellular and molecular developmental alteration in multiple cell populations, including neural progenitors, beyond motor neurons. This was ascertained using bulk transcriptomics, single cells RNAseq, and multi-electrodes array analysis, along with immunophenotypic characterization. Our preliminary results on treatment demonstrated that 1) Risdiplam-like compound modulates at least 15% of disease affected genes; 2) long-term *in vitro* treatment is well-tolerated; 3) ratio between full length *SMN2* and Δ7 is robustly restored; 4) pathological hallmarks are reverted, all in all supporting the idea that SMA organoids represent a reliable model to explore drug kinetics and therapeutic consequences. Moreover, our study highlights the early-onset and pervasive developmental nature of SMA pathogenesis, which can be further disentangled exploiting organoids. Our study precisely contributes to the optimization of Risdiplam therapy and to the identification of targets for complementary treatment intervention in SMA patients.

CN04 | Human iPSC-based cellular systems to model Autosomal dominant leukodystrophy

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Autosomal dominant leukodystrophy (ADLD) is a slowly, progressive, genetic, and fatal neurological disorder. The genetic cause of ADLD is Lamin B1 (LMNB1) overexpression due to coding duplications or noncoding deletions at the LMNB1 locus. Lamin B1 is a component of the inner nuclear membrane of cells and although LMNB1 is ubiquitously expressed, it appears that neurons and glial cells are particularly sensible to LMNB1 dosage. Currently, only symptomatic and palliative treatments are available for this fatal disease. Since its discovery, human induced pluripotent stem cell (hiPSC) technology has open to the generation of novel and pathological-relevant *in vitro* models for Central Nervous System human diseases, for which no appropriate model systems were available. In this work, we describe the reprogramming of peripheral blood mononuclear cells and fibroblasts from ADLD patients carrying different genetic mutations into hiPSCs by Sendai Virus-based method. These hiPSC lines were characterized to assess their pluripotency state by means of qRT-PCR and immunofluorescence assay. Also, embryoid bodies formation assay was used to evaluate their functional pluripotency. In parallel, we set up a procedure for the differentiation of hiPSCs into neuronal and astrocyte 2D cultures, and 3D cell models including hiPSCs derived forebrain and cerebellum organoids. These cultures were characterized to assess the expression of stage-specific markers and potential ADLD-relevant phenotypic and functional alterations. In conclusion, patient-derived ADLD hiPSC lines coupled with hiPSC-based technologies might represent valuable tools for studies aiming to investigate ADLD-specific alterations at molecular and cellular levels and develop potential target-specific drugs.

NI07 | Interplay Between Microglial Receptor TREM2 and Maternal Immune Challenges in Schizophrenia

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Schizophrenia (SZ) is a neurodevelopmental disorder and its onset in the offspring has been associated to viral infections during pregnancy. Microglia play key roles in neurodevelopment and express the Triggering Receptor Expressed on Myeloid Cell 2 (TREM2) which regulates microglial phagocytosis and synapse elimination. Our bulk RNAseq analysis in hippocampi and pre-frontal cortices of *Trem2+/+* and *Trem2-/-* mice, revealed that the differentially expressed genes in *Trem2-/-* displayed a significant association with SZ. Based on these findings, we investigated the molecular and transcriptional pathways connecting maternal viral infections and TREM2 dysfunction in SZ. We used a model of Maternal Immune Activation (MIA) by exposing pregnant dams to the viral mimetic PolyIC. *Trem2+/+* offspring derived from PolyIC injected dams (here referred as PolyIC offspring) and analyzed at post-natal day (P) 18 showed defective excitatory synapses, a hallmark of SZ, together with decreased microglial synaptic engulfment in the hippocampus. Also, PolyIC *Trem2+/+* offspring displayed decreased TREM2 protein and mRNA brain levels. Notably, the same analyses performed in *Trem2+/-* and *Trem2-/-* offspring, revealed that the lack of one or two copies of *Trem2* prevented the synaptic dysfunction induced by MIA, thus suggesting a protective role of TREM2 in this context. To define the mechanisms involved, we run bulk RNAseq analysis on *Trem2+/+*, *Trem2+/-* and *Trem2-/-* microglia treated with PolyIC *in vitro* and we observed that *Trem2-/-* microglia show defective cytokines, proteasome and interferon responses upon PolyIC challenge. Our data highlight that TREM2 receptor is involved in synapse homeostasis upon MIA, opening the possibility that modulating its expression could have a positive impact. Unveiling the role of the receptor and the downstream pathways will shed light on new mechanisms and will help identifying new targets in neurodevelopmental pathologies like SZ.

NI08 | Elucidate the role of microglia in fmr1ko mice model

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Fragile X Syndrome (FXS) is the most common neurodevelopmental disorder associated with intellectual disabilities. In FXS, an increased CGG triplet repetition (more than 200) in the *fmr1* gene, cause the hypermethylation of the 5'UTR and, in turn leads to the gene silencing and the absence of the Fragile X mental retardation protein (FMRP). FMRP is an RNA binding protein, able to regulate the expression of different RNAs, and fundamental for synapses formation and function. FMRP is expressed in neurons but also in astrocytes and microglia, the other key cells of the brain. Despite many studies have focused their attentions on the role of FMRP in neurons and locally at synapses, only a few pieces of evidences have reported the role of the protein in other brain cells types. In preliminary results, performed in *fmr1*ko mice, revealed an hyperactivated state of microglia, measured with phagocytic marker CD68, and an increase of microglia-specific transcriptor factor PU.1/Spi1. Interestingly, activated microglia is present in many neurodegenerative disorders, such as Alzheimer's disease (AD), Huntington's disease, and Amyotrophic Lateral Sclerosis (ALS). Based on this rationale, we aim to clarify the role of FMRP in microglia through a deep transcriptional profiling of *fmr1*ko microglial cells compared to wild type (WT). Furthermore, increase level of transcriptional factor PU.1 in *fmr1ko* mice suggest an unbalance of epigenetic mechanism in absence of FMRP and a Chip-Seq analysis to study a PU.1-regulated genes in *fmr1ko* microglia will be fundamental. This approach will be instrumental to identify new molecular pathways responsible for the activated-state of microglia in *fmr1*ko mice and suggest that modulating PU.1 expression may be a valid therapeutic target to prevent microglial-mediated hyperactivation in *fmr1ko* mice model.

NI09 | dCK intracellular localization is regulated by serine 11 phosphorylation and predicts the response to cladribine treatment in T cells

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Activation of cladribine (2CdA), a drug approved for multiple sclerosis, is driven by a high deoxycytidine kinase (dCK)/5'nucleotidase (5NT) ratio in the cytoplasm. Due to their high dCK/5NT ratio, lymphocytes are a preferential target for 2CdA. We have previously demonstrated that 2CdA-induced apoptosis in stimulated T cells is correlated with enhanced dCK expression and activity and that cladribine treatment of lymphocytes affects the phosphorylation of dCK in activated T cells. Little is known regarding how phosphorylation of dCK at different sites affects its activity and intracellular localization.

The objective of this work was to assess the composition of differently phosphorylated dCK isoforms in healthy donor peripheral blood mononuclear cells and T cells.

For this aim, protein lysates from CD4+ T cells treated with or without anti-CD3/CD28 dynabeads and cultured for 72h, were digested with trypsin after protein purification. Phosphopeptides were enriched using High-SelectTM Fe-NTA enrichment kit and analyzed using Q Exactive™ Plus Hybrid Quadrupole-Orbitrap™ mass spectrometer. Activated/not activated CD4+ T cells were mounted on microscope slides and, after staining with dCK, CD4+ and SYTO™ 16, confocal images were acquired with a Leica Stellaris confocal microscope to observe dCK nuclear Vs cytoplasmic localization.

Activation of T cells led to phosphorylation of dCK at Ser11 residue. In Ser11-P-enriched, activated CD4+ T cells, dCK was localized mostly in the cytoplasm, whereas in unstimulated cells the highest dCK signal density was in the nucleus. In conclusion, these results suggest that T cell activation leads to phosphorylation of dCK at Ser11 leading to dCK preferential localization in the cytoplasm. Assessing baseline phosphorylation of Ser11 in T cells from untreated patients with MS may permit to identify those who are more likely to respond to treatment.

NI10 | Pathogenic insights for reducing thalamic hemorrhage-induced pain and depression by regulating microglia and MED1/BDNF/TrkB signaling

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Central post-stroke pain (CPSP) is a neuropathic pain syndrome occurring after ischemic or hemorrhagic stroke involving somatosensory system. Studies reported maladaptive plasticity of the thalamocortical network, which early involves neuroinflammation, underlying a pivotal role of microglia. The goal of this study was to investigate the pharmacological efficacy of PEALut, a co-ultramicronized combination of N-palmitoylethanolamide (PEA) and luteolin (an antioxidant flavonoid), in a model of thalamic hemorrhage (TH)-induced CPSP. TH was made through collagenase IV injection in thalamic ventral posterolateral (VPL) nucleus. The effectiveness of PEALut in CPSP-associated behavioral tasks (allodynia and depression) was evaluated during a period of 28 days. We studied the effect of PEALut on microglia through immunofluorescence staining. TH-induced CPSP led depression development in the late phase. We also investigated the role of Brain-derived neurotrophic factor (BDNF) and its conjugate receptor (TrkB) into the hippocampus, through biomolecular analysis and the correlation of BDNF/Trkb pathway to MED1 (Mediator Complex subunit 1), that negatively regulate BDNF levels through the up-regulation of the miR-191, a small non-coding RNA post-transcriptional gene regulator. Neurotransmitters release and neuronal activity has been investigated, analyzing hippocampal circuits involved in depressive behavior development. The results showed that PEALut significantly reduced tactile allodynia up to 28 days after TH compared to vehicle-treated mice by reducing perilesional microglial activation in the early phase. The repeated administration of co-ultra PEALut significantly reduced the depressive behavior at 21- and 28-days post-stroke. MED1 gene expression significantly increased in TH mice compared to sham, similarly to TrkB, 21 days post-TH.These results pave the way for better investigating depression in hemorrhagic stroke and propose PEALut as an adjuvant treatment of CPSP.

NI11 | Alginate displays anti-inflammatory properties in the ibotenic-lesioned rat brain

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Neuroinflammation involves different populations of brain-resident or peripheral immune cells, which may be part of innate or adaptive immunity. The integrity of the blood-brain barrier is crucial to ensure the normal inflammatory framework of the brain, and its damage leads to increased recall of periphery immune cells. This condition is known to be related to several neurological and neurodegenerative diseases. Alginate is a marine biopolymer capable of gelation and is used for drug delivery, wound healing, and tissue engineering. While its structural, pro-differentiation, and neuroprotective properties have been shown in vitro, the in vivo anti-inflammatory effects have not been deeply described. The main purpose of this study is to highlight the anti-inflammatory and protective properties of alginate in the brain lesioned with ibotenic acid (IBO), an excitotoxic agent. Sprague-Dawley adult male rats were bilaterally injected in ventral *Cornus Ammonis 3* with IBO. Four days later, a subgroup of these rats received a further injection of saline or alginate. After ten weeks, neuronal damage and the presence of resident and infiltrating immune cells were evaluated by immunofluorescence using immune response biomarkers. Behavioral impairments in the different groups were also investigated. Our experiments revealed that alginate hydrogel is still present and filling the lesion ten weeks after the injection, suggesting that it is neither dissolved nor washed out by the liquor. Moreover, we found a low immune response *in vivo*, which makes it a promising candidate to support cellular differentiation and neuronal maturation in cell-based therapy. Our results shed light on the future application of alginate in innovative therapeutic interventions for neurological diseases aiming at repairing brain lesions.

NI12 | Neuroprotective effect of butyrate in Friedreich's Ataxia models

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Friedreich's Ataxia (FA) is an inherited autosomal neurodegenerative disorder caused by a mutation in the gene encoding for mitochondrial protein frataxin (FXN) leading to the decrease of FXN content. Neurological diseases are usually associated with microbiota dysfunction. Analysing the microbiota of FXN knock-in/knock-out (KIKO) mice, we found that KIKO mice show a decrease of bacteria producing the short chain fatty acid butyrate. This is a small, short chain fatty acid physiologically produced by gut microbiota and has been studied for its neuroprotective and anti-inflammatory role. Our preliminary data show that the cerebellum of KIKO mice has signs of inflammation and an increase of microglial population. We generated an *in vitro* model of FA consisting in a microglial cell line (BV2) with FXN downregulation (FXN-). We observed that FXN- cells are more susceptible to a pro-inflammatory stimulus (i.e., lipopolysaccharides, LPS) if compared to cells with normal FXN levels and that butyrate treatment can counteract the increase of pro-inflammatory cytokine production. By supplementing butyrate through diet for 16 weeks, we observed that KIKO mice underwent an amelioration of neuro-mobility as assessed by rotarod test. Overall, these data suggest the use of butyrate as a safe and valuable molecule to counteract neuroinflammation in FA.

NI13 | BTK inhibitors modulate microglial extracellular vesicles release to regulate remyelination in Multiple Sclerosis

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It is a common finding that myelin repairing mechanisms in people with MS are altered and ineffective. As myelin offers protection against stressors, demyelinated axons are exposed to damaging factors, leading to neurodegeneration commonly found especially in the progressive MS phenotypes. To date, remyelination is one of the greatest unmet needs in MS. Since its underlying mechanisms are still unclear, their deeper comprehension may help in understanding the disease modifying treatments (DMT) effects on myelin repairing mechanisms and offers food for thought for new therapeutic approach. Extracellular vesicles (EVs) represent a way of intercellular communication thanks to their contain of proteins, nucleic acids and more on. EVs derived from human mesenchymal stem cells and from neural stem cells have already shown the potential therapeutic ability to ameliorate both progressive and relapsing remitting models of MS. Bruton's tyrosine kinase inhibitors (BTKi) are a new DMT class primary acting on myeloid cells as microglia, the immune cells of the central nervous system. A recent study has shown that ibrutinib, one BTKi, is able to modulate EVs release from BV2 cells, a microglial murine cell line. Our first aim is to analyze EVs released by microglia (untreated, proinflammatory, anti- inflammatory and BTKi-treated cells) in single culture and in a complex system (brain cortex and spinal cord cultures), both in de- and a re-myelinating *in vitro* models. Second aim is to evaluate the role played by EVs released from microglial cells in a remyelination *in vitro* model of MS. First aim will provide further insights about microglial response to BTKi, mainly acting on myeloid cells and currently tested on MS patients. Second aim will clarify microglial role, exerted through EVs, in remyelination.

NP08 | Sub-toxic glyphosate treatment alters GABAergic synapses in murine hippocampal neurons

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Glyphosate (Gly)-based herbicides (GBH), that are widely used worldwide, act by inhibiting the enolpyruvylshikimate-3-phosphate synthase of the shikimate pathway, an enzyme expressed in plants but not in mammals. We recently found that chronic *exposure* to toxic doses of GBH produces: reduced expression of neurotransmitters, increased cellular oxidative stress, augmented anxiety/depression-like behaviors, and impairments of learning and memory. Although the safety of Gly is actively investigated, so far very little is known on the mechanisms underlying Gly neurotoxicity. Importantly, only few studies addressed the neuronal consequences of the acceptable daily intake (ADI) dose. To fill this gap, we investigated the effects of an acute Gly (3µM) treatment on both structural and functional organization of synaptic connections by patch-clamp recordings, immunofluorescence and confocal microscopy of primary hippocampal neuronal cultures. The measure of evoked excitatory and inhibitory postsynaptic currents showed that Gly administration reduces the amplitude of inhibitory GABAergic neurotransmission without affecting glutamatergic responses. Moreover, both the amplitude and frequency of miniature inhibitory postsynaptic currentswere affected by Gly administration, suggesting that GABAergic transmission is affected both pre- and post-synaptically. In line with this, we found that Gly reduces the number of postsynaptic $GABA_A$ receptors channels, the size of the readily releasable pool of synaptic vesicles and the number of release sites. Consistently, morphological analyses showed that the density of both pre- (vGAT-positive) and post-synaptic (gephyrin-positive) inhibitory compartments are reduced in Gly-treated neurons while excitatory contacts are unaffected. In sum, these data disclose novel neuronal and synaptic abnormalities caused by the ADI dose of Gly, which strongly prompts for further investigations to assess the underlying molecular pathways.

NP09 | Exposure to interleukin 15 modulates hippocampal synaptic transmission and interfere with episodic memory formation via serotonin receptor activation

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Cytokines are potent immunomodulators exerting several pleiotropic effects also in the CNS. They influence neuronal functions and circuit activity with effects on memory processes and behaviours. In particular, the interleukin 15 (IL-15) play a pivotal role in the differentiation, activation and viability of innate and adaptive lymphocytes. Here, we unravel the neuromodulatory function of the IL-15 in the brain. In *ex vivo* hippocampal slices, acute exposure to IL-15 enhances GABA release and reduces glutamatergic currents, while chronic delivery of IL-15 in vivo into the hippocampus alters synaptic transmission and impairs memory in the novel object recognition test. Moreover, we describe the involvement of serotonin receptor in the neuromodulatory effect of IL-15: indeed the pre-treatment with a selective 5HT3A receptor antagonist prevents the IL-15-mediated effects on inhibition and ameliorates performance in NOR. These findings provide new insights into the complex interaction between cytokines and CNS at the functional levels with implications on behaviour.

NP10 | 5-HT7R Stimulation Modulates Synaptic Protein Synthesis in Autism Spectrum Disorders

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Autism Spectrum Disorders (ASD) is a complex neurodevelopmental disorder characterized by persistent deficits in social interaction and restricted patterns of behavior. The etiology of ASD remains poorly understood, but emerging evidence suggests a potential involvement of the serotonin system. Serotonin signaling, mediated by the serotonin receptor 7 (5-HT7R), plays a crucial role in neurodevelopment, synaptic plasticity, and social behavior. Our research focused on local synthesis at central nervous system synapses using synaptosomes, an *in vitro* model mimicking synaptic contacts in living organisms. We used a novel, non-radioactive protein labeling technique named SUnSET (Surface and Sensing of Translation) to investigate the synaptic system of protein synthesis. We examined the effects of a selective 5-HT7R agonist named LP-211 on protein synthesis of the synaptosomal fraction from the brain cortex of BTBR mice, a well-established animal model of ASD. We observed that LP-211 treatment significantly increased synaptic protein synthesis. These results suggest that the activation of 5-HT7R by LP-211, in an ASD animal model, promotes the molecular processes involved in protein synthesis at the synapses. To further investigate the specificity of this effect, synaptosomes were co-incubated with LP-211 and the selective 5-HT7R antagonist SB-269970. This treatment successfully restored protein synthesis levels to the baseline, indicating a 5-HT7R modulatory role in synaptic protein synthesis. These results shed light on the involvement of 5-HT7R in the molecular mechanisms underlying synaptic plasticity in ASD, opening a new perspective in the identification of therapeutic targets for ASD.

NP11 | Natural Killer cells modulate sleep pressure via Interferon-gamma

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Sleep is a fundamental physiological process regulated by several mechanisms. In the last years it has been demonstrated that not only neurons, but also glial cells and immune system cells participate in in the sleep process through the release of cytokines, regulating neuronal activity. In this study, we investigate the involvement of Natural Killer (NK) cells in the regulation of the sleep process. Through a combination of EEG analysis and behavioral assessments, we demonstrate that NK cells exert a significant influence on sleep process. In particular, NK cells depletion in mice induces a decrease in the sleeping time during the resting phase and a reduction in the sleep pressure, a mark of the necessity to sleep, during the active-to-resting phase transition. We, also, identify the interferon (IFN)-γ as key molecular mediator responsible for this regulation. Using XMG1.2 antibody, which specifically targets and blocks IFN-γ, we demonstrated that the blockade of the cytokine mimics the effect of NK cell-depletion in mice. Moreover, we demonstrated that IFN-γ and NK cells modulate the activation of $nNOS^+$ (neuronal nitric oxide synthase) inhibitory interneurons, that are directly responsible in the generation of the sleep pressure. These findings provide important insights into the complex network of factors involved in the regulation of sleep, and highlights the intricate interplay between the immune system and neuronal circuits in physiological processes.

NP12 | Voluntary running ameliorates brain development and behavioral performance in a mouse model of CDKL5 deficiency disorder

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Cyclin-dependent kinase-like 5 (CDKL5) deficiency disorder (CDD) is a rare neurodevelopmental disease caused by mutations in the X-linked CDKL5 gene. CDD is characterized by a broad spectrum of clinical manifestations, including early-onset refractory epileptic seizures, intellectual disability, hypotonia, visual disturbances, and autism-like features. The Cdkl5 knockout (KO) mouse recapitulates several features of CDD, including autistic-like behavior, impaired learning and memory, and motor stereotypies. These behavioral alterations are accompanied by diminished neuronal maturation and survival, reduced dendritic branching and spine maturation, and marked microglia activation. There is currently no cure or effective treatment to ameliorate the symptoms of the disease. Aerobic exercise is known to exert multiple beneficial effects in the brain, not only by increasing neurogenesis, but also by improving motor and cognitive tasks. To date, no studies have analyzed the effect of physical exercise on the phenotype of a CDD mouse model. In view of the positive effects of voluntary running on the brain of mouse models of various human neurodevelopmental disorders, we sought to determine whether voluntary daily running, sustained over a month could improve brain development and behavioral defects in *Cdkl5* KO mice. Our study showed that long-term voluntary running improved hyperlocomotion and impulsivity behaviors, and memory performance of *Cdkl5* -/Y mice. This is correlated with increased hippocampal neurogenesis, neuronal survival, spine maturation, and inhibition of microglia activation. These behavioral and structural improvements were associated with increased BDNF levels. Given the positive effects of BDNF on brain development and function, the present findings support the positive benefits of exercise as an adjuvant therapy for CDD.

NO06 | Astrocytes-derived small extracellular vesicles hinder glioma growth by the regulation of the volume-regulated anion channel (VRAC)

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Small extracellular vesicles (sEVs) represent a way used from all cells of the body, including brain, to exchange biological information (lipids, proteins and nucleic acids as mRNA, miRNA, ctDNA) during neural functional processes but also in pathological conditions (such as inflammation, neurodiseases or brain cancer).
sEVs mediate a bidirectional crosstalk between healthy and cancer cells in the most common and malignant primary brain tumor, the glioblastoma (GBM). GBM has a high rate of invasiveness, migration and chemoresistance. In the peri-tumoral environment, astrocytes act as pro-tumoral or antitumoral cells depending on the stage of GBM progression.
In this study, we demonstrated that astrocytes-derived sEVs (ADEVs) have a defensive mechanism against tumor invasion and cell growth. sEVs response is mediated by the transfer to tumor cells of factors that hinder glioma growth, reducing both tumor volume and tumor cell proliferation and prolonging survival of glioma-bearing mice. Among many factors transported by ADEVs, we identified miR124 that is enriched in these vesicles. Among downregulated target genes of miR124, we found the volume-regulated anion channels (VRACs). VRACs regulate GBM cell migration and invasion. We demonstrated that ADEVs reduce migration and invasion of GBM cells by translocating miR124.
In summary, astrocytes exert an anti-tumor response in the context of GBM by releasing ADEVs enriched in miR124.

NO07 | Dissecting the ERAP1/ACP2 interplay in Hedgehog signaling control and Hedgehog-dependent medulloblastoma

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The Hedgehog (HH) pathway is crucial for embryonic development; it is deregulated in several cancers, including medulloblastoma (MB, the most common paediatric brain tumor). Given the high heterogeneity of MB, the identification of novel players involved in the control of HH signaling is essential to advance innovative and more effective therapeutic approaches. We previously identified the endoplasmic reticulum aminopeptidase 1 (ERAP1, a key regulator of immune responses) as a strong activator of the HH pathway and promising druggable target for HH-dependent MB (HH-MB). We hypothesized that ERAP1 may drives HH-MB tumorigenesis by cooperating with still unknown molecular partners. To this end, we performed a proteomic screening of ERAP1 binding partners and identified the acid phosphatase 2 (ACP2) as interesting candidate. ACP2 is a soluble luminal hydrolase dynamically regulated during mouse cerebellum development whose expression gradually increases as development proceeds; *Acp2-/-* mice exhibit cerebellar hypoplasia with a striking reduction in the number of granule cells and severe defects in cerebellar functions. We demonstrated that Erap binds Acp2 in murine cerebellar granule cell precursors (GNPs, the cells of origin of HH-MB), and that ACP2 counteracts ERAP1 enzymatic activity working as a negative regulator of HH signaling. Interestingly, Acp2 is strongly downregulated in MB tissues from HH-MB mouse models, and its ectopic expression reduces the proliferation of both murine GNPs and primary HH-MB cells by impairing HH signature genes. In human, low *ACP2* levels correlate with reduced overall survival in HH-MB patients. Our work firstly investigates the ERAP1/ACP2 interplay in the control of the HH pathway, shedding the light on the complex dynamic networks underlying the regulation of HH signaling useful for the development of novel and tailored anti-cancer interventions.

NO08 | Phospholipases as potential prognostic biomarkers and targets in the development of new therapeutic strategies for glioblastoma

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Phospholipases (PLCs) are the hydrolyzing enzymes of phospholipids, which represent the most abundant species contributing to the biological membranes of nervous cells of the healthy human brain. Several studies have shown the importance of PLCs in the regulation of different mechanisms in the central nervous system as well as in glioblastoma, the most lethal brain tumor in adults. Nowadays, despite the progress made in understanding the molecular pathogenesis of glioblastoma, the survival rate of patients remains unsatisfactory. Consequently, a better understanding of the molecular mechanisms underlying tumor transformation could help to find new effective therapeutic strategies. Our studies suggested a potential role of PLCβ1 and PLCg1, in regulating the phenotypic characteristics of this tumor. It was demonstrated that PLCβ1 expression was relatively lower in glioblastoma patients compared to their healthy/low-grade counterparts. PLCβ1 silencing, in both immortalized and primary cell lines, led to increased cell migration, invasion, proliferation, cell survival and induced the upregulation of mesenchymal markers and metalloproteinases. Contrariwise, data collected on patients' biopsies and engineered cell models, proposed a strong association between PLCγ1 expression and the acquisition of a more aggressive tumor phenotype. This trend was deepened using patient derived glioma stem cells (GSCs), which represent a specific tumor population that drives aggressiveness and recurrence in glioblastoma. Transcriptomic analysis of GSCs confirmed that PLCγ1 downregulation led to the activation of pathways that negatively regulate cell motility and migration and led to reduced expression of genes involved in cancer development and progression. All these data highlight the importance of further investigating phospholipases as potential prognostic biomarkers and targets in the development of new therapeutic strategies for glioblastoma.

NO09 | Enhancing Natural Killer cell cytotoxicity to counteract Glioblastoma growth

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Glioblastoma (GBM) is the most malignant brain tumor. Extensive invasiveness within the surrounding parenchyma and intra-tumoral cellular heterogeneity are GBM's distinctive traits, along with a highly immunosuppressive tumor microenvironment (TME). Among the peripheral immune cells infiltrating into the TME, the invasion of Natural Killer (NK) cells is weak, and glioma cells attenuate NK-mediated tumor killing. NK cells are able to exert anti-tumoral function by direct cytotoxic activity, mediated by activating and inhibitory receptors. Signalling mediated by inhibitory receptors, such as NKG2A and PD-1, is an immunological checkpoint that can negatively regulate NK cell-mediated immune response and it is exploited by tumor cells to achieve immune evasion. Despite the advancement of knowledge regarding the interactions between immune cells and the microenvironment of brain tumors, several aspects need further investigation. Here, we investigated the possibility to enhance NK cells cytotoxicity against glioma cells, taking advantage of RNA interference technology. Using amphiphilic dendrimers, we delivered *klrc1*(NKG2A) and *pd-1*(PD-1)small interfering RNA (siRNA) in NK cells isolated from mice spleens, reducing the expression of these receptors. Then, we performed cytotoxic assay *in vitro* between *klrc1/pd-1*-siRNA-transfected NK cells co-cultured with GBM cells, increasing tumor killing. We aim to investigate if enhancing NK cell activity, modulating the expression of the inhibitory receptors with specific siRNA, could be a promising targeting strategy for improvement of treatment of GBM patients.

NO10 | The use of animal proteins in the diet: impact on gut microbiota and glioma growth in a preclinical murine model

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Glioblastoma multiforme (GBM) is the most common and deadly malignant brain tumor, with a low life expectancy (around 14-17 months) and poor efficacy of first-line therapies such as maximal surgical resection and chemotherapy, leading to a high relapse rate. However, it is already known that lifestyle changes, such as restricted diets and fasting, have an impact on GBM tumor growth. Less clear is the effect of eating red meat on the tumor. One observational study found that people who consume a diet high in animal protein and fat have changes in the composition of their microbiota, in particular an increase in common hydrogen sulfide (H_2S) producing bacteria. This metabolite appears to counteract GBM growth in both in vitro and in vivo models. Recent studies have highlighted the role of the gut-brain axis in altering the GBM tumor microenvironment and growth. Our aim is to investigate the effect of a standard animal protein diet on tumor growth by the possible involvement of the gut microbiota. To assess such a hypothesis, we fed mice two isocaloric diets with different percentages of proteins derived from red meat (protein diets) or animal-derived proteins (control). After two weeks on the diets, we orthotopically injected murine glioma cells (GL261). We allowed the tumors to grow for a further three weeks. At the end of the experiment, we collected stool for each group to assess H_2S concentration and brains to assess tumor volume. Preliminary data showed an increased concentration of H_2S in the faeces and a decreased tumor volume in mice fed the animal protein diet compared to controls. These results are consistent with our in vitro data showing a reduction in GL261 viability following treatment with two doses of sodium hydrosulfide (NaHS), an H_2S donor. These preliminary results suggest that a standard red meat diet may have an antitumor effect on GBM compared to a standard animal-derived protein diet.

NIM03 | Investigating Cerebral Lateralization during Visual Stimulation using Functional Transcranial Doppler: A Preliminary Study

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The Transcranial Doppler (TCD) is a noninvasive neuroimaging technique used to assess the cerebral blood flow velocity (CBFV) in the major cerebral arteries, which closely correlates with cerebral metabolism and function. Compared to other neuroimaging methods, TCD is portable, cost-effective, and robust in assessing brain area activation. Functional Transcranial Doppler (fTCD) employs TCD to analyze brain activity during specific tasks and to detect brain lateralization. Brain activity lateralization refers to the preferential increase in blood flow to one hemisphere compared to the other when performing a particular task or function. This study presents a preliminary exploration of local cerebral perfusion by monitoring bilaterally Posterior Cerebral Arteries (PCA), the suppliers of the visual cortex, using fTCD. The study focuses on selective stimulation of the visual field using a black and white checkerboard pattern with a inversion frequency of 10 Hz. The brain lateralization of nine healthy subjects was evaluated during three visual stimuli: bilateral, left hemifield only and right hemifield only, obtained by extending the checkerboard over the full screen or limiting it to left or right half-screen, respectively. Based on the hemodynamic changes exhibited by the two PCAs a Lateralization Index (LI) was calculated, quantifying the asymmetry (right-left) of the response during each stimulus.
The results demonstrated a low LI during bilateral stimulation. Conversely, a larger and positive (negative) LI was observed in response to the left (right) hemifield stimulations, in agreement with the expected functional splitting of visual pathways at the optic chiasm. The assessment of cerebral lateralization through fTCD presents an additional potential in supporting and monitoring post-stroke rehabilitation, as well as facilitating comprehension of cerebral reorganization subsequent to cerebrovascular accidents, particularly among patients experiencing hemianopia.

NIM04 | Evaluation of prodromal markers of Parkinson's disease in a progressive neurotoxic mouse model using multi-tracer PET-CT imaging

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by progressive loss of dopaminergic neurons affecting the nigrostriatal system. Diagnosis of PD relies on the clinical manifestation of motor symptoms, when neurodegeneration is already advanced, thus compromising the efficacy of disease-modifying treatment approaches. Therefore, research has been focusing on the study of prodromal PD, the stage preceding the appearance of motor signs. Here, we characterized the prodromal stage using a mouse model of PD obtained by sub-chronic treatment with the neurotoxin MPTP and the clearance inhibitor probenecid (MPTPp), by combining in-vivo PET-CT imaging and immunohistochemistry.

A group of 10 mice were injected with 100 mg/kg of probenecid followed by 25 mg/kg of MPTP, twice a week, for a total of 5 weeks. They were monitored longitudinally with PET-CT imaging before treatment and after 1, 3 and 10 MPTPp injections using two radiotracers: [^{18}F]-FP-CIT, a marker of dopamine transporter (DAT) and [^{18}F]-FDG, to assess brain glucose metabolism and metabolic connectivity. They were then sacrificed and brains were collected for post-mortem immunohistochemical analysis of DAT and tyrosine hydroxylase.

We found that both striatal DAT binding in-vivo assessed with [^{18}F]-FP-CIT PET and the density of striatal DAT-positive fibers observed post-mortem started to decrease significantly from the third MPTPp injection. [^{18}F]-FDG uptake was significantly decreased in the striatum and thalamus already at the first administration, while at 10 MPTPp injections [^{18}F]-FDG uptake was increased in the somatosensory and somatomotor cortex. Metabolic connectivity analysis revealed that already after 1 MPTPp injection all significant connections between cortical and subcortical regions were lost, while almost all connections were lost after 10 injections.

Our results suggest that glucose metabolism is an earlier marker than DAT-binding in detecting neurodegeneration in PD mice model.

ND15 | Nanoparticles used for the targeted delivery of PACAP peptide through an in vitro blood-brain barrier

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Nanoparticles can be used as carriers to transport many therapeutic molecules as they are able to improve bioavailability, assisting their release without enzymatic degradation. Furthermore, nanoparticles can be modified on their surface by cell-penetrating peptide (CCPs) to enhance drug concentration in the brain parenchyma. gH-625 is a CCPs deriving from the glycoprotein H of *Herpes simplex* virus type I, used to delivery many types of molecules across the in vitro and in vivo cell membrane without endosomal entrapment and lysosome degradation. Pituitary adenylate cyclase-activating polypeptide (PACAP) has anti-inflammatory and neuroprotective properties, but it has a low half-life in the bloodstream (minutes). The goal of this research work is to use liposomes, whose surface are functionalized with gH-625, to deliver PACAP through a fluid dynamic in vitro model of blood-brain barrier (BBB). We have reconstructed a BBB in vitro dynamic model using a bioreactor with double flow independent chambers: murine endothelial brain cells (bEnd.3) are seeded on the porous membrane in the upper chamber and lower chamber contains spheroids made of 3D neuroblastoma cell lines (SH-SY5Y) enriched to the neural fraction from human brain. We tested the passage of gH-625-lipo-PACAP through this reliable BBB in vitro dynamic model and we also evaluated, through different molecular analysis, cell viability. Results show the effects of our delivery nanosystem on the cell lines used.

ND16 | Brain organoids: a promising approach to investigate neurodegeneration in MSA-C and SCA2

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The complexity of the human brain has made the study of brain disorders difficult, thus highlighting the need to generate *in vitro* models for human brain degeneration to overcome the limitations of *in vivo* animal studies. The generation of a three-dimensional model is one of the most promising approaches to study human diseases of the central nervous system (CNS), as it provides robust and consistent phenotypes with clinical translatability. Here, we take advantage of optimized iPSC-derived cortical organoids to study the pathological mechanisms underlying two rare neurological diseases, Multiple System Atrophy Cerebellar type (MSA-C) and Spinocerebellar Ataxia type 2 (SCA2), associated with the expansion of Ataxin-2. First, we generated iPSC lines for MSA-C subjects (n=3), age-matched controls (n=3), SCA2 individuals (n=3) and respective isogenic controls. Further, we cultured and deeply characterized via immunofluorescence, RT-PCR, calcium imaging and electrophysiology cortical organoids derived from subject-specific iPSCs lines. Finally, we also explored the use of antisense oligonucleotides (ASOs) designed with Morpholino chemistry to modulate the expansion of *ATXN2*. Indeed, although there are currently no approved disease-modifying treatments for these two diseases, RNA-targeted therapies are promising for neurodegenerative disorders. Regarding SCA2, creating new reliable human models may help optimize this antisense therapeutic strategy. On the other hand, the pathogenesis of MSA is still puzzling, and a comparison of data obtained in 3D models of MSA-C with those from SCA2 may provide insights into understanding the pathogenesis of this complex disorder.

ND17 | Specific alterations of circulating and cellular lipids levels in Parkinson's disease patients carrying TMEM175 mutations

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Parkinson's disease (PD) is a progressive neurodegenerative disease characterized by a complex genetic background. Large-scale sequencing studies identified several risk genes including *TMEM175* encoding for a lysosomal K^+ channel. We recently demonstrated that mutations in this gene occur in a wide number of Italian PD patients (about 6 %) and are associated with altered channel activity, increased $Ca2^+$ release from endoplasmic reticulum, impaired lysosomal-autophagic pathway, ER stress and activation of Unfolded Protein Response.
To better investigate the impact of *TMEM175* mutations on cellular metabolism we performed a multi-omics approach including genomic, metabolomics, lipidomic and proteomics in dermal fibroblasts and circulating biofluids from 40 PD patients and 16 healthy subjects, matched for sex and age. The group of PD patients was matched for sex, age, therapy, and clinical features (age at onset, motor symptoms, cognitive impairment, and non motor symptoms). At genetic level PD patients were classified in two sub-groups, one carrying *TMEM175* mutation (n=26) and one carrying mutations in genes not related to the lysosomal pathway (n=14). Omics data and meta-data were first analysed as independent omics layer. Preliminary data showed a general deregulation of lipids and metabolites in PD patients compared to controls. Interestingly, we observed a specific lipidomic and metabolomic signature associated with *TMEM175* mutations both in dermal fibroblasts and in plasma. Particularly, we found a dysregulation of Sphingomyelin, Phosphatidylcholine and Acylcarnitine lipid classes which are closely related to lysosomal and mitochondrial functioning. Systems biology approaches is ongoing to integrate data from different omics levels to improve our understanding of their interrelation and combined influence. These results might lead to identify novel deregulated pathways and druggable targets associated to *TMEM175* mutations to better address therapeutic strategy.

ND18 | Identification of a Novel Class of Small Molecules for the Treatment of TREM2-Based Diseases

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The triggering receptor expressed on myeloid cells 2 (TREM2) is a member of immunoglobulin superfamily mainly expressed by microglia. This receptor promotes microglial proliferation and survival, as well as regulating phagocytosis and metabolism, and is proposed to mediate a novel form of microglial anti-inflammatory activation. A number of TREM2 variants have been identified as risk factors for a wide array of neurodegenerative diseases (NDs), including Nasu-Hakola disease, Alzheimer's disease and Parkinson disease (PD). In AD-related conditions, TREM2 interacts with lipoproteins, anionic lipids, and Aβ, which contributes to microglial metabolism remodelling, as well as promotes microglial phagocytosis of cell debris and Aβ. These effects support the hypothesis that TREM2 might play a protective role through regulating microglia polarization and be a potential target for AD prevention and treatment.
Many proteins or compounds that bind to TREM2 have been reported, but the natural signal-transducing ligands of TREM2 present in the brain have not been identified. We recently reported a novel immunomodulatory sulfolipid named Sulfavant A (SULF-A), which primes maturation of dendritic cells (DC) towards a novel homeostasis-determining phenotype (homeDCs) by engagement of TREM2. Preliminary results suggested the ability of this molecule to activate also microglial cells towards an unconventional non inflammatory state, prompting the idea that the investigation of TREM2 pathway may lay the groundwork for the development of a new class of drugs with therapeutic potential in neurodegenerative diseases, chronic-inflammation and cancer.

ND19 | The role of G2019S LRRK2 in excitatory/inhibitory imbalance of Parkinson's disease

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The excitatory/inhibitory (E/I) balance of neural circuits is tightly regulated by an appropriate ratio of excitatory to inhibitory synaptic signals, which falls in several neurodevelopmental and neurodegenerative disorders. Parkinson's disease (PD) is associated with general modifications in the circuitry, resulting in E/I imbalance in the striatum caused by an aberrant excitatory input responsible for the excitotoxicity phenomenon. Recently, *Leucine-rich repeat kinase 2* (LRRK2) has been discovered to play a role in both monogenic and sporadic forms of PD, in which the substitution Gly2019Ser has been observed at a high frequency. It has been demonstrated that G2019S LRRK2 takes part in glutamatergic reuptake process by regulating the activity of glutamate transporter EAAT2 and its membrane localization. On the contrary, the role of LRRK2 on GABAergic transmission is poorly understood. By two-electrode voltage-clamp technique, we investigated the possible modulation of LRRK2 on inhibitory neurotransmission. Taking advantage of micro-transplantation technique, striatal membranes derived from G2019S LRRK2-associated mouse model were be injected in *Xenopus laevis* oocytes.
Our data showed a significant reduction of GABA evoked current amplitude in G2019S LRRK2 striatum compared to the wild-type tissue, indicating that LRRK2 affects GABAergic transmission. The reason behind this reduction is still unclear as the $GABA_A$ receptors are functionally unaltered. Notably, our findings suggest an altered distribution of different $GABA_A$ receptor isoforms in G2019S LRRK2 striatum membrane compared to the wild-type counterpart, demonstrated by both slower desensitization of $GABA_A$ receptor and reduced phasic GABA current.
Overall, our results highlight a critical role of LRRK2 on GABAergic signalling, participating in E/I imbalance of PD LRRK2-associated.

ND20 | Effect of 3D Synthetic Microscaffold Nichoid on the Morphology of Cultured Hippocampal Neurons and Astrocytes

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The human brain is the most complex organ in biology, being composed of an extraordinary number of synapses. Considering that the brain pathologies are bound to rise, there is an essential need to establish effective in-vitro system of Central Nervous System that could be applied to test new therapeutic avenues. To this aim, we set up a new model of hippocampal neurons and astrocytes co-culture taking advantage of the Nichoid technology, a 3D scaffold microfabricated by two photon laser polymerization technology, to generate brain micro-tissues of 30 µm thickness. After 21 days in-vitro, by confocal microscopy, we morphologically characterized the co-cultures comparing 2D and 3D conditions. We observed that astrocytes as well as and neurons had become well-differentiated and colonized the entire volume of the Nichoid. This was further elaborated with the use of Drebrin, PSD-95, and Synaptophysin antibodies that labelled most neurons, both in the 2D as well as in the 3D co-cultures. Interestingly, in the Nichoid, astrocytes displayed a more physiological morphology, closer to the in-vivo condition, appearing more starry compared to 2D cultures. Lastly, using Scanning Electron Microscopy, we found that neurons co-cultured with astrocytes in the 3D environment showed more dendritic spine protrusions compared to the 2D culture, suggesting they could be more prone to form connections. Our results show that the Nichoid can be used as a 3D device to investigate the structure and morphology of neurons and astrocytes in-vitro as well as the complex cell-cell interactions within the brain. In addition, it may serve as a tool to study mechanisms governing synaptic plasticity/dysfunction and to drug discovery.

ND21 | MicroRNA as potential circulating biomarkers for AD diagnosis and novel therapeutic targets

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Alzheimer's Disease (AD) is the most frequent cause of dementia and a steadily growing global epidemic. Such disappointing situation indicate the need for new paradigms in the identification of diagnostic biomarkers and novel therapeutic targets. Recently, a considerable effort has focused on the dysregulation of small non-protein-coding microRNA (miRNA) and the associated post-transcriptional gene alteration in AD. miRNAs are small RNAs involved in the post-transcriptional control of gene expression and are the most important fine-tuning regulators for many cellular processes (cell proliferation, differentiation, senescence). Several miRNA families, affecting Ab deposition or tau phosphorylation, are dysregulated in AD patients. Biofluids, such as serum, and cerebrospinal fluid (CSF), contain miRNAs that can be identified and quantified. The goal of the project was to validate circulating miRNAs within blood and CSF which can be used as non-invasive, diagnostic biomarkers that facilitate the early detection of disease and potentially therapeutic targets. In this study, 40 patients, 20 clinical AD, and 20 healthy controls were recruited and plasma samples were obtained at the same time as the lumbar puncture. Total RNA was isolated for miRNA sequencing, and the differential gene expression analysis in serum and CSF samples allowed to identify differentially expressed miRNAs, such as hsa-miR-197-3p, hsa-miR-191-5p, hsa-miR-223-3p, hsa-miR-150-5p, hsa-miR-125b-5p, hsa-miR-101-3p. These miRNAs are involved in the regulations of important pathways of the pathology, such as synaptic transmission, cell metabolism, trafficking, and lipid metabolism, so they could be relevant diagnostic biomarkers as well as therapeutic targets. Such information may not only support disease diagnosis but provide the opportunity to evaluate therapeutic interventions earlier in the disease process.

ND22 | What about astrocytes? Elucidating the new role of astrocytes in Autosomal Dominant Leukodystrophy

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Autosomal Dominant Leukodystrophy (ADLD) is an ultra-rare and fatal late-onset neurodegenerative disorder that affects the central nervous system myelination and lacks effective therapy. The disease is caused by lamin B1 (*LMNB1*) gene alteration that leads to demyelination with the disease mechanisms remaining unknown. Although oligodendrocytes are responsible for myelination, astrocytes and ADLD patients' cells overexpressing *LMNB1* have displayed nuclear alterations with activation of proinflammatory and oxidative stress mechanisms that were absent in oligodendrocytes. The present study involved the characterization of astrocytes overexpressing lamin B1 and the elucidation of their new role in demyelination. Human astrocytes (HA) were transfected with *LMNB1* and sorted for two assays: 1) characterization of astrocytes for expression of proinflammatory markers, and 2) myelination assay on 3D microfiber co-cultures with oligodendrocyte precursor cells (OPCs). For the characterization, immunocytochemical analysis displayed nuclear localization of NFAT4 (nuclear factor of activated T cells 4) and NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) suggesting astrocytic activation and inflammation. Additionally, proteome array of the transfected HA supernatants revealed increased levels of serpin E1, osteopontin and Dkk-1 which are markers present in activated astrocytes during CNS injury. For the myelination assay the sorted HA were co-cultured with human OPCs on a microfiber scaffold for two weeks. It was displayed that OPCs were unable to produce myelin basic protein when grown with HA overexpressing *LMNB1* indicating the crucial role of astrocytes in supporting myelination. Overall, the study elucidated that *LMNB1* overexpression leads to astrocyte activation that consequently triggers inflammatory states and hinders myelination. Thus, these novel findings could place astrocytes at the epicenter of ADLD demyelination and drug development studies.

ND23 | Increased apoptotic cell death and autophagy alteration in Riboflavin Transporter Deficiency

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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 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. As this disorder lacks dependable *in vivo* models, we took advantage of iPSC technology to recapitulate human neuronal features of RTD. More specifically we perform combined confocal and immunoblotting analyses aimed at characterizing the pathomechanisms associated to RTD. Patient-specific iPSCs and iPSC-derived MNs have been analysed by Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) that demonstrates mitochondrial ultrastructural alterations, involving shape, number, and intracellular distribution of organelles. Increased apoptosis was observed in RTD cells, confirmed by the presence of vesicles and blebs budding from the cell surface of RTD cells and by activated caspase-3 immunofluorescence, WB and TUNEL assays. Consistent with these results and due to the high cell mortality associated with RTD, we investigated survival mechanisms, such as autophagy, an essential process for cell viability that ensures an effective turnover of impaired cytoplasmic components and organelles. Immunoblotting experiments and confocal analyses demonstrate an abnormal activation of autophagic process associated with alteration of the lysosomal compartment. Overall, our work contributes to the knowledge on the multiple cellular features associated to RTD phenotype, supporting a central role played by apoptosis in its pathogenesis, thus suggesting potential targets for future therapies.

ND24 | Boosting peripheral nerve regeneration in ALS by the CXCL12-CXCR4 axis

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The peripheral nervous system can regenerate upon injury. Regeneration relies on the intrinsic ability of motor neurons and the contribution of various cell types in the milieu. Nerve terminal regeneration at the neuromuscular junction (NMJ), the synapse controlling movement, involves a signaling axis composed of the chemokine CXCL12α, released by Schwann cells, and its neuronal receptor CXCR4. Nerve injury triggers CXCR4 expression, while its inhibition delays the process of nerve repair.

In Amyotrophic Lateral Sclerosis (ALS), a severe neurodegenerative condition where initial impairments arise at the NMJ before symptom manifestation, the NMJ undergoes cycles of denervation and re-innervation till degeneration overcomes the regenerative capability of the system. We hypothesize that in ALS the regenerative competence can be restored by stimulating CXCR4 using an agonist molecule. In the SOD1G93A ALS mouse model, CXCR4 expression increases at early disease stages and during disease course, and its engagement by a receptor agonist (NUCC-390) improves motor performance and the respiratory function, and prolongs survival.

Currently I'm testing if this axis is involved in peripheral neurorepair in human samples. I am conducting experiments on biopsies obtained from ALS patients and additionaly I'm using MNs derived from patients' iPSCs to test the translational potential of boosting repair pathways to counteract degeneration in ALS.

The present research holds significant translational value. On one hand, it provides experimental evidence that supporting nerve regeneration may be a new exploitable strategy to improve therapeutic protocols and regenerative outcomes in ALS. On the other hand, it identifies NUCC-390 as a potential candidate molecule to counteract ALS as well as other neurodegenerative diseases.

ND25 | Impaired Bioenergetic Profile and Proliferation in Neuron Progenitor Cells from iPSCs of Patients Affected by AGC1 Deficiency

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Background – AGC1 deficiency is a rare encephalopathy (DEE39, OMIM# 612949) that manifests in infants with neuromuscular delay, hypotonia, epilepsy, and hypomyelination associated with reduction of brain N-acetyl-aspartate, the precursor of the myelin lipids in CNS. AGC1 deficiency is caused by mutations of *SLC25A12* gene, encoding the isoform 1 of the mitochondrial aspartate/glutamate carrier (AGC1). AGC1 catalyzes a Ca^{2+}-stimulated entry of glutamate into mitochondria in exchange for aspartate and is essential for the correct oxidation of glucose in neurons and the import of the glycolysis-derived reducing equivalents in the mitochondrial matrix since it is a component of the malate-aspartate NADH shuttle.

Materials and methods – To study the pathogenetic mechanisms of AGC1 deficiency, we re-programmed fibroblasts of two patients carrying different missense mutations in *SLC25A12* into induced Pluripotent Stem Cells (iPSCs) for the subsequent differentiation in neuron progenitor cells (NPCs).

Results – NPCs of patient appeared characterized by reduced dimensions and higher tendency to aggregate. Both patient NPCs revealed a proliferation deficit, in particular when deprived of glutamine, with higher cell death and increased expression of apoptotic markers, as compared to control NPCs from healthy individuals. Along with higher lactate production, both patient NPCs showed a higher glycolytic activity and a significant increase of total ATP production, suggesting an important deficit in mitochondrial pyruvate oxidation in patients' neurons. Since the administration of ketogenic diet appears to improve myelination and more in general the clinical features of AGC1 deficiency patients, we evaluated the effect of ketone bodies on NPCs mitochondrial respiration. OCR measurements revealed a significantly enhanced mitochondrial respiration, but only in the absence of glucose and in combination with glutamine.

ND26 | Intensive exercise training counteracts nigrostriatal degeneration and striatal structural changes in an alpha-synuclein based experimental model of Parkinson's disease

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Parkinson's disease (PD) is characterized by Lewy-body aggregates formation, and dopaminergic neuronal loss. Clinically, PD is associated with motor slowing, rigidity, and tremor. Intensive physical exercise has beneficial effects on PD patients at early stages, but the underlying mechanisms are not completely understood. In this study, we investigated whether an intensive treadmill training program, at an early phase, counteracts nigrostriatal neurodegeneration in intrastriatal alpha-synuclein (α-syn) preformed-fibrils (PFFs)-injected rats. To evaluate the effects of treadmill on nigrostriatal morpho-functional changes, we assessed the dopaminergic neurons survival, counting tyrosine hydroxylase (TH)-positive neurons of the Substantia Nigra *pars compacta* (SNpc); and the functional integrity of their striatal terminals, by dopamine active transporter (DAT) expression level. Furthermore, we analyzed the dendritic spine density of the striatal spiny projection neurons. Comparisons were made between sedentary and active α-syn-PFFs-injected animals compared to control animals. Interestingly, in active parkinsonian animals we found increased number of SNpc neurons with a higher density of DAT+ - terminal-fibers in the dorsolateral-striatum, compared to sedentary α-syn-PFF-injected rats. Furthermore, active animals showed increased spine density in striatal projection neurons with a greater proportion of young immature spines. These structural changes were also associated with a functional outcome as active animals displayed better performances in motor coordination and visuospatial learning tests. In conclusion, we demonstrate that intensive exercise training in parkinsonian animals, at presymptomatic stages of disease, has effects in counteracting neurodegeneration of the nigrostriatal pathway. Physical activity induces striatal adaptive responses to the α-syn-PFFs intoxication, associated with improved motor and cognitive performances compared to the parkinsonian sedentary group.

ND27 | Neuroprotective activity of the new metabotropic Glutamate Receptor 3 Positive Allosteric Modulator in Parkinson's Disease in vitro and in vivo models

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Parkinson's Disease (PD) is a complex and progressive neurodegenerative disorder associated with the gradual loss of dopaminergic neurons in the nigrostriatal circuit, which leads to the development of motor dysfunctions, such as rest tremors and rigidity, and non-motor symptoms, including dementia. Currently, no disease-modifying pharmacological treatments are available, and dopamine-based therapies typically help to mitigate symptoms but also cause side-effects such as dyskinesia. In this context, indirect pieces of evidence have suggested that activation of metabotropic Glutamate Receptor 3 (mGluR3) is able to exert neuroprotective effects in animal models of PD. However, so far, the lack of selective agonists/ligands for this receptor has hindered more in-depth investigations. Recently, a new Positive Allosteric Modulator (PAM) selective for mGluR3 has been synthesized. In this study, we demonstrate that the novel mGluR3 PAM is able to protect SH-SY5Y cells, a human neuroblastoma cell line, against the degeneration induced by 6-hydroxydopamine (6-OHDA) exposure through the modulation of Mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) and phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathways. Furthermore, *in vivo* treatment with mGluR3 PAM up-regulates the expression of Glial cell line-derived neurotrophic factor (GDNF) and Brain-derived neurotrophic factor (BDNF), and modulates the activation of MAPK/ERK and PI3K-Akt pathways in several mouse brain regions. Overall, though preliminary, our results suggest the therapeutic potential of the new mGluR3 PAM in the context of PD management.

EBN08 | Metabolic supplementations and epigenetic alterations in in vitro AGC1 deficiency models

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AGC1 deficiency is an ultra-rare demyelinating disease caused by mutations in the SLC25A12 gene, which encodes for isoform 1 of the mitochondrial aspartate-glutamate carrier (AGC1). The main pathological features are secondary hypomyelination, along with impaired proliferation of brain cells. Probably, abnormal myelin production is due to a reduced synthesis of N-acetyl-aspartate (NAA), from which the acetyl groups mainly derive. This, in turn, leads to epigenetic alterations in the brain precursor cells and resulting in transcriptional dysregulation, causing proliferation and differentiation defects, as demonstrated by previous data on our *in vitro* AGC1 deficiency models (precursor cells of mouse oligodendrocytes -OPCs- where SLC25A12 is silenced by a shRNA and neurospheres by mouse model of AGC1 deficiency) of our laboratory.
Along with epigenetic alterations, lower production of NAA leads specifically to reduced levels of acetyl-CoA, involved in a large number of biological activities, including the synthesis of fatty acids, major components of the myelin sheath. Thus, an alteration of their production leads to hypomyelination. This is confirmed by RNA-seq analysis on OPCs, which show altered expression of transcriptional factors and enzymes involved in the fatty acid synthesis pathway.
Firstly, we would like to verify the *in silico* data of the RNA-seq analysis. Moreover, to try to compensate for the lack of acetyl-CoA, and the consequent epigenetic and metabolic alterations, supplementations with amino acids and ketone bodies -directly involved in the synthesis of acetyl-CoA- will be carried out to induce a potential recovery of differentiation/proliferation defects in both our *in vitro* models.

EBN09 | Auditory-hippocampal alterations and behavioural fluctuation during development in a mouse model of autism and epilepsy

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Autism spectrum disorders (ASDs) are heterogeneous neurodevelopmental disorders characterized by two main symptoms: social deficits and repetitive behaviors. Autistic children often exhibit several secondary symptoms including epilepsy. Mutations in the Synapsin2 (SYN2) gene, associated with ASD and epilepsy in humans, are causative for an imbalance between excitatory and inhibitory systems. Synapsins are a family of neuron-specific phosphoproteins which control synaptic vesicle trafficking and modulate neurotransmitter release at the presynaptic terminal. Mice lacking SYN2 (SynII KO) display autistic-like traits with a strong reduction of ultrasonic vocalizations (USV) and social sniffing, repetitive behaviors (self-grooming) and mild cognitive impairments (deficit in social memory and recognition). Furthermore, SynII KO mice display epileptic seizures that appear at 2-3 months of age. Interestingly, we recently showed that the impaired USV phenotype strongly correlates with a reduced functional connectivity in the auditory cortex and hippocampus, two brain regions playing an important role in social behavior.
Our goal was to clarify how synaptic alterations in these regions impact on the social brain circuitry generating pathological conditions. We characterized the synaptic alterations in the auditory cortex and hippocampus using western blotting analysis and immunohistochemistry.
Our results show a significant reduction in presynaptic and postsynaptic markers of the GABAergic system together with alterations in synaptic density in these areas of Syn II KO mice. These results indicate that developmental changes in the neural connectivity of specific cortical areas may underlie the epileptic and social phenotype of SynII KO mice.

EBN10 | Dopaminergic alteration triggers autistic-behaviours in lysosomal storage disorders

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Mucopolysaccharidosis IIIA (MPS-IIIA) is a severe inborn errors of metabolism caused by mutations of the sulfamidase gene (*SGSH*), a lysosomal enzyme that participates in the metabolism of the heparan sulfate (HS). Defective sulfamidase activity leads to the lysosomal HS accumulation which results in defective autophagosomal/ lysosomal degradative capacities and build-up of primary and secondary storages, ultimately leading to neurodegeneration and dementia in children. However, dementia is preceded by severe and incapacitating autistic-like behaviours, including self-injury, stereotypic behaviours and social impairment. We recently discovered that young MPS-IIIA mice show autistic-like behaviours due to increased proliferation of mesencephalic dopamine neurons originating during embryogenesis. This hyperdopaminergia is not due to lysosomal dysfunction, but to altered HS function as co-receptor of growth factors (*De Risi et al., 2021, Nature Communications*). We have also shown that pharmacological inhibition of D1 dopamine receptors, but not D2, rescues autistic-like behaviours in young MPS-IIIA mice. We are therefore testing in preclinical and clinical settings FDA-approved dopaminergic drugs that might act, through different biochemical pathways, on the same dysfunctional mechanism responsible for autistic-behaviour in MPS-IIIA.

EBN11 | Further insights into Allan-Herndon-Dudley syndrome: characterization of two genetic variants in SLC16A2 gene

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Genetics variants in SLC16A2 gene encoding for the monocarboxylate transporter 8 (MCT8) cause a severe X-linked intellectual deficit and neurological impairment known as Allan-Herndon-Dudley syndrome (AHDS). MCT8 promotes cellular uptake and efflux of thyroid hormone and its mutations provoke elevated serum T3 levels in children. Iodothyronine deiodinases (DIO) 1 and 2 are implicated in the conversion of T4 into biologically active T3, while DIO3 converts T4 into the inactive hormone reverse T3 (rT3). Active T3 and retinoid X receptors (RXR) can form heterodimer complexes which bind to hormone response elements (HREs) that leads to activate or repress transcription. Our aim is to investigate the impact of MCT8 mutations on the pathogenetic mechanisms of AHDS. Fibroblasts were obtained from skin biopsies of 2 AHDS and matched controls. To evaluate both MCT8 and thyroid hormone signaling pathway related genes expression, RNA was extracted with TRIzoL™ and assessed by Real-Time PCR. Protein expression was valuated via western blot and immunofluorescence. MTT assay was used to compare cell viability. Live and dead assay was used to discriminate live and dead populations. Lipids were detected via oil red o staining. MTT assay demonstrated a reduced cell viability as consequence of mutations in SLC16A2. We report that SLC16A2 RNA expression in AHDS patients was extremely reduced in comparison with total RNA from healthy controls. Additionally, DIO2, progastricsin, HR and KLF9 RNA expression resulted upregulated, whilst DIO1, DIO2-AS1, DIO3 and TH were downregulated influencing T_3 cell entrance. Myelin related genes were significatively reduced. The lipid staining revealed an increasing presence of lipid droplets in AHDS patients. Taken together, our preliminary data emphasize an impairment in AHDS fibroblasts in relation to mutations in MCT8 transporter, increasing our understanding in the pathogenic mechanism of mutation in two patients affected by AHDS.

EBN12 | Characterization of the molecular mechanism underlying GABAA-receptor defects in CDKL5 deficiency disorder

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Cyclin-dependent kinase-like 5 (CDKL5) is a serine/threonine kinase highly expressed in the brain. The role of CDKL5 appeared fundamental when CDKL5 mutations were associated to CDKL5 deficiency disorder (CDD), a severe neurodevelopmental encephalopathy characterized by early-onset epilepsy, intellectual disabilities and autistic features. Although CDKL5 functions in the excitatory synapse have been widely studied, its possible involvement in regulating inhibitory neurotransmission is still unexplored. The inhibitory activity in the central nervous system is mainly mediated by γ-aminobutyric acid type A receptors ($GABA_AR$) and is crucial for the regulation of major neurodevelopmental processes. In particular, $GABA_AR$ receptors are heteropentameric ligand-gated ion channels consisting of two α (α_{1-6}), two β (β_{1-3}) and either a γ (γ_{1-3}) or a δ subunit. Considering the involvement of $GABA_AR$ dysfunctions in neurodevelopmental disorders, we decided to study how CDKL5 deficiency influences $GABA_AR$ expression. To this aim, we performed cell-surface biotinylation assays and immunohistochemical analyses to investigate the surface exposure of $GABA_ARs$ in hippocampi of *Cdkl5*-WT and -KO male mice along a developmental timeline. Our data show that while total $GABA_AR$ levels are unaltered in hippocampi of *Cdkl5*-KO mice, CDKL5 loss negatively influences the surface expression of synaptic $GABA_ARs$. This might suggest a defect in receptor membrane stabilization and/or internalization. Accordingly, we have found reduced phosphorylation of the $GABA_AR$ subunit b_3. We are currently investigating whether CDKL5 loss causes an alteration in $GABA_AR$ recycling. These data reveal a novel role of CDKL5 in regulating GABAergic synaptic inhibition, which may allow unveiling the molecular mechanism underlying epileptic seizures in CDD patients and pave the way for the design of drug based therapeutic approaches.

EBN13 | CACNA1A mutations impair neuronal induction and function

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CACNA1A encodes the pore-forming α1A subunit of the voltage-gated CaV2.1 calcium channel. This channel is found primarily on presynaptic terminals, dendrites, and neuroendocrine cells in the brain, and it is critical on regulating synaptic function. Many mutations have been identified in *CACNA1A*, causing different neurological disorders, as various forms of ataxia, epilepsy, and migraine. However, the molecular mechanisms underlying these disorders are little known, and specific therapeutic approaches are lacking.

The aim of this study was to investigate the effects of *CACNA1A* loss-of-function mutations on neuronal development and function by using human-derived *in vitro* models. Accordingly, iPSCs carrying two *CACNA1A* variants causing episodic ataxia type 2 (Y1854X, selectively affecting CaV2.1[EFa] isoform, and F1491S, affecting both CaV2.1[EFa] and [EFb] isoforms) have been produced by CRISPR/Cas9 methods. Mutated and isogenic control iPSC lines were used to generate neuronal cultures by differentiation protocols passaging through embryoid bodies, neural rosettes, neural progenitors, and neuronal network stages. Morphological, molecular, and functional tests highlighted different neurodevelopmental defects caused by the two mutations. Cells carrying F1491S showed an impaired neuronal induction at very early stages, with mutated neural progenitors appearing with an altered morphology, reduced expression of neural markers, and enhanced migration. Instead, cells carrying Y1854X behaved apparently normal in terms of neuronal specification and maturation but showed a reduced spontaneous electrophysiological activity with lack of synchronous network events.

Our findings highlighted novel roles of CaV2.1 in neuronal induction, besides confirming its relevance in synaptic communications. Importantly, the iPSC-derived neuronal models developed in this study will pave the way for future therapeutics testing for neurological disorders involving *CACNA1A*.

EBN14 | Dopamine Transporter DNA Methylation modulation evoked by stress in university students

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Stress can be defined as a physiological and psychological response to environmental changes that can affect well-being. We examined how stress can modulate the transcriptional regulation of key signalling pathways. Perceived Stress Scale-10 (PSS-10) at two time points within a year of each other was adopted as the measure of perceived stress level of university students and DNA methylation status at specific CpG sites of Oxytocin Receptor (OXTR), Dopamine transporter (DAT), and Serotonin transporter (SERT) genes was analysed at the same time points by pyrosequencing in DNA collected from salivary samples. PSS scores ranging from 0 – 13 are considered as low self-perceived stress, those ranging from 14 – 26 as moderate self-perceived stress, and those ranging from 27–40 as high self-perceived stress. At the first time-point, significant increases have been observed for DNA methylation at DAT CpGs 1, 5, and 7 and in the average of all the CpG sites under study when comparing subjects with low and medium PSS scores to those with high scores. When we analysed DAT DNA methylation considering the PSS scores one year later, we observed that in people showing a reduction from high to medium or low scores, the alterations in the epigenetic mark disappeared, resulting also similar to the levels of controls. No changes between the two time points were observed for OXTR and SERT DNA methylation among students with different PSS scores. This study focuses on the dynamic nature of stress and DNA methylation patterns, biological markers that might have potential applications in stress management and interventions.

EBN15 | Investigating the role of rare missense variants in RAB11B in Autism Spectrum Disorder

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Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by a distinct set of social and communication deficits, repetitive behaviors and restricted interests. The genetic architecture is complex, with multiple types of variants involved. Approximately 30% of cases stem from high impact *de novo* variants, which have led to the identification of hundreds of genes implicated in ASD. Because 30-50% of ASD individuals have also intellectual disability (ID) and/or other neurodevelopmental disorders (NDDs), many genes are associated with both disorders.

From the Whole Exome/Genome Sequencing (WES/WGS) data analysis of 116 families with at least one ASD individual, we identified 37 rare *de novo* potentially damaging variants. Among these, we identified a case with a novel *de novo* missense variant in *RAB11B*, a gene recently implicated in a NDD including ID and white matter anomalies. To date, 2 pathogenic variants have been functionally described in this gene. Thorough a review of public databases and the existing literature, we found two additional uncharacterized SNVs in the same gene in two individuals with ASD/NDDs.

RAB11B is one of 3 genes (*RAB11A*, *RAB11B, RAB25*) encoding for the Rab11 subfamily of Rab GTPases, molecular switches involved in intracellular transport and endosomal trafficking. The precise coordination of vesicular cargo is essential for numerous neurodevelopmental processes, including ciliogenesis, neurite outgrowth, dendritic spine formation, and synaptogenesis.

To better understand *RAB11B* role in the ASD phenotype, we functionally characterized the three missense variants identified in this and other studies by immunofluorescence analyses on ARPE-19 cells, a retinal pigment epithelium cell line. Studying the co-localization of RAB11B mutants with key cellular compartments involved in primary cilium differentiation will elucidate the effects of such variants on endosomal trafficking, and ultimately clarify their role in the disorder.

EBN16 | The functional role of CDKL5 at the inhibitory synapse and its interaction with the cytoplasmatic collybistin-gephyrin complex

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Mutations in the cyclin-dependent kinase-like 5 gene (*CDKL5*) are responsible for a severe neurodevelopmental disorder, namely CDKL5 deficiency disorder (CDD), characterized by early-onset epileptic encephalopathy, severe intellectual disability and intractable seizures. So far, the role of CDKL5 in excitatory synapses has been widely explored; on the contrary, more has still to be investigated regarding its influence on the inhibitory compartment.
Our recent data showed that CDKL5 loss impacts the number of synaptic $GABA_A$Rs, which may be explained by its interaction with the postsynaptic scaffolding complex containing gephyrin and collybistin. Gephyrin is the core scaffolding protein of the inhibitory synapse, while CB is a brain specific GEF, involved in recruiting gephyrin to postsynaptic sites. CB is retained in a folded-inactive conformation by its auto-inhibitory SH3 domain; when this domain interacts with other proteins, CB switches in the open-active conformation.
Interestingly, through various biochemical and immunofluorescence approaches based on different derivatives of both proteins, we have demonstrated that CDKL5 can release CB from its inactive conformation allowing the distribution of gephyrin to sub-membranous sites in expressing cells. The interaction of CDKL5 with CB requires the SH3 domain of the latter as well as the catalytic activity of CDKL5. We are currently investigating whether CB is phosphorylated in CDKL5 dependent manner through the PhosTag™ approach.
In conclusion, our results seem to place CDKL5 as a novel key regulator of the postsynaptic scaffolding complex of inhibitory synapses. Furthermore, elucidating the synaptic networks regulated by CDKL5 from a molecular point of view are likely to pave the way to understand the molecular mechanisms underlying CDD and, possibly, to develop target-based therapeutic approaches.

POSTER SESSION

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POSTER SESSION

CN05 | CRISPR/Cas9 and piggyBac Transposon-Based Conversion of a Pathogenic Biallelic TBCD Variant in a Patient-Derived iPSC Line Allows Correction of PEBAT-Related Endophenotypes

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Induced pluripotent stem cells (iPSCs) have been established as a reliable *in vitro* disease model system and represent a particularly informative tool when animal models are not available or do not recapitulate the human pathophenotype. The recognized limit in using this technology is linked to some degree of variability in the behavior of the individual patient-derived clones. The development of CRISPR/Cas9-based gene editing solves this drawback by obtaining isogenic iPSCs in which the genetic lesion is corrected, allowing a straightforward comparison with the parental patient-derived iPSC lines. Here, we report the generation of a footprint-free isogenic cell line of patient-derived *TBCD*-mutated iPSCs edited using the CRISPR/Cas9 and *piggyBac* technologies. The corrected iPSC line had no genetic footprint after the removal of the selection cassette and maintained its «stemness». The correction of the disease-causing *TBCD* missense substitution restored proper protein levels of the chaperone and mitotic spindle organization, as well as reduced cellular death, which were used as read-outs of the *TBCD* KO-related endophenotype. The generated line represents an informative *in vitro* model to understand the impact of pathogenic *TBCD* mutations on nervous system development and physiology.

CN06 | Identification of novel antibodies in patients with small fiber neuropathy

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Small fiber neuropathy (SFN) is a peripheral neuropathy that manifests clinically with pain and autonomic disturbances. Over 50% of cases are idiopathic SFN (iSFN). Some studies suggest a possible autoimmune etiology; in particular, the presence of antibodies against FGFR3 has been identified in about 20% of patients with iSFN. In addition, a recent study suggested a possible role of antibodies to MX1, DBNL, and KRT8. Our aim was to evaluate the frequency of antibodies against FGFR3, MX1, DBNL, and KTRT8 in patients with suspected SFN. We included consecutive patients undergoing skin biopsy at the leg and thigh for suspected SFN afferent to the *UOC Neurological Clinic* of Bologna. Serum was tested for the presence of antibodies (Ab) against FGFR3, MX1 and DBNL by *in-house* cell-based assay. Small-fiber pathology was confirmed by a reduction of intraepidermal nerve-fiber (IENF) density. A total of 315 patients were investigated. Antibodies against the targets of interest were identified in 4/315 cases. Specifically, FGFR3-Ab were detected in 1/298, MX1-Ab in 2/296 and DBNL-Ab in 1/309. All positive patients had small fiber neuropathy on skin biopsy. Case 1 with MX1-Ab (M, 86 years old) showed somatic and autonomic SFN, whereas case 2 (M, 79 years old) showed only somatic fiber involvement. The patient with FGFR3-Ab (F, 46 years old) presented a somatic SFN with minimal autonomic fiber involvement distally. Finally, the patient with DBNL-Ab (F, 45 years old) showed somatic and autonomic SFN. In conclusion, antibodies against FGFR3, MX1, DBNL, and KRT8 are rare in patients with iSFN.

NI14 | Nerve growth factor influences microglial activity in vivo via TrkA receptors

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The Nerve Growth Factor (NGF), in addition to supporting neurons, has pleiotropic actions on non-neuronal cells, especially in the peripheral immune system. Moreover, our lab discovered potent immunomodulatory properties of NGF via TrkA on the brain immune cells, microglia: NGF steers them toward an anti-inflammatory phenotype *in vitro*. Here, we provide *in vivo* evidence of a (neuroprotective) role for NGF signaling in brain microglia, both in (1) pathological and (2) physiological conditions. (1) We first considered Rett syndrome (RTT) as a neurodevelopmental disease that could benefit from NGF. Thus, we tested the therapeutic effects of a mutein of NGF, called *human NGF painless* (hNGFp), via a non-invasive intranasal delivery in female RTT model, the MeCP2$^{+/-}$ mice. We reveal a deficit in microglial morphology in MeCP2$^{+/-}$ mice, completely reversed in treated animals. To understand the immunomodulatory activity of hNGFp, we also analyzed the cytokine profile after hNGFp treatment in MeCP2$^{+/-}$ mice, to discover that the treatment recovered the altered expression of key neuroimmune-communication molecules. Thus, hNGFp can ameliorate symptoms in the MeCP2$^{+/-}$ model, by exerting strong neuroprotection also via microglia. (2) Secondly, to directly assess the functional role of the microglial NGF-TrkA signaling, we generated a novel inducible transgenic mouse line, in which TrkA can be specifically deleted in microglia. We report that knocking out the microglial NGF-TrkA signaling leads to a reduction of microglial density in primary somatosensory cortex. We also found differences in microglial morphology and phagocytosis of dendritic spines. Lastly, at behavioral level, microglial NGF-TrkA signaling deletion affects memory and learning. Altogether, these overall data demonstrate that TrkA signaling influences pivotal microglia activities. Modulating the NGF-TrkA axis on microglia *in vivo* might be harnessed as a broad neuroprotective therapeutic strategy.

NI15 | A comprehensive molecular imaging study in a mouse model of CMT1B neuropathy

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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 demyelination. Considering that previous studies described a potential role of innate immunity in the pathogenesis of neuropathy in CMT animal models, and molecular imaging has proved to be a valuable non-invasive means to explore the development of micro-inflammatory processes *in vivo*, we aimed at verifying the presence of immune/inflammatory cell infiltration in the nerves and muscles of this novel CMT1B model, and correlating the imaging findings with disease progression. To do this, we subjected MPZ-D61N heterozygous ($MPZ^{D61N/+}$) and homozygous ($MPZ^{D61N/D61N}$) mice to a sequential protocol including evaluation of motor performance, ultrasonography, magnetic resonance imaging (MRI) before and after administration of Ferumoxytol (a contrast agent classically used to label macrophages), and whole-body micro-PET imaging, after administration of a tracer for the TPSO protein. Finally, animals were sacrificed, and sciatic nerves and muscles were collected for histological analyses. Both MRI and micro-PET imaging indicated that macrophage infiltration is absent in our models. However, histological analysis revealed a marked increase in the number of neutrophils in the sciatic nerve of $MPZ^{D61N/D61N}$ mice. Moreover, our MRI scanning protocol was sensitive enough to detect structural alterations in the sciatic nerves of both $MPZ^{D61N/+}$ and $MPZ^{D61N/D61N}$ mice. Even if this study represents a pilot investigation of inflammatory response in the muscle and nerve in a severe form of CMT1B neuropathy, our results suggest that low-grade inflammation is present in the sciatic nerve of D61N-homozygous mice and may exacerbate the neuropathic phenotype.

NI16 | Butyrate decreases the regulatory function of human natural killer cells and promotes their maturation

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An increased amount of evidence suggest that bacteria-derived metabolites play a role in shaping immune cell function. We and others have shown dysfunction in regulation of the T-cell response by NK cells in multiple sclerosis (MS). The objective of this work was to assess whether microbial-derived metabolites affect the regulatory function of NK cells. We cultured NK cells from healthy controls (HC) in presence/absence of the butyrate and of different tryptophan derivatives in presence of activating cytokines and then co-cultured them with autologous T cells. We cultured PBMCs from HC in presence/absence of the butyrate and tryptophan derivatives and assessed the phenotype of NK cells through a 14-marker flow cytometry panel. We performed Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) on NK cells sorted from PBMCs cultured in presence of butyrate. ATAC-Seq data were analyzed by GUAVA tool starting from raw data to the calls of the identified peaks and their functional characterization in Gene Ontology (GO). Further, data were compared to those from a public single-cell RNA sequencing dataset of NK cell clusters. We found that butyrate decreased the percentage of $CD56^{bright}$ NK cells, increased the percentage of $CD69^{+}$ NK cells and decreased the suppressor function of NK cells. Tryptophan derivatives did not affect the function, nor the phenotype of NK cells. ATAC-seq revealed that butyrate affects chromatin modifications in 218 genes causing demethylation in the promoter region of most (> 80%) genes, involved in (i) the regulation of specific populations of T cells within the immune system, (ii) the modulation of cellular processes in neurons and (iii) to inflammation. The epigenetic signature of butyrate-treated NK cells increased similarly to terminally differentiated $CD56^{dim}$ NK cells. In conclusion, we describe a novel effect of butyrate on human NK cells, whereby butyrate induces their maturation and decreases their regulatory function.

NI17 | Transcranial magnetic stimulation restores glial response and microvasculature integrity in experimental Parkinson's disease

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Transcranial magnetic stimulation (TMS) is a form of non-invasive brain stimulation used to induce neuroplasticity in the brain and a non-pharmacological treatment in different neuropathological conditions. Although TMS has been shown to modulate several aspects of neuronal plasticity, there is still limited information on how TMS works at the cellular and molecular levels, as well as its impact on non-neuronal cells, like astrocytes and microglia and other different components of the Blood-Brain-Barrier (BBB), the pericytes and the endothelial cells (EC).This study investigated changes in RNA and protein levels of the main components of the BBB following different treatments of theta-burst stimulation (TBS), repetitive TMS patterns, such as continuous (cTBS) and intermittent (iTBS) protocols, used in a 6-hydroxydopamine (6-OHDA)-lesioned hemiparkinsonian rat model. In the striatum of parkinsonian animals, mRNA and protein levels of markers of pericytes, such as PDGFβ, decreased significantly after the lesion compared to control animals. Notably, in 6-OHDA-lesioned animals, the decrease of pericytes markers correlated with a reduction of PECAM-1 (also known as CD31), a marker of vessel endothelial cells, and these changes were associated with impairments in brain microvasculature. After the injury, treatment with iTBS protected both pericytes and endothelial cells of the striatal microvasculature from 6-OHDA-induced damage. Similarly, cTBS treatment restores microvessels integrity by restoring lectin and PECAM-1 expression, thus improving microvasculature integrity, that was found similar to what was observed in unlesioned animals.Collectively, these findings demonstrate the ability of TMS to modulate specific aspects of non-neuronal cell phenotype and restore the microvasculature integrity, highly impaired after 6-OHDA lesion, potentially contributing to the known effects of TMS on neuroprotection.

NI18 | NLRP3-inflammasome inhibition by Leishmania-derived factors in the neuropathogenesis of Alzheimer's Disease (AD): assessing the molecular and therapeutic role

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The hygiene hypothesis suggests that certain aspects of modern life are linked to lower rates of exposure to pathogens and lower immune system stimulation, being in turn positively related to Alzheimer's-disease risk. Amazonian tribes exhibit an exceptionally high prevalence of infections in individuals of all ages. It results in a better-regulated degree of inflammation and improved cognitive functions after infection with Leishmanias (L) parasite. Indeed, parasitic infections recognized the same receptors that sense misfolded-Aß in microglia; it is followed by subsequent regulation of the NLRP3, a cytosolic multiprotein complex mainly expressed in myeloid cells and composed of the Nod-like receptor (NLR), the adaptor apoptosis-associated speck-like protein (ASC), and the pro-caspase1, leading to the cleavage in bioactive IL1ß and IL18.
Since the N is considered a therapeutic target against neuroinflammation in AD, herein we investigated a possible connection between L *tarantulae (t)*, nonpathogenic for humans to reduce IL1ß, IL18, Caspase1 release and ASC-speck formation. THP1cells wild-type and ASC-knockout were cultured in unstimulated or Lypolisaccaride+Aß-treatment post *Lt-p10* infection. L phagocytosis were quantified by confocal mycroscopy, cytokines by ELLA and inflammasome formation and by AMNIS FlowSight. ASC-speck formation as well as IL18 and caspase1 were significant ($p<0.05$)downregulated in LPS+Aß THP1 wild-type and post L-infection compare to unstimulated cells; no significant differences were found for IL1ß and NLRP3 proteins between wild-type and ASC-knockout cells.
L is able to modulate the inflammasome-assembly suppressing the inflammatory response. Given this ability, we propose a protective role of L in Alzheimer's disease as a consequence of the molecular inhibition of the NLRP3-inflammasome.

NI19 | Potential role of the hydroxyl carboxylic acid receptor type 2 (HCAR2) in microglia pathophysiology

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Following insults or injury, microglia cells are activated contributing to the cytotoxic response or by promoting an immune-mediated damage resolution. The inhibition of microglia activation or the shifting of proinflammatory toward to the other alternatively activated phenotypes, may represent therapeutic approach to prevent central nervous system (CNS) disorders. Microglia cells express a hydroxy carboxylic acid receptor (HCAR2), mediating neuroprotective and anti-inflammatory effects. Given the crucial role of microglia in the induction and maintaining of neuropathic pain states, was investigated in this study the effects of HCAR2 stimulation on primary microglia cell. Was tested MK1903, a full agonist of HCAR2, on Lipopolysaccharide (LPS)-primed microglia cells, by measuring cells viability, morphological activation and pro/anti-inflammatory mediator's production. In addition, the activity of HCAR2 was evaluated through electrophysiological recordings in vivo by measuring the spinal nociceptive neurons (NS) activity after MK1903 administration. To this end, was promoted microglia activation by topically applying the neuronal chemokine fractalkine (CX3CL1) which signals through its unique receptor CX3CR1 exclusively expressed on microglia. The results showed that HCAR2 expression levels were increased in LPS-primed microglia cells. In a similar way, the treatment with MK1903, a potent full agonist of HCAR2, increased receptor protein levels. Moreover, was observed HCAR2 stimulation prevented morphological activation and pro/anti-inflammatory mediator's production in LPS-treated cells. Finally, was found that spinal application of MK1903, before CX3CL1, prevented cells viability the spinal fractalkine-induced hypersensitivity, indicating the HCAR2/CX3CR1 involvement in the cross-talk between neuron and glia. The study paves the way for further investigations aimed at understanding the role HCAR2 as potential target in neuroinflammation-based CNS disorders.

NI20 | Microglia-released extracellular vesicles counteract age-related cognitive impairment and restore microglia homeostasis in the aging brain of male and female mice

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Aging is a time-related deterioration of physiological functions, associated with oxidative stress, chronic inflammation and high production of inflammatory compounds. During brain aging, among the first changes, there are modifications of microglia (MG), the resident immune cells of the central nervous system (CNS), which become hyper-responsive and nonfunctional. These cells undergo the most prominent aging-related changes in both the morphological and functional phenotypes; their progressive loss of neuroprotective functions results in chronic neuroinflammation (i.e., increase of inflammatory markers and dystrophic morphology), which impacts the whole brain homeostasis. All these alterations occur differently in aged males and females and cause cognitive functions deterioration, lack of motor coordination and memory loss. Extracellular vesicles (EVs) are key players of the inter-cellular communication between donor and target cells; EVs are exploited by the cells to exchange a package of molecular information consisting of lipids, proteins and nucleic acids. Given their properties, EVs are emerging as a promising tool to develop revolutionary non-invasive therapies for a wide range of diseases. We investigated the effect of EVs released by anti-inflammatory MG and intranasally administered to both male and female mice during the old age (16-18 months). We evaluated *in vivo* memory and anxiety-like behavior and *ex vivo* inflammatory and homeostatic MG profile by RT-qPCR and cellular morphology analysis. EVs treatment ameliorated brain functionality in terms of reduction of anxiety; we observed a reduction of pro-inflammatory genes (*Il-6, Tnfα, Il1β, Cd86*) and a recovery of the homeostatic MG morphology. The effects on microglia were dissimilar in male and female mice pointing out sex-differences in the anti-aging effects of EVs treatment. The findings indicate EVs as an innovative strategy to slow down the effects of aging on brain functioning.

NI21 | Resolution of inflammation is impaired in Parkinson's disease and is rescued by specialized pro-resolving lipid mediator RvD1 through targeting microglia

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Uncontrolled neuroinflammation is associated with many widely occurring diseases such as Parkinson's disease (PD). Indeed, during PD neuroinflammatory mechanisms seem to play a role in the selective degeneration of dopaminergic neurons, suggesting that brain inflammation is important in the pathogenesis of PD. Recent studies suggest that neuroinflammation could be due to a failure in resolving inflammation, the resolution of which is mediated by a newly characterized pathway that involves specialized pro-resolving lipid mediators (SPMs), that include resolvins, protectins and maresins. By means of targeted lipidomics we found impairments of lipid classes in plasma that were associated to disease severity. Thus, we boosted resolution of inflammation *in vivo* in a rat model of PD overexpressing human alfa-synuclein (a-Syn) by treating them with resolvin D1 (RvD1) starting at an early stage of the disease and until the symptomatic phase. By means of multiparametric flow cytometry, we evaluated the infiltration of $CD45^+$ leukocyte cell populations ($CD3^+$ T-cells, $CD45RA^+$ B-cells, $CD161^+$ NK-cells and CD45-CD11b) within substantia nigra. We found that a-Syn rats showed a higher degree of all leukocyte subsets compared to wild-type rats and RvD1 treatment reduced their infiltration with the exception of macrophages whose infiltration was differentially modulated. Although the percentage of microglial cells remained unchanged between the different experimental groups, we observed that microglia of a-Syn rats shifted from a pro-inflammatory M1-like to a pro-resolving/anti-inflammatory M2-like immunophenotype upon RvD1 treatment, in terms of modulation of their respective M1 (CD68, CD86, MHC-II) and M2 (CD206, TREM2) markers. These results suggest that early potentiation of the resolution of inflammation pathway might represent a novel approach to blunt neuroinflammation and to eventually design potential disease-modifying treatment for PD based on targeting innate immune cells.

NI22 | Role of Pentraxin3 in neurodevelopmental disorders

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Control of synapse number and function is critical to the formation of neural circuits. Recently we showed that the innate immune molecule Pentraxin3 (PTX3) interacts with Thrombospondin1 (TSP1) to promote the proper formation and function of excitatory synapses; TSP1 induces the formation of silent synapses, while PTX3 leads to their correct maturation. PTX3 activity is regulated by TSP1, which exerts a negative regulation of PTX3 itself suggesting that the relative amount of these proteins and their complex are crucial to set the balance between synaptic growth and synapse maturation. Prenatal inflammation is a recognized risk factor in neurodevelopmental disorders. PTX3 expression is strongly stimulated by inflammation and as a consequence PTX3 and TSP1 levels may change upon immune challenge during brain development affecting synaptogenesis. Starting from these premises, we aim to investigate how prenatal immune stimulation affects PTX3 and TSP1 levels and how this impacts synaptogenesis and brain circuit development. Using a mouse model of Maternal Immune Activation (MIA) we analyzed the effects of prenatal inflammation on the production of PTX3 and TSP1 in the cortex of the offspring. We found that prenatal inflammation disrupts the physiological developmental pattern of expression of PTX3 and alters the PTX3:TSP1 ratio in in the cerebral cortex during the postnatal period of synaptogenesis. Moreover, we observed morphological and functional alterations in astrocytes, which are the main source of PTX3. We are currently investigating the impact of altered PTX3 levels during brain development on astrocytes maturation.
Furthermore, we are investigating the impact of PTX3 alteration in a human cohort of premature neonates (ELGAN study) whereas the pattern of PTX3 production and release under inflammatory conditions is also investigated in human iPSC-derived astrocytes.

NP13 | The effect of daily exercise on improving post-injury symptoms in a mild traumatic brain injury mouse model

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Traumatic brain injuries (TBI) refer to multiple acquired dysfunctions arising from damage to the brain caused by an external force, including rapid acceleration/deceleration and concussion. Among them, mild TBI (mTBI) accounts for most cases (up to 90%) of injuries. It is responsible for a variety of symptoms, including anxiety, depression, and cognitive impairments that remain difficult to be treated. It has been reported that regular physical activity, as well as, improving life quality, displays a neuroprotective function, suggesting a possible role in post-traumatic rehabilitation. In this study, we investigated the effects of treadmill exercise in a mice mTBI model by behavioral, electrophysiological, and neurochemical analysis. Daily exercise decreased anxiety, aggressive behavior, and depression in mTBI mice. Accordingly, electrophysiological and neurochemical maladaptive rearrangement occurring in the hippocampus of mTBI mice was prevented by the exercise. We discovered that daily exercise can significantly reduce anxiety, aggression, and depression at specific time points after trauma by performing behavioral tests. We noticed meaningful differences between the groups by performing electrophysiology on the dentate gyrus and nucleus accumbens pathways, and finally, microdialysis results by focusing on neurotransmitter changes (GABA and Glutamate) proved our findings.

NP14 | Early-life interference with the attachment bond induces depressive-like behaviour and potentiated Ih current in VTA dopaminergic neurons of adult DBA/2J females

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Early stages of postnatal life are critical for the development of brain areas connectivity. Stressful events occurring in this period can induce alterations potentially lasting into adult life and leading to either stress-related depression or well-adaptation to adversities - a behavioural phenotype termed “resilience”. We have previously shown that Repeated Cross Fostering (RCF; an early-life stress protocol recapitulating the interference with the attachment bond) determines the appearance of a resilient phenotype in adult (60-90 days old) C57BL/6J female mice. At the cellular level, this behavioural profile was causally related to the reduction of the I_h current in dopaminergic neurons of the Ventral Tegmental Area (VTA). Interestingly, such behavioural modulation appears to be sensitive to the individual’s genetic background: thus, DBA/2J females exposed to RCF show a depression-like (as opposite to resilient) behaviour in adult life. To follow this up, here we asked whether at the cellular level an analogous opposite modulation was present in dopaminergic neurons. To investigate this, we exposed DBA/2J newborns to RCF and tested adult females using behavioural protocols and brain slice patch-clamp. We found that indeed in these mice both behavioural and neurophysiological phenotypes were inverted compared to C57BL/6J animals, with adult DBA/2J females showing depressive-like behaviour and potentiated I_h current in VTA dopaminergic neurons. Last, as the presence of an alternative stable caregiving figure can prevent the negative consequences of an insecure attachment bond in humans, we asked whether the introduction of a stable Alternative Caregiver (SAC) during RCF protocol could ‘neutralise’ the early-life stress in susceptible mice and found that indeed both depressive-like behaviour and potentiation of the I_h current in VTA neurons were prevented.

NP15 | Astrocyte diversity across mammals: a comparative analysis on distribution and single cell morphology

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Astrocytes play a crucial role in brain functions, they show primate-specific features, and they are relevant in several diseases. In this context, a comprehensive comparison of astrocytes in a diverse range of mammals is pivotal for understanding the morphology across species and the related modifications in gene expression and functions. We have recently described primate-specific features of two subtypes of astrocytes, such as the Interlaminar astrocytes (ILAs) and the Varicose Projection astrocytes (VP-As), which can be of particular interest in the context of astrocyte evolution. Our project will expand from previous data and aims to investigate astrocytes' diversity across mammals, by characterising the distribution and the single-cell morphology of different subpopulations of astrocytes within different cortical layers across mammals. In details, we analyzed samples from prefrontal cortex of: Primates (chimpanzee, rhesus macaque, human), Carnivora (tiger, lion, leopard), Artiodactila (cow, tursiops), Rodentia (mouse) and Chiroptera (Seba's short-tailed bat). We immunostained these samples with various astrocyte markers (*i.e.*, GFAP, ALDH1L1, S100β, GLAST) to compare: (1) the distribution of different astrocyte subpopulations, and (2) the single-cell astrocyte morphology reconstructed with an algorithm-driven segmentation and Image-J plugin Neurotracer analyzer. Our previous results showed an increase of ILA morphological complexity and density in primates. With this project, we will unlock unprecedented details of the distribution and the single-cell morphological complexity of different astrocyte's subtypes across different layers and different mammals, with a special focus on primates and, in particular, humans. Data obtained from this research have the potential to lead to new fascinating hypotheses on the role of astrocytes in primate neuroanatomical, behavioural and cognitive complexity.

NP16 | Homeostatic plasticity in response to short-term monocular deprivation in the visual cortex of adult mice

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In adult humans, short-term monocular deprivation boosts the deprived eye responses (measured as increased predominance in binocular rivalry), an effect due to homeostatic plasticity mechanisms. To date, this phenomenon has never been studied in animal models. Here, we characterized for the first time the effects of short-term occlusion of one eye in adult mice. Awake head-fixed mice underwent electrophysiological recordings from the binocular zone of the primary visual cortex (V1), before and after a short-term session (120 minutes) of occlusion of the eye ipsilateral to the recorded brain side. We observed that short-term deprivation led to marked changes in favor of the deprived eye in visual evoked potential (VEP) amplitudes and in the contralateral to ipsilateral eye (C/I) VEP ratio, a paradigmatic measure of ocular dominance properties of cortical neurons. These results show a previously undescribed residual potential for homeostatic plasticity in the visual cortex of adult mice and may have translational implications for the treatment of visual disorders such as amblyopia.

NP17 | Investigating Cerebellar Abnormalities in a mouse model of lysosomal lipid storage disease: Implication for Social Behavior

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The cerebellum is a versatile brain region that regulates various motor/non-motor behaviors. Thus, impairments in its architecture and circuitry lead to a wide range of neurodevelopmental/ neuropsychiatric disorders. During postnatal development, the cerebellum undergoes changes in its cellular arrangement, guided by the Brain-derived Neurotrophic Factor (BDNF), which plays a role in appropriate development, synaptogenesis, and maintenance of cerebellar connectivity. In Niemann-Pick C1 disease (NPC1), a rare lysosomal lipid storage disease, we have previously shown that a decline in Sonic hedgehog (Shh) and BDNF expression in the first weeks of postnatal development disrupts cerebellar granule cell (GC) migration and maturation, influencing the final cerebellar cytoarchitecture. In *Npc1* mice, through immunohistochemistry/ Neurolucida analysis at various stages of early postnatal life, we observed a significant decrease in the amount, size and tortuosity of glomeruli, the main synaptic contact between GC dendrites and axons of mossy fibers. These results prompted us to investigate the presence of functional abnormalities in mature glutamatergic synapses. Therefore, by subcellular protein fractionation, we examined the expression levels of specific presynaptic (Syntaxin 1A, VAMP2, SNAP-25) and postsynaptic (Drebrin, Shank3) proteins during different stages of postnatal development, finding a general SNAP-25 deficiency in *Npc1* mice compared to *wild type* (wt) mice. Furthermore, through Golgi-Cox staining analysis, we characterized the density and morphology of GC dendritic spines in the internal granular layer, both in *wt* and mutant mice, to identify abnormalities in synapse maturation and pruning processes, during critical stages of cerebellar development. Finally, *Npc1* male mice showed no preference for social/nonsocial cues in a typical task used to study autistic-like behavior, consistent with studies indicating reduced levels of cerebellar BDNF in autistic patients.

NP18 | The Brain Anti-Reward Center In Autism Spectrum Disorder

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Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by deficits in social interaction, communication and cognitive flexibility. In the search of possible neuropathological abnormalities linked with ASDs, the disruption of reward processing has been identified as a common pathomechanism shared across the genetic and phenotypical heterogeneity of ASD. Although multiple studies linked aberrant mesolimbic reward pathway to dysfunction in social interaction and restricted behaviors, a key element has been neglected so far: the lateral habenula (LHb), also known as the anti-reward center of the brain. LHb activity encodes aversive responses and inhibits the physiological dynamics of ventral tegmental area (VTA), interfering with reward perception. My hypothesis is that aberrant activity in the LHb-VTA pathway, resulting in the altered coding of reward signals, might engender a dysfunctional control of motivated behaviors that translates into the ASD phenotype. A detailed analysis of neuronal projections using viral tracing showed that LHb is tightly connected to the VTA, but also target other neuromodulatory systems involved in the regulation of motivational and cognitive functions. Moreover, preliminary results show that increased activity of LHb obtained through a chemogenetic approach significantly reduces social aptitude of wild-type (WT) mice in the three-chamber social test, suggesting that a heightened activation of LHb-VTA network might arise social alterations comparable to ASD traits in WT animals.

NP19 | Dissecting the mechanism of action and neuroplastic potential of molecules targeting NMDA or 5-HT receptors

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Major depressive disorder (MDD) is a debilitating illness characterized by depressed mood, anhedonia, and impaired cognitive function. In the last few years, compounds targeting serotonin receptors 5-HT_{2A} or N-methyl-D-aspartate (NMDA) receptors have emerged as promising rapid-onset antidepressant agents. These drugs, collectively known as fast-acting antidepressant, may show clinical efficacy within 24 hours after the first administration and their effects may last long after the compounds have been cleared from the body. Fast-acting antidepressants have been shown to promote structural and functional neuroplasticity in the prefrontal cortex (PFC), which is one of the brain areas that have been documented to undergo neuronal atrophy in MDD patients. Despite evidence for clinical efficacy is growing, the molecular mechanism underlying the neuroplastic effect of these drugs is not fully understood. Intriguingly, neuroplasticity-promoting molecules seem to share similar downstream pathways that recapitulate in the increase of dendritogenesis and spinogenesis; nonetheless, their upstream binding partners and interactors might be distinct. Our aim is to investigate the mechanism of action of fast-acting antidepressants targeting the serotonin receptor 5-HT_{2A} or the NMDA receptor, such as psilocin and esmethadone, respectively. By combining pharmacological, genetic, and imaging interventions on primary cortical neurons and neuron-differentiated cells, we aspire at dissecting the intracellular pathways underlying the neuroplastic potential of fast-acting antidepressants. Our approach may be useful to characterize the neuroplastic activity of new 5-HT_{2A} agonists or NMDA receptor antagonists.

NO11 | Identification of a Novel KDM5C-Related Signature in Glioblastoma Multiforme

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Glioblastoma multiforme (GBM) is the most aggressive brain tumour without an effective pharmacological treatment. We analyzed the expression levels of the cancer driver gene Lysine (K)-specific demethylase 5C (KDM5C) in a series of GBM tissues. KDM5C belongs to the Jumonji C domain-containing histone demethylase family involved in various cancer types. It catalyzes the removal of the methyl groups from di- and tri-methylated lysine 4 on histone H3 in a Fe (II)- and α-ketoglutarate-dependent manner. By using real-time quantitative PCR and Western blotting analysis, we found an altered abundance of KDM5C transcript and protein in GBM samples identifying patients with higher (KDM5CHigh) and lower (KDM5CLow) levels compared to control samples. By exploring the impact of the defective KDM5C quantity, a positive and negative relationship with hypoxia -inducible transcription factor-1α (HIF-1α) and BDNF levels were found in $KDM5C^{High}$ patients. KDM5C overexpression and hypoxic studies performed in glioblastoma cell line (T98G) suggest that the stimulation of KDM5C expression is preceded by the induction of HIF-1α. High levels of HIF1α-KDM5C axis was also found associated with high levels of NANOG, SOX2 and NESTIN in GBM tissues isolated from conventional and 5-aminoleveulinic acid (5-ALA) fluorescence-guided surgery (FGS). A pro-inflammatory condition was also detected in 5-ALA FGS highlighting differences across the GBM microenvironment. Taken together, our study reveals for the first time a correlation between the HIF-1α-KDM5C axis and GBM opening a new field of investigation to validate KDM5C as a new GBM biomarker.

NO12 | Developing a Mouse Brain Organoid Model of Glioma Progression

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Gliomas are common malignant brain tumors in adults that progress rapidly and have a poor prognosis. Once they progress from low to high grades, these tumors become highly heterogeneous and infiltrate deeply into the brain parenchyma, inevitably leading to death. Therefore, understanding the mechanisms involved in the progression of gliomas is fundamental. We studied glioma progression using a mouse model in which we overexpressed PDGF-B in E14.5 mouse embryos. Although our study highlighted a crucial role for the immune system during tumor progression, we demonstrated that gliomas can progress even in an immunocompromised environment. Therefore, the interaction between glioma and surrounding cell types, other than the immune system, also influences progression. To investigate this interaction, we needed a more accessible and manipulable model than the mouse model. A solution is to use mouse brain organoids as an in vitro platform to model glioma progression. In this work, we present a protocol for generating unguided mouse brain organoids adapted from the well-established protocol by Lancaster and Knoblich, which uses human pluripotent stem cells. We found that modifying a single step of the protocol ensures the correct differentiation of the organoids. To shorten the time required for the generation of mouse brain organoids, we attempted to differentiate neural precursor cells isolated from E14.5 mouse embryos. We found that the culture medium of these cells is critical to ensure the pluripotency suitable for the correct differentiation of mouse brain organoids. Our work contributes to the generation of unguided mouse brain organoids and lays the foundation for future work in which this model will be used to establish an in vitro glioma progression model.

NO13 | Cancer-neuronal cross talk in Glioblastoma: how neurons sustain tumor progression

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Glioblastoma (GBM) is an aggressive brain tumor that interacts with the surrounding microenvironment and brain cells. The communication between neurons and glioma cells plays a role in tumor growth and invasion. However, the specific mechanisms and contributions of brain cells in this interaction are still unclear. To this end, we developed an in vitro model of the neuro-tumoral unit where primary human GBM Stem-like Cell Lines (hGSCs) derived from different patient samples were co-cultured with murine primary neurons. We found that hGSCs displayed higher rate of proliferation after 7 days of co-culture, regardless of the tumor molecular subtype. To note, neuronal conditioned medium was effective in enhancing cancer cell proliferation, indicating the putative contribution of secreted factors. The enhancement of neuronal activity by KCl or Bicuculline/4-Aminopyridine further promoted hGSC proliferation. Since glutamate is one of the neurotransmitter released from neurons, we used Glutamate-Sensitive Fluorescent sensor (iGluSnFR) to study the ability of hGSCs to sense glutamate released by neurons. We found that hGSCs can detect neuron-released glutamate with kinetics similar to neuron-to-neuron synapses, despite no synaptic-like activity was detected in hGSCs co-cultured with neurons. Concurrently, we measured neuronal network activity using High Density-Multi Electro Array, and we observed increased firing rates in the presence of tumor cells, indicating that hGSCs promote network excitability. These data indicate a GBM-neurons vicious cycle in which neurons boost hGSCs proliferation and cancer cells trigger neuron hyperexcitability. Given the capacity of hGSCs to sense glutamate released by neurons, our data suggest a possible role played by glutamate in such a cycle. These results provide a suitable tool for investigating if and how standard treatments influence the formation and maintenance of neuro-tumoral unit.

NIM05 | RUBIK: a fluorescent reporter for combinatorial Cre and Flp recombination

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Current technologies for precise genetic manipulation of neurons mostly rely on the use of site-specific recombinases (SSR) and CRISPR-Cas9 gene editing tool. Together, these systems are routinely used to generate conditional transgenic models for studying gene functions within specific tissue and/or at specific time points, both *in vivo* and *in vitro*. In this context, the use of drug-inducible SSR is a widely diffused approach to obtain fine spatiotemporal control of specific gene expression. Here we present RUBIK, a fluorescent tool specifically tailored for the reporting of the combinatorial action of Cre and Flp recombinases depending on the spatiotemporal sequence of recombination events. To test this reporter, we generated and isolated a stable knock-in HeLa cell line using the CRISPR-Cas9 technology and single cell-sorting. Our preliminary data show proper functioning of our system upon recombination with both Cre and Flp recombinases alone or in combination. Additionally, we optimized a variant of the Trimethoprim (TMP)-inducible Flp recombinase (FlpDD) designed to decrease the activity of the recombinase without TMP. Overall, we were able to set up a system that could be exploited in the field of neuroscience by the generation of knock-in mice expressing RUBIK. These transgenic mice could be resourceful to study and precisely define the roles of specific neuronal populations depending on the activity of Cre and Flp.

NIM06 | Gradient of Dentate-Thalamo-Cortical Tract Microstructural Disruption: Applying Diffusion MRI Profilometry in Friedreich Ataxia

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The dentate-thalamo-cortical tract (DTT) is the main cerebellar efferent pathway. Microstructural changes of the DTT are considered core features of Friedreich ataxia (FRDA). Nonetheless, whether some areas of the DTT are more impacted than others, possibly determining a gradient of disruption, is still a matter a debate. This study aimed to investigate microstructural integrity along the DTT in FRDA using a profilometry diffusion MRI (dMRI) approach.

MRI data from 45 FRDA patients (mean age: 33.2±13.2, M/F:26/19) and 37 healthy controls (HCs; mean age: 36.5±12.7, M/F:18/19) were included in this cross-sectional multicenter study. A profilometry analysis was performed on dMRI data by first using tractography to define the DTT as the white matter tract connecting the dentate nuclei to the contralateral motor cortex. The tract was then divided into 100 segments and diffusion tensor metrics of microstructural integrity (fractional anisotropy [FA], mean diffusivity [MD], and radial diffusivity [RD]) were extracted at each segment and compared between FRDA and HC groups. The process was replicated on the arcuate fasciculus for comparison.

The profilometry analysis indicated that, across all diffusion metrics, the region of the DTT connecting the dentate nucleus and thalamus was more impacted in FRDA than downstream cerebral sections from the thalamus to the cortex. The arcuate fasciculus was minimally impacted.

Our study further expands the current knowledge about brain involvement in FRDA, showing that the presence of microstructural abnormalities within the main cerebellar-cerebral tract are weighted to early segments of the tract (i.e., the superior cerebellar peduncle). These findings are in line with the hypothesis of the DTT undergoing anterograde degeneration arising from the dentate nuclei and progressing to the primary motor cortex.

ND28 | Investigating the role of large microglial extracellular vesicles carrying pathogenic misfolded proteins in Alzheimer's disease and their interaction with neurons

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Extracellular vesicles (EVs) are lipid-encased nanoparticles that convey bioactive signals from a donor to specific target cells, influencing their functions, and can transfer misfolded pathological proteins. Our previous work demonstrated that large (>200 nm) microglial EVs carrying Aβ (Aβ-EVs) are more prone to motility and move faster on neuronal surface (optical manipulation) and can propagate synaptic dysfunction in the mouse brain compared to EVs from non-treated microglia (Ctr-EVs). Here we investigated the effects of Tau on microglia and the interaction of large EVs derived from tau-treated microglia (Tau-EVs) and neurons. Microglial primary culture has been exposed to recombinant tau protein (200nM o/n) and EVs have been isolated from the cell supernatant by differential centrifugation after ATP stimulation. Analyses of EV production and size distribution by Tunable Resistive Pulse Sensing (TRPS) technique didn't highlight any difference between Tau-EVs and Ctr-EVs. Tau-EVs ability to interact and move at the surface of axonal projections has been tested *in vitro* by optical manipulation, finding no differences compared to Ctr-EVs. However, calcium imaging experiments showed that Tau-EVs increase basal calcium levels in neurons, and stereotaxic injection of Tau-EVs but not Ctr-EVs caused LTP impairment at the enthorinal cortex and in its target region, the dentate gyrus of the hippocampus, suggesting the presence of detrimental EV cargoes. We are currently investigating whether the detrimental action of Tau-EVs on neurons and synaptic plasticity depends on tau or other pathogenic molecules. Our preliminary data indicate decreased proliferation (EdU^+ cells %Ctr=26.71%; $\%_{Tau}$=1.99%) and decreased expression of activation markers (CLEC7A, CD11c) in tau-treated microglia, suggesting that tau can drive a microglial senescent phenotype. Further experiments are needed to clarify whether tau-treated microglia may sort ageing/senescent signals into EVs.

ND29 | The interplay between Rab proteins and mitochondrial dysfunction in PD pathology

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Mitochondria undergo processes of fusion, fission and selective degradation via mitophagy, collectively referred as mitochondrial dynamics. Alterations in the fine tuning of these processes negatively impact on neuronal cells, which are characterised by a high energetic demand. In fact, dysfunctional mitochondrial dynamics have been widely associated to neurodegenerative diseases, like Parkinson's disease (PD). Mutations in mitophagy-related *PRKN* gene, which encodes the E3 ubiquitin ligase Parkin, have been linked to autosomal recessive juvenile PD. Recently, also genetic/functional alterations of Rab proteins, involved in vesicles trafficking in the endosomal-lysosomal pathway, have been implicated in PD pathogenesis. In order to investigate the interplay between Rab proteins and dysfunctional mitophagy in the context of PD, we evaluated levels and sub-cellular localization of a subset of Rab proteins in different models: 1) human neuroblastoma SH-SY5Y cells treated with dopamine (DA), to recapitulate the impaired DA homeostasis of early PD stages, 2) SH-SY5Y cells after carbonyl cyanide 3-chlorophenylhydrazone (CCCP) treatment, as a positive control of mitophagy induction, and 3) patient-derived primary skin fibroblasts carrying *PRKN* mutations. After CCCP-induced mitophagy, Rab5 levels were increased, while in DA-induced impaired mitophagy, Rab5 and Rab7 proteins were up-regulated. In *PRKN*-mutated fibroblasts, Rab7 and Rab11 protein levels were increased with respect to controls. Moreover, preliminary data suggest Rabs colocalization with the mitochondrial network. Thus, our results suggest that Rab proteins may be an interesting molecular target to unveil early pathogenetic events related to mitochondria dynamics in PD.

ND30 | Developing a localised GDNF gene therapy to treat neurodegenerative diseases

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GDNF (glial cell line-derived neurotrophic factor) is a neurotropic factor produced and secreted by glial cells and neurons in the brain, where it has been found to exert potent neuroprotective effects. Based on these data, GDNF has been employed as a drug candidate for the treatment of Parkinson's disease (PD), a progressive neurodegenerative disorder characterized by loss of dopaminergic neurons. However, while promising results were observed in preclinical studies, GDNF-based therapies failed in patients. To fully explore the therapeutic potential of GDNF, we plan to employ integrating lentiviral vectors (LVs) to achieve its prolonged expression in the *substantia nigra (s. nigra)*. At variance with previous studies, we plan to perform the *in vivo* testing in a recently developed genetic animal model of Juvenile PD that resembles most of the features of the human disease. In particular, we will engineer a bicistronic LV containing the GDNF sequence and a reporter gene (GFP) and we will inject it directly in *s. nigra* of mice before the symptoms onset. With the aim to identify an ideal vector design, we performed *in vitro* experiments using the two different isoforms of GDNF (alpha and beta) and investigated possible differences in their expression levels and secretion efficiency. In parallel, to investigate the ability of the LV to reach the target area and express the transgene *in vivo,* we injected wild type animals with a control, GFP-expressing, LV. Confocal microscopy confirmed local transduction of neurons in the *s. nigra* but revealed poor transduction of the dopaminergic population. Stereological counting highlighted potential toxicity of the vector, likely related to the dose employed. Further studies are now ongoing to better characterise this toxicity and to understand whether GDNF production by neurons and/or glial cells in the *s. nigra* might prevent symptoms onset in the mouse model of PD.

ND31 | Analysis of astrocyte calcium activity in alpha7 nicotinic receptor KO Alzheimer's disease mouse model

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Alzheimer´s disease (AD) is a neurodegenerative condition which affects more than 40 million people worldwide, as reported by the World Health Organization. AD patients develop cognitive decline and learning issues. Two important hallmarks for AD diagnosis are i) the presence of amyloid plaques, formed by aggregation of amyloid beta (Aβ) peptides and ii) neurofibrillary tangles, characterized by hyper-phosphorylation of microtubule associated protein tau. It is widely believed that accumulation of Aβ peptides triggers the disease arise. Indeed, it was shown that Aβ oligomers are prone to aggregation which induces cell death and inflammation. Furthermore, Aβ oligomers are able to bind the alpha7 nicotinic receptor (A7-nAChR) inducing glutamate release at the pre-synaptic terminal. With the progression of AD disease and the increasing levels of Aβ, the complex A7-nAChR/Aβ gets internalized. This reduces the amount of glutamate released, contributing to a loss of synaptic plasticity. Several studies demonstrate that A7-nAChR is expressed by several cells types in the central nervous system, including astrocytes and microglia. In astrocytes, the activation of A7-nAChR by Aβ oligomers triggers Ca^{2+} elevation and glutamate release. In this project we aim to investigate the role of astrocytic A7-nAChR in the AD pathophysiology. By employing Ca^{2+} sensitive fluorescent probes, such as GCaMP6f, we are characterizing astrocytic spontaneous and evoked Ca^{2+} signal in hippocampal slices from young and aged mice. The same approach will be used in hippocampal slices of A7-nAChR KO AD mouse model to investigate the possible changes of astrocyte Ca^{2+} activity and the influence of astrocytic A7-nAChR on AD progression. Furthermore, we plan to analyse how astrocytic Ca^{2+} events change in presence of pathological levels of Aβ1-42 oligomers, to possibly find a new therapeutic target to treat AD.

ND32 | The UPR response and ER stress in a mouse model of Alzheimer's disease obtained by intracerebroventricular injection of β-amyloid oligomers at different ages

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Alzheimer's disease (AD) is emerging as the most prevalent and socially disruptive illness of aging populations. The major organelle involved in protein folding and quality control, the endoplasmic reticulum (ER), is dramatically affected in AD neurons. The abnormal levels of misfolded proteins at the ER engage the unfolded protein response (UPR) that in turn activates a quick response to restore proteostasis. The present work aims to study the role of UPR an ER stress in AD by using an integrated approach of behavioral test, bioinformatics and biomolecular analysis in a murine model of AD induced. Murine model (mice C57BL76) of AD was obtained by intracerebroventricular injection of β-amyloid oligomers ($A\beta_{1-42}$) at different ages, 3 and 18 months. After 10 days, mice underwent behavioral assessment and then were sacrificed to collect hippocampal sample. RNA sequencing was carried out. The expression of each gene was assessed for different age and treated and not treated mice (3 Aβ1-42/18 Aβ1-42, 3/18 Sham, 18 Sham/18 Aβ1-42, 3 Sham/18 Aβ1-42) by their log2 fold change (log2FC) from the basal-state to investigate the UPR, oxidative stress, inflammation and cell death on hippocampal samples. Our data showed as the impairment induced by Aβ1-42 injection worsen in aging, underlying the involvement of UPR and inflammatory response. RNA-seq data highlighted the presence of 125 common genes between all group, 47 of them involved in important pathways as "Neurodegenerative disorder" and "Inflammation mediated by chemokine and cytokine signaling pathway". The injection of $A\beta_{1-42}$ in mice of different age turn on cellular pathways involved in the cellular response to stress and in the regulation of cellular death. Among them, the involvement of the ER stress and the UPR seem to play a role not only in relation to the presence of Aβ1-42 oligomers but also to the aging process. These results are preliminary and point out the close relation between ER stress,AD and aging process.

ND33 | The role of astrocytic Ca2+ dynamics in Alzheimer's disease associated neuroinflammation

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Alzheimer's Disease (AD), a progressive neurodegenerative disease, is the leading cause of dementia worldwide. Histopathological hallmarks of AD include brain region-specific extracellular deposition of Amyloid-β (Aβ) plaques and intracellular Tau neurofibril aggregation, accompanied by neuronal loss. Current treatments for AD, focussed mainly on addressing Aβ plaques, have had limited success at controlling the cognitive decline associated with the disease. Compared to plaque deposition, neuroinflammation in the brain is known to better correspond with AD-related cognitive decline, and understanding inflammatory mechanisms in AD could reveal potentially more effective therapeutic targets. While microglia are the main initiators of inflammatory responses in the central nervous system, the active role of astrocytes in propagating and sustaining the response has been elaborated in recent years. Astrocytes possess several receptors that respond to extracellular stimuli, including neurotransmitters and inflammatory molecules, causing elevations in cytosolic levels of calcium ion (Ca^{2+}). Intracellular Ca^{2+} responses are an important secondary signal in the physiological function of astrocytes. In this project, we analyse the changes in astrocytic Ca^{2+} handling during Alzheimer's disease-linked neuroinflammation and the associated functional changes of astrocytes which might promote neurodegeneration. We investigate the role of these Ca^{2+} responses in inducing and amplifying inflammatory responses of astrocytes, including the release of inflammatory cytokines. Further, we investigate the involvement of astrocytic mitochondria in these changes, since they localize to the regions of Ca^{2+} elevation and, by actively taking up or releasing Ca^{2+}, can tune the cytosolic Ca^{2+} signal. We assess whether manipulating mitochondrial Ca^{2+} dynamics can modulate the neuroinflammatory markers of astrocytes in AD.

ND34 | Modeling Tauopathy-associated neurodegeneration in human iPSC-derived 2D and 3D retinal cultures

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Tau is a crucial protein that plays a significant role in maintaining neuronal structure and function. During retinogenesis, tau is involved in the development and maturation of retinal tissue. Previous studies have identified pathological hallmarks of tauopathies, such as Frontotemporal dementia (FTD), in the retina. However, the precise molecular mechanisms and the sequence of events underlying this process remain poorly understood. In this study, we investigated the changes associated with tau during retinogenesis using a human induced pluripotent stem cell (iPSC) line harboring an intronic IVS10+16 tau mutation, associated with FTD. By utilizing both 2D and 3D retinal models derived from tau-mutant iPSCs and their isogenic controls, we examined the spatiotemporal development of retinal progenitor cells and post-mitotic neurons, considering the role of tau in regulating the growth and differentiation of retinal neurons. Through gene expression and immunofluorescence analysis, we observed that mutant cultures exhibited delayed neurogenesis which disrupted the balance between the proliferation and differentiation of neuronal progenitor cells. Furthermore, we confirmed an early and increased expression of 4R tau isoforms in the mutant cultures, leading to an altered 4R/3R isoform ratio. Since tau is known to promote neuronal survival and prevent apoptosis, we also monitored the pathological hallmarks of tau throughout the differentiation process, and we identified phosphorylated site during the early stage of IVS10+16 retinal differentiation. Additionally, we investigated the formation of functional neural circuits and observed that the IVS10+16 tau mutation deeply affects synaptic maturation, influencing the organization of pre and postsynaptic components. Moreover, functional analysis in 2D retinal cultures showed that tau mutation impaired neuronal activity reducing both the frequency and synchronicity of Ca2+ oscillations.

ND35 | Identification of Sex-specific autophagy enhancers for dementia

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Aging is a process characterized by the accumulation of biological changes leading to the functional decline of the organism over time. This process leads to the accumulation of misfolded proteins, such as beta amyloid and alpha synuclein, which are believed to underly the onset of neurodegenerative disorders and dementia.
Nearly all therapy for dementia has been designed based on a “one-size-fits-all” approach; this has penalized women. Most of the treatments for brain disorders come from pre-clinical evidence in males, although women are more vulnerable than men to Alzheimer’s disease (AD) (Alzheimer’s Association Report, 2021), which is the first cause of age-related dementia. Sex-differences regulate genes expression and responses to treatments, including behavioral ones, such as exercise training which is more effective in rescuing cognitive decline in women than men. This makes urgent the need to develop sex-specific treatments for AD.
Using a recent mouse model of early aging and a genetic animal model of AD we found that autophagy enhancers, which clear protein aggregates responsible for neuronal death and dementia, had completely different molecular and behavioural effects in male and female rodents. Our preliminary data, suggest that these sex-specific effects were due to sex-differences on the transcription factor EB (TFEB) expression, a master gene regulator of lysosomal/autophagy pathways. We are currently investigating on sex-specific treatments capable to induce autophagy to identify drugs that can be directly used to slow-down AD in women, filling a major knowledge and treatment gap in gender medicine for AD.

ND36 | A novel neural stem cell therapy targeting upper motor neurons provides better outcomes in Amyotrophic Lateral Sclerosis mice models

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that leads to muscle atrophy and paralysis due to progressive degeneration of both lower (LMNs) and upper motor neurons (UMNs), with no effective therapy nowadays. Even if most studies have mainly focused on LMNs, it is clear that also UMNs are equally involved in the pathogenesis of the disease and could be a target for future therapy to protect the motor cortex as well as extend the possible positive effect on the whole MN circuitry. Here, we investigate the mechanisms both at molecular and phenotypic levels of a novel therapeutic approach based on the transplant of human neural stem cells (NSCs) from cerebral organoids within the central nervous system of a murine ALS model, SOD1G93A, through intracerebroventricular (ICV) or cortex administration. Behavioral and *ex-vivo* tests were performed to assess the efficacy of the treatment and showed improved outcomes in cortex-treated animals compared to ICV-treated and control mice. Thus, to predict the mechanism responsible for the amelioration of the phenotype, bulk-sequencing was performed on total RNA extracted from cortical motor area sections of cortex-treated and untreated animals, and the sequencing information was subdivided into human-derived and mouse-derived genes. The improvements observed in the different phenotypic analyses prompted the selection of some genes, among those that emerged from RNA-seq analysis, to be evaluated *in vitro* in 2D cultures as human proteins likely produced by engrafted NSCs and responsible for the rescued pathological phenotypes.

ND37 | Corroboration of Stathmin-2 in human and murine models of Spinal Muscular Atrophy as potential therapy target

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Spinal Muscular Atrophy (SMA), one of the most common infantile inherited neurological diseases characterized by impairing function and survival of lower motor neurons (LMNs), is caused by mutations in the *Survival Motor Neuron 1* gene (*SMN1*). The recently approved SMA therapeutic approaches are focused on increasing the levels of full-length SMN protein. However, some critical issues still remain and research aims to identify novel SMN-independent targets as future complementary strategies, to improve the therapeutic opportunities for all SMA treated patients. To develop this complementary approach, one possibility is to identify downstream genes responsible for selective motoneurons (MNs) dysfunction. Recent studies showed how STATHMIN-2 (STMN2), a protein involved in neurite outgrowth and axonal regeneration, is dysregulated in different neurodegenerative disorders and that its overexpression rescues axonal defects *in vitro* Amyotrophic Lateral Sclerosis (ALS) models. Remarkably, our group also observed STMN2 deregulation in SMA mice spinal cord and in human SMA MNs, suggesting a potential involvement of STMN2 in SMA pathogenesis and hinting at STMN2 as a new therapeutic target. In this study, we investigate for the first time the therapeutic impact of STMN2 modulation on *in vitro* and *in vivo* SMA models. Interestingly, the overexpression of STMN2 ameliorated the typical pathological features in SMA MNs, especially the axonal integrity and complexity. Similarly, in severe SMAΔ7 mouse models the treatment with AAV9 encoding *Stmn2* improved motor phenotype and histologic features related to muscle and neuromuscular junction (NMJ) alteration. Therefore, the capability of STMN2 to ameliorate the effects of reduced SMN levels on axon growth supports the view that axon biology is crucial for SMA pathogenesis and that the modulation of axon stabilizing proteins can modify the disease phenotype. Overall, our data provide evidence that STMN2 may act as a protective modifier of SMA.

ND38 | The Role of Intracellular Calcium in GBA2-Linked Hereditary Spastic Paraplegia

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Calcium (Ca^{2+}) is a key second messenger involved many cellular processes. The interplay between endo-lysosomal system, mitochondria and the endoplasmic reticulum (ER) is important in the regulation of intracellular Ca^{2+} homeostasis. Alteration of Ca^{2+} concentration and signalling impact on neuronal function and survival and it can play a key role in the onset and progression of several neurodegenerative diseases. A recent study demonstrated that Ca^{2+} signalling is altered also in forms of Hereditary Spastic Paraplegia (HSP). HSP refers to heterogeneous group of inherited disorders characterized by limb weakness and spasticity. Severe forms of HSP may include also ataxia, epilepsy, cognitive impairment, peripheral neuropathy. To date, mutations in more than 80 genes have been linked to the pathogenesis of HSP. Among them, mutations in the *GBA2* gene cause a particular form of HSP with ataxia. *GBA2* gene encodes the non-lysosomal β-glucosylceramidase 2 (GBA2), a membrane associated protein located in the cytoplasmic surface of the ER and the Golgi, which hydrolyzes the glucosylceramide (GlcCer) to glucose and ceramide. GBA2 mutations cause the loss of function of the enzyme with alterations in the cellular lipid environment. In the present work GBA2-inhibited mice cerebellar neurons are used to model HSP in order to investigate the impact of GBA2 reduced activity on neuronal function. After stimulation with non-toxic glutamate concentration (10µM), intracellular Ca^{2+} levels were increased compared to control neurons. Changes in the expression of mitochondrial and lysosomal markers like TOM20, LC3 and cathepsin D, evaluated by western blot, and live cell imaging experiments investigating mitochondrial function and lysosomal proteolytic activity, suggest the involvement of mitochondrial and lysosomal defects in Ca^{2+} dysregulation. Further studies will be needed to define the molecular mechanisms by which GBA2 dysfunction impact on these organelles.

ND39 | An in vivo protocol for screening and readily assessable neuro-behavioral investigation in early CRISPR-Cas9 zebrafish mutants of a rare neurodegenerative condition

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Morphological and behavioral assessments relevant to mechanism understanding and pre-clinical studies of human encephalopathies with childhood neurodegeneration can be performed in zebrafish starting from embryonic stages. Many *pan-embryo* techniques are being established requiring minimal manipulation and feasible within a short developmental time window and experimental period, which minimizes animals' distress. For instance, effective mutant generation already in G0 (*i.e.* CRISPR-Cas) and soft imaging modalities, which do not require surgery or disrupt animal physiology have advanced particularly for zebrafish lately and are suitable to study delicate neurodevelopmental processes affected by genetic diseases. Answering the need of an equally gentle genotyping method to improve animal welfare and select readily assessable genetic models, here we show our recent advancement in minimally invasive single live embryo genotyping as early as 24 hours-post-fertilization. We demonstrate that, compared to untreated fish, the technique established does not artificially impair embryo survival, long-term fitness and normal locomotor behavioral traits even in a delicate model of motoneuron disease and is compatible with early embryo genotype-phenotype screening. We apply the method to conduct and correlate a detailed morpho-anatomical and behavioral investigation in CRISPR-Cas9 loss of function models established in the lab of a recently identified neurodevelopmental disease with early-onset neurodegeneration. The genotyping method combined with multi-stage behavioral assessment from early embryos to adults allowed us to follow and describe the degenerative stages and progressive loss of motor abilities in homozygous mutants and to map the neurological alterations underlying the behavioral decay.

ND40 | Emerging value of olfactory neuronal Prokineticin-2 as novel target in Parkinson's disease

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Prokineticin 2 (PK2) is an inducible chemokine that is overexpressed in response to pathological perturbations to promote neuroprotective or neurotoxic responses. Remarkable neuroprotective effects have been observed in animal models of Parkinson's disease (PD). Specifically, PK2 increases in dopaminergic nigral cells in early stages of neurodegeneration or immediately after toxic exposure, triggering positive bioenergetic and anti-inflammatory cascades. Although a preliminary study measured higher levels of PK2 in the blood and substantia nigra of PD patients, the actual contribution of PK2 to PD in humans remains to be elucidated. Because PK2 signaling is also critical for the proper development and survival of the olfactory system, which is affected early and has the specific neuropathological features of PD, we examined the mRNA and protein expression of PK2 and its receptors (PKRs) in the olfactory neurons (ONs) of 38 PD patients at different stages of disease and 26 healthy controls (CTRLs). PK2 protein expression was also correlated with the expression of different α-synuclein species (total and oligomeric) and with the clinical parameters of the patients. We found that PK2 expression was significantly increased in the ONs of PD patients compared with CTRLs. PK2 expression was higher in early disease stages, proportional to motor severity, and associated with accumulation of pathological α-synuclein forms. Conversely, PKR1 and PKR2 expression levels remained unchanged, suggesting that PK2 increase serves as a mediator and does not compensate for loss of receptors due to neurodegeneration and cell depletion. In later stages of disease, in patients receiving dopaminergic therapy, PK2 expression instead decreased and did not correlate with key clinical features. These data, consistent with preclinical findings, support PK2 as a potential target in the early stages of PD and confirm the reliability of olfactory neurons in reflecting PD pathological changes.

EBN17 | Human iPSC derived micropatterning models as a new in vitro system for GNAO1 disease modelling and drug testing

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GNAO1 encephalopathy is a very rare genetic disease caused by different mutations in GNAO1 gene which encodes for the α subunit of a heterotrimeric guanine nucleotide-binding protein (Go), the most abundant membrane protein in the mammalian nervous system. GNAO1 plays pivotal roles both in adult brain function and during neurodevelopment and children affected by this pathology show different combinations of clinical symptoms, including developmental delay, hypotonia, epilepsy and movement disorder. Go protein is known to be a transducer of Wnt, a morphogen that plays crucial role in embryonic pattern and neural development, however it is still not clear if and how mutations in this gene affects early stage of development. Thus the aim of this study is to highlight defects that occur during embryogenesis taking advantage of micropattering models of gastrulation, also called gastruloids, in which colony confinement and BMP4 stimulation are sufficient to induce the *in vitro* formation of three germ layers with a spatial organization that resemble the embryonic pattern *in vivo* (Warmflash et al, 2018). Thus we have generated gastruloids starting from a collection of GNAO1-mutated iPSCs compared with their isogenic controls, in order to reproduce the complex clinical scenario of this syndrome. Performing imaging analysis and convolutional neural network, gastruloids provide the chance to analyse how alterations of Goα activity affect early phase of development and whether there is a correct fate specification and spatial arrangement of the three germ layers. This strong and reliable system would also allow to perform high throughput drug screening in a fast and highly reproducible way.

EBN18 | Targeting adenosine A2A receptor in FXS-patient derived human cortical organoids and cortical culture for animal free drug discovery and repositioning

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Fragile X Syndrome (FXS) is a heritable cognitive impairment caused by the expansion of CGG repeats in the FMR1 gene, resulting in the loss of fragile X mental retardation protein (FMRP). The absence of FMRP leads to excessive protein synthesis in dendrites and synapses, resulting in hyperactivity, autism, and seizures. Recent research has suggested a connection between FXS and the over-activation of metabotropic glutamate 5 receptor (mGlu5R)-mediated signaling, which is associated with adenosine A_{2A} receptors ($A_{2A}R$). However, the limited understanding of FXS pathophysiology and the differences between human and animal models have hindered the approval of specific pharmacotherapies. In our study, we aimed to characterize FXS using induced pluripotent stem cells (iPSCs) derived from patients. We generated 3D cortical brain organoids and 2D cortical cultures different iPSC line. These models exhibited neurodevelopmental alterations observed in FXS at the molecular, cellular, and functional levels. Specifically, we observed increased neuronal excitability in FXS neurons during maturation. To explore potential treatments, we investigated the effects of Istradefylline (KW6002), an $A_{2A}R$ antagonist used in Parkinson's treatment, in our model systems. Previous studies demonstrate promising results of KW6002 in ameliorating synaptic and cognitive abnormalities in Fmr1 knockout mice. Therefore, using iPSC-derived 2D and 3D cultures, we evaluated the impact of KW6002 treatment at the molecular, cellular, and functional levels. Our findings revealed that KW6002 treatment reduced spontaneous network activity, restoring normal neuronal excitability in FXS. Furthermore, the treatment influenced gene expression in FXS cultures, restoring the expression levels of differentiation-related genes comparable to the control group. These results highlight the potential of utilizing humanized models in the discovery of treatments for FXS and other neurodevelopmental disorders.

EBN19 | Pol III-related Leukodystrophy -affected patients present a profound transcriptional dysregulation and an impairment in protein synthesis

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Leukodystrophies (LDs) are genetic disorders which impact the white matter in the central nervous system, leading to a deficiency in myelin development. Mutations in RNA Polymerase III (PolIII) subunits cause POLR3-related LDs, such as those present in POLR3A, POLR3B and POLR1C genes. PolIII is involved in the transcription of small ribosomal units, tRNAs, 7SL RNA, U6 spliceosomal RNA, and more. tRNAs are implicated in translation, whilst small RNAs can perform regulatory functions on mRNA transcripts. The work hereby presented aimed to identify the transcriptional dysregulations present in primary fibroblasts of POLR3-mutated patients and to also assess alterations in the translation process. Fibroblasts were obtained from skin biopsies of 2 POLR3A and 1 POLR3B mutated patients and matched controls. RNA was extracted with Trizol reagent and Total RNA sequencing was performed with the CORALL Total RNA-Seq Library Prep Kit using Illumina NextSeq 500 Sequencing. Differential expression analysis was performed with DESeq.2 package and enrichment analyses were performed on differentially expressed genes (DEGs). Click-iT Protein Labeling approach was used to analyze newly synthesized proteins. Trascriptomic profiling highlighted a strong dysregulation in POLR3 patients, identifying 418 DEGs when comparing LDs patients to controls. Moreover, when comparing each patient to its matched control, a personalized DEGs signature was also observed, suggesting caution when grouping patients harboring different mutations in the same gene. Moreover, nascent protein synthesis analysis highlighted a down-regulation in nascent protein synthesis, suggesting an impaired translation possibly due to alterations in tRNA metabolism. In conclusion, our results highlight a profound impact on transcription and translation processes, in patients' specific primary cells used as pre-clinical experimental model of the disease.

EBN20 | A loss of function zebrafish model of a new disease gene involved in cargo sorting and autophagy recapitulates patients' axonopathy and cerebral atrophy and provides insights into disease mechanism

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The endosomal sorting complex for transport (ESCRT) machinery includes many subunits belonging to the complex I-III essential for multivesicular body cargo sorting and transport of ubiquitinylated transmembrane proteins to lysosomes for degradation, participating in autophagy. Manifesting the importance of these complexes in neurodevelopment, a number of knockout mouse models of various genes belonging to ESCRT complexes have demonstrated the causative link with several neurodevelopmental and neurodegenerative conditions, often associate to early embryo lethality. Here, we discuss the establishment of a transient zebrafish model of one of the subunits of the ESCRT complexes generated to model and study loss of function (LoF) mutations recently identified by whole exome sequencing in a new form of rare leukoencephalopathy with neurodevelopmental arrest, optic nerve and cerebral atrophy. Defects show varying severities in a court of patients collected internationally and harboring different gene variants. We show the early brain phenotyping of the zebrafish LoF model, established through a transient antisense oligonucleotide approach. The model recapitulates the global developmental delay, axonopathy and optic/cerebral atrophy observed in the disease, which is not rescued by the variants found in the patients, conversely to the wild-type form of the gene. The work allowed a quick validation of the pathogenicity of the candidate variants identified, useful for clinical purposes, such as disease identification and stratification. In addition, we show preliminary data on autophagy alteration and a first analysis of precursor cell behavior providing evidence that deficiency of this ESCRT subunit leads to impaired forebrain neurogenesis. In summary, our zebrafish disease model offers a tool to unveil mechanistic insights into a new leukoencephalopathy caused by a new disease genes leading to defective ESCRT function.

EBN21 | Novel Frontiers in Aicardi-Goutières Sydrome: Characterization of a RNU7-1 Mutation

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Aicardi-Goutières Syndrome (AGS) is a rare and genetically determined pediatric disorder majorly defined by chronic lymphocytosis and raised levels of type I interferon-alpha (IFN-α) in the absence of demonstrable brain infections. Considering genetic aspects, AGS is associated with mutations in 9 genes (*TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR, IFIH1, LSM11* and *RNU7-1*) which all encode for proteins involved in metabolism and nucleic acids uptake. Mutations in the *RNU7-1* gene (AGS9) lead to the least characterized form of the disease. This gene encodes for a small nucleolar RNA which is a member of the small nuclear ribonucleoprotein complex (U7 snRNP). It has been demonstrated that U7 snRNP is essential during the maturation of pre-mRNA of replication-dependent histones (RDH) as it leads to the cut of the Poly-A tail in these transcripts. The main aim of this work is to dissect the role of *RNU7-1* mutation in AGS pathogenesis. Specifically, we investigated canonical AGS features such as the upregulation of IFN-α, interferon-stimulated genes (ISGs) and specific outcomes of *RNU7-1* mutation in primary fibroblasts obtained from AGS patients and compared to healthy controls. Total RNA was extracted using TRIzol reagent and the genes' expression levels were determined by Real Time PCR. ELISA, Western Blot analysis and immunofluorescence were also performed to assess protein expression levels. Our results confirm the upregulation of ISGs and the typical "interferon signature" in AGS patients which cause an increase in IFN-α production. Moreover, we confirmed the modulation of this "interferon signature" through hydroxychloroquine treatment. Lastly, we assessed the increased presence of enriched Poly-A RDH transcripts confirming the role of U7 snRNP in the cleavage of Poly-A tail. In conclusion, our work describes the molecular mechanisms involved in AGS9 mutation which lead to the upregulation of ISGs, IFN-α overproduction, and misprocessing of RDH mRNAs.

EBN22 | Mutations in the stretch-activated ion channel TMEM63B associate with developmental and epileptic encephalopathies and progressive neurodegeneration

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By converting physical forces into electrical signals or triggering intracellular cascades, stretch-activated ion channels (SACs) allow the cell to respond to osmotic and mechanical stress. Knowledge of the pathophysiological mechanisms underlying associations of SACs with human disease is limited. Here we describe 17 unrelated patients, with severe early onset developmental and epileptic encephalopathy (DEE), intellectual disability, and severe motor and cortical visual impairment, associated with progressive neurodegenerative brain changes, carrying ten distinct heterozygous variants of *TMEM63B*, encoding for a highly conserved SAC. The variants occurred *de novo* in 16/17 patients for whom parental DNA was available and either missense, including the recurrent V44M in 7/17 patients, or in-frame, all affecting conserved residues located in transmembrane regions of the protein. In 12 patients, haematological abnormalities co-occurred, such as macrocytosis and haemolysis, requiring blood transfusions in some. We modelled six variants (V44M, R443H, T481N, G580S, R660T, and F697L), each affecting a distinct transmembrane domain of the channel, in transfected Neuro2a cells and demonstrated leak inward cation currents across the mutated channel even in isotonic conditions, while the response to hypo-osmotic challenge was impaired, as were the Ca^{2+} transients generated under hypo-osmotic stimulation. In conclusion, TMEM63B-associated DEE represents a novel clinicopathological entity in which altered cation conductivity results in a severe neurological phenotype with progressive brain damage and early onset epilepsy, associated with haematological abnormalities in most patients.

EBN23 | scRNA transcriptomics reveals a defective corticogenesis in a mouse model of ARX-DEE

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Developmental and Epileptic Encephalopathy (DEE) is a pediatric epilepsy characterized by abundant epileptiform activity resistant to traditional anti-epileptic therapies. A severe form of DEE, affecting only male children, is caused by expanded runs of consecutive GCN repeats in Aristaless-related homeobox (*ARX*) gene. This is an X-chromosome gene encoding a bifunctional transcription factor with a key role in mammalian corticogenesis. This *Arx* knockin mutant develops severe tonic-clonic seizures in a phenotype that recapitulates the chronic epilepsy associated to the c.304ins (GCG)7 mutation detected in DEE male patients. We examined the cellular diversity and the transcriptome landscapes of the epileptogenic neocortex in the *Arx* polyalanine mouse $Arx^{(GCG)7/Y}$ at the embryonic day 15.5 compared to the male control by a single-cell RNASeq approach (scRNASeq). scRNASeq data revealed an altered cell composition in the diseased neocortex with lower proportion of radial glia cells (RGCs) and higher proportion of immature neurons (INs). Analysis of differentially expressed genes (DEGs) involved in cell cycle and immunofluorescence studies upon *in vivo* BrdU pulse-chase assay confirmed that neurogenesis and corticogenesis are both damaged in $Arx^{(GCG)7/Y}$ developing cortex. Enrichment analysis showed altered pathways in RGC and IN populations implicated in chromatin remodelling and RNA metabolism, neuronal motility and structure, and synapse organization. Concerning the alterations in morphology-related genes, immunocytochemistry followed by morphometric analysis revealed a defective neurite arborization with hypoconnectivity in $Arx^{(GCG)7/Y}$ primary cortical neurons. Taken together, our scRNAseq and functional studies disclose a complex cell-type-specific dysregulation of cortical projections and neuronal morphology that potentially underlies DEE pathogenesis.

EBN24 | Epileptiform activity in a non-epileptic control rat: spontaneous syndrome or lesion-induced epilepsy?

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Epilepsy is a pathological condition characterized by recurrent seizures that affects around 50 million people worldwide. Based on human clinical data, we hypothesize that spontaneous pathology may occur even in animals. Here, we report the case of a non-epileptic control (NEC) rat that showed electrographic ictal and interictal patterns, similar to what was described in the animal model of epilepsy. While some cases of seizures were reported in NEC rats, interictal activity has never been described in non-epileptic rodents. We carried out a video-EEG analysis in NEC adult Sprague Dawley male rats as a control group of rats with temporal lobe epilepsy of a larger project. NEC rats were injected intraperitoneally with saline; 2 weeks later epidural electrodes in the frontal cortex and depth electrodes in the hippocampal areas were implanted. Video-EEG recordings were performed over a period of 14 weeks, and recordings were 24-h long to monitor the circadian cycle of the electrical brain activity. Brains were then extracted, sliced, and used for histological staining to verify the correct position of the electrodes and the presence of any damage due to technical problems during the surgery. While 3 NEC rats showed normal electrographic activity in all the recorded traces, 1 animal exhibited interictal and ictal activity starting from week 5 post-injection, often correlating to scratching or facial automatisms corresponding to stages 1 and 2 of the Racine scale. We present here a descriptive analysis of the ictal and interictal activities from this rat. According to our preliminary results, we have hypothesized that this rat may have spontaneous epilepsy; however, histological data must demonstrate that there is no damage due to a suboptimal electrode implantation.


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