Brayn conference

VERONA 2024

9 • 10 • 11 OCTOBER

BOOK OF ABSTRACTS

7th BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

Polo Didattico Zanotto, Università di Verona
Viale Università, 4 • 37129 Verona

www.braynconference.com

Dear Young Neuroscientists,

The BraYn Association and the BraYn Conference team warmly welcome you to the **7th Brainstorming Research Assembly for Young Neuroscientists**, the BraYn conference.

Inspired and organized by young researchers from various scientific backgrounds, the BraYn conference aims to foster brand-new collaborative connections between the future leaders of Neuroscience. The conference philosophy is simple: to **meet**, **connect**, **collaborate**, and **share**. We believe that cooperation among different research groups is essential to expanding our horizons and elevating the quality of our research.

We are thrilled to announce that we have received a record-breaking **234 abstracts** this year — an incredible achievement that reflects the growing interest and active participation within our community.

In line with our commitment to innovation, we are excited to introduce a new initiative: the **Speed Talks**. This dynamic format will allow participants to present their research in concise, focused sessions, providing a unique opportunity to engage with peers, exchange ideas, and receive rapid feedback.

As always, our core sessions will cover key areas such as **neurodegeneration**, **neuro-oncology**, **neuroinflammation**, and **neurophysiology & neural plasticity**. We've also expanded to include sessions on **neuroimaging**, **epilepsy, brain development & neurogenetics**. Furthermore, we've added a special focus on **clinical neuroscience**, addressing patient-related observations, the role of biomarkers, and emerging treatments for neurological diseases.

We look forward to seeing you at the **7th BraYn Conference** and to another year of vibrant discussions, inspiring connections, and groundbreaking research.

The BraYn Staff

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NEUROIMAGING & CLINICAL NEUROLOGY

Neuroimaging & Clinical Neuroloy is a comprehensive scientific session exploring the intersection of advanced neuroimaging techniques and clinical neuroloy applications. This session delves into the utilization of various neuroimaging methodologies to probe the structure, function, and physiology of the nervous system, alongside the translational aspects of clinical neuroloy. The session covers two primary neuroimaging approaches: structural imaging, which aids in the diagnosis of large-scale intracranial diseases and injuries, and functional imaging, crucial for diagnosing metabolic diseases like Alzheimer's and facilitating neurological and cognitive psychology research. Techniques such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Electroencephalography (EEG), and Positron Emission Tomography (PET) will be discussed in the context of their applications alone or in combination to investigate neurological diseases. Moreover, the session emphasizes the integration of neuroscience data and basic research with clinical neurology to enhance understanding and treatment of nervous system disorders. The session invites submissions showcasing translational significance and real-world clinical applications, focusing on patient-related observations derived from experimental research, clinical trials, and clinical cases. Special attention will be given to discussions on the potential role and use of biomarkers in clinical settings, as well as novel therapies for neurological diseases. Join us to explore the latest advancements in neuroimaging techniques and their pivotal role in shaping clinical neuroloy, bridging the gap between bench and bedside for improved patient outcomes..

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.

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.

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.

NEUROPHYSIOLOGY & NEURAL PLASTICITY

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.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

Epilepsy, Brain development & 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.

9 OCTOBER • Day 1

10:00 Registration

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

BRAYN STARTING GRANT SESSION

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

11:15 **Ludovica Lospinoso Severini** (Starting Grant 2023 Winner)
Defining the role of the oncofetal protein SALL4 in Hedgehog-dependent medulloblastoma.

11:30 Lectio Magistralis | **Martin Chalfie** (Chairman: M. Cambiaghi) • *GFP: Lighting up Life.*

12:30 Lunch Box with Poster Session 1

SESSION 1 • NEUROIMAGING & CLINICAL NEUROLOGY ORAL COMMUNICATIONS

Chairpersons: A. Bombaci, G. Borgonovo, S. Cocozza, S. Bosticardo

14:00 Teresa Giannattasio • *Assessing wireless implantable microbots using high-resolution 3D imaging techniques.*

14:15 Marco Micali • *PULSAR: an in silico model to predict ultrasound treatments in preclinical settings.*

14:30 Martina Greselin • *Instance-level explanations in multiple sclerosis lesion segmentation: a novel localized saliency map.*

14:45 BraYn Educational Symposium ▶ **Evident**
Luca Cevenini: *Transforming Precision Imaging: empower your microscopy imaging experiment with quantitative image data.*

15:00 SpeedTalk | Sara Cabras • *Role of 2-[18F]FDG-PET as a biomarker of upper motor neuron involvement in Amyotrophic Lateral Sclerosis.*

15:05 SpeedTalk | Martina Greselin • *Exploring the relationship between volume and microstructural changes in multiple sclerosis lesions using advanced quantitative MRI.*

15:10 SpeedTalk | Ilaria Gabusi • *MRI analysis of white matter in spastic ataxia: insights from the PROSPAX cohort.*

SESSION 2 • NEUROINFLAMMATION ORAL COMMUNICATIONS

Chairpersons: M. Bottero, S. Angiari, F. Caratis

15:15 Lecture | **Kiavash Movahedi** (Chairman: S. Angiari)
Macrophage-Brain Symbiosis in Health and Disease: From Basic Understanding to New Therapeutic Paradigms.

15:45 Elena Ellmeier • *Coenzyme A fueling with pantethine limits autoreactive T cell pathogenicity in experimental neuroinflammation.*

16:00 Sara Balletta • *Effects of anti-CD20 therapy on T lymphocyte-dependent synaptic excitotoxity in Multiple Sclerosis.*

16:15 BraYn Educational Symposium ▶ **Novartis**
Roberta Magliozzi: *The compartmentalized inflammatory response in multiple sclerosis: looking for biological biomarkers.*

16:45 BraYn Educational Symposium ▶ **Alexion**
Valentina Camera: *The innate immune system: the protective power of the complement system and its role in NMOSD and gMG.*

17:00 Alessandra Colamatteo • *Calorie restriction as a novel therapeutic tool to modulate immune system during multiple sclerosis.*

17:15 Giulia Borgonovo • *Modulation of the Nerve Growth Factor signaling impacts on microglial phenotype.*

17:30	SpeedTalk | **Anastasia Lechiara** • *Role of immune system and axonal damage: serological biomarkers in neurological sequelae post-SARS-CoV-2 infection.*
17:35	SpeedTalk | **Enrica Marzani** • *Pharmacological inhibition of CDK9 in sepsis-associated encephalopathy: impact on microvascular endothelial function.*
17:40	SpeedTalk | **Leen Ali** • *Emerging roles of brain border macrophages in brain homeostasis and disease.*
17:45	SpeedTalk | **Francesca Ciarpella** • *Neural stem cells in meninges interact with immune cells and are modulated during the progression of experimental autoimmune encephalomyelitis (EAE).*
17:50	SpeedTalk | **Eleonora Terrabuio** • *Tissue resident memory leukocytes alter neuronal functionality during neurodegenerative diseases.*
17:55	Closing remarks

10 OCTOBER • Day 2

SESSION 3 • NEURODEGENERATION
ORAL COMMUNICATIONS

Chairpersons: S. Amoretti, F. Anastasi, S. Tessitore

9:15	**Paola Pacifico** • *Epidermal Langerhans Cells promote Painful Diabetic Neuropathy through neuroimmune-mediated mechanisms.*
9:30	**Ayla Lievens** • *Microglia replacement as a therapeutic tool for neurological disorders.*
9:45	**Antonia Wenger** • *Myelin content in major white matter tracts is associated with clinical disability and serum neurofilaments in patients with Multiple Sclerosis.*
10:00	**Elena Fontana** • *Detection of TDP-43 seeding activity in the olfactory mucosa from patients with frontotemporal dementia.*
10:15	BraYn Educational Symposium ▶ **Revvity** **Reynald Herteaux**: *How accurate cell counting can ease your long and complex experiments.*
10:30	**Sebastiano Antonio Rizzo** • *Injectable glucose-releasing materials to rescue cells from oxygen-glucose deprivation and its implication for neurotransplantation: a novel approach to an old problem?*
10:45	**Fabiana Miraglia** • *Targeting the Enteric Nervous System to halt the progression of Parkinson's Disease*
11:00	**Enrico Frigerio** • *Stromal Vascular Fraction-laden hydrogel for the treatment of spinal cord injury.*
11:15	SpeedTalk | **Matteo Bordoni** • *Spinal cord organoids generation for the study of amyotrophic lateral sclerosis.*
11:20	SpeedTalk | **Nikolaos Vareltzakis** • *Alteration of circadian clock genes in brain-infiltrating leukocytes in Alzheimer's disease.*
11:25	SpeedTalk | **Federica Carrillo** • *Multi-omics approach in Parkinson's disease: a comprehensive study of TMEM175 mutations effect on lipid and metabolic pathway in PD patients at cellular and circulating level*
11:30	SpeedTalk | **Federica Anastasi** • *Plasma p-tau and Amyloid biomarkers discrimination accuracy of biologically-defined Alzheimer's disease in a memory clinic setting: a head-to-head study.*
11:35	SpeedTalk | **Barbara Parisi** • *APache: a novel neuronal autophagic marker regulating autophagosome retrograde trafficking.*
11:40	Lecture | **Bianca A. Silva** (Chairman: M. Cambiaghi) • *Brain circuits for memory update.*
12:10	**Domenico Arenella, Maria Chiara Bassi** (Chairman: G. Ferrara) ▶ *How to effectively communicate the results of our scientific research. Journals, citations, bibliometrics, open access and other strange things.*
13:00	Lunch Box with Poster Session 2

PARALLEL SESSION (10:45-12:15)
• For scheduled groups only •

BraYn Open Lab (Giovanni Ferrara, Roberta Magliozzi)
From lab to clinic: the role of neurofilaments as biomarkers in neurological diseases.

PARALLEL SESSION (14:30-16:30)
• For scheduled groups only •

BraYn meets Martin Chalfie

SESSION 4 • NEURO-ONCOLOGY
ORAL COMMUNICATIONS

Chairpersons: E. Vannini, E. Stanzani, J. Maqbool

14:15	Lecture | **Giorgio Seano** (Chairwoman: E. Stanzani) *Adaptive cell and microenvironment plasticity in residual post-surgery glioblastoma.*
14:45	**Marta Ibañez** • *Development of novel immunotherapeutic approaches for pediatric CNS tumors.*
15:00	**Eugenia Guida** • *BRAFV600E mutation and PTEN deletion in neural stem precursor cells give rise to glioma and neurofibromatosis.*
15:15	BraYn Educational Symposium ▶ **Euroclone** **Stephen Hague**: *Spatial Biology Advancement in Neuroscience*
15:30	**Chiara Riviera** • *Modeling glioma progression in mouse and human neural organoids.*
15:45	**Elisabetta Mori** • *Preclinical testing of a novel therapeutic approach to counteract Glioblastoma Multiforme.*
16:00	BraYn Educational Symposium ▶ **AIRC** **Laura Galbiati:** *AIRC funding opportunities for a career in cancer research.*
16:30	SpeedTalk | **Pablo Blanco Carlón** • *The Force Awakens: PIEZO1 as a novel oncogene in glioma.*
16:35	SpeedTalk | **Mariassunta De Luca** • *Plasma derived EVs of glioma-bearing mice contain promising biomarker for an early Glioblastoma diagnosis.*
16:40	SpeedTalk | **Noemi Marino** • *A pan-sigma receptors modulator as a novel therapeutic strategy to fight glioblastoma.*
16:45	SpeedTalk | **Elena Cerutti** • *Involvement of DNA repair in high-grade glioma recurrence: mechanistic insights into the nucleotide excision repair pathway in glioma stem cells.*
16:50	SpeedTalk | **Alice Reccagni** • *The role of hydrogen sulfide on glioblastoma growth: a gut-brain approach.*

16:55 Closing remarks

11 OCTOBER • Day 3

SESSION 5 • NEUROPHYSIOLOGY & NEURAL PLASTICITY
ORAL COMMUNICATIONS

Chairpersons: M. Cambiaghi, G. Sansevero, P. Lippiello, I. Di Marco

9:00 Janina Leonie Röckner • *Quantifying muscle activation in Octopus vulgaris stereotypical motion.*

9:15 BraYn Educational Symposium ▶ **Beckman Coulter**
Nunzio Iraci: *From cell-to-cell communication to nanomedicine the secret(ed) spread of extracellular vesicles.*

9:30 Alessandra La Terra • *Spinogenesis of cerebellar Purkinje cells is locally repressed in an activity-dependent way.*

9:45 Federica Marchiotto • *Transcranial direct current stimulation (tDCS) promotes myelin repair and plasticity in the mouse motor cortex.*

10:00 Lecture | **Christiaan Levelt** (Chairman: G. Sansevero)
The effect of visual experience on visual and auditory processing throughout the mouse visual cortex.

10:30 Lecture | **Alessandro Gozzi** (Chairwoman: F. Alvino) • *Brain map decoding via cross-species fMRI*

11:00 SpeedTalk | Marta Carè • *Advancing Stroke Rehabilitation: Personalized Neurostimulation Using Spiking Neural Networks.*

11:05 SpeedTalk | Carola Dolci • *Deciphering the interactions across different attentional control mechanisms during target selection: Insights from Behavioural and EEG Experiments.*

11:10 SpeedTalk | Ilaria Barone • *Tumor-associated macrophages-educated promote neuronal regeneration in vivo and in vitro.*

11:15 BraYn Educational Symposium ▶ **Miltenyi**
Luca Lorenzini: *Experimental colitis in young Tg2576 mice accelerates the onset of an AD-like clinical phenotype.*

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

12:00 Poster Session 3 and Lunch Box.

SESSION 6 • EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS
ORAL COMMUNICATIONS

Chairpersons: M. Rasile, E. Tagliatti, L. Fusar Bassini

14:00 Lecture | **Stephanie Schorge** (Chairwoman: E. Tagliatti)
Brain hacking with viruses: Bringing gene therapy for epilepsy out of science fiction and into clinical trials.

14:30 Stephana Carelli • *Ketogenic diet treatment in GLUT1-DS patients: identification of ion channel signaling deregulation related to both epigenetic changes and splicing events.*

14:45 Letizia Esposito • *Novel frontiers in Aicardi-Goutières syndrome: association between a RNU7-1 variant and histone dysfunctions.*

15:00 Alessio Balzerano • *A novel regulatory role of NBS1 at the primary cilium highlights impinges on cerebellar development and medulloblastoma insurgence.*

15:15 SpeedTalk | Mariam Marie Chellali • *Upregulation of Negr1 converges into core impaired processes in autism spectrum disorders.*

15:20 SpeedTalk | Francesca Ciarpella • *Brain organoid platform for discovering new therapeutic strategies to promote neural maturation in Allan-Herndon-Dudley Syndrome (AHDS).*

15:25 SpeedTalk | Martino Bonato • *Unraveling the roles of oligodendrocyte progenitor cells in the development of the cortical inhibitory system.*

15:30 SpeedTalk | Wenjie Liao • *Unveiling the molecular mechanism of intestinal metabolite para-cresol in modulating neuroinflammation and synaptic dysfunction: implications for autism spectrum disorder.*

15:35 SpeedTalk | Chiara Tesoriero • *SLC6A1 KO Zebrafish model: an innovative tool to identify new therapeutical approaches for myoclonic-astatic epilepsy.*

15:40 Closing remarks (Chairpersons: G. Ferrara, M. Romeo, N. Iraci, P. Infante, C. Calì, V. Chiurchiù)
BraYn Awards (Best Oral & Poster Presentation, BraYn Starting Grant, Creative BraYns)

SESSION 1 • NEUROIMAGING & CLINICAL NEUROLOGY

SESSION 2 • NEUROINFLAMMATION

SESSION 3 • NEURODEGENERATION

SESSION 4 • NEURO-ONCOLOGY

SESSION 5 • NEUROPHYSIOLOGY & NEURAL PLASTICITY

SESSION 6 • EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

POSTER SESSION 1 • October 9, 12:30-14:00

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

NEURODEGENERATION

NEUROINFLAMMATION

NEUROIMAGING & CLINICAL NEUROLOGY

NEURO-ONCOLOGY

NEUROPHYSIOLOGY & NEURAL PLASTICITY

POSTER SESSION 2 • October 10, 13:00-14:15

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

NEURODEGENERATION

NEUROINFLAMMATION

NEUROIMAGING & CLINICAL NEUROLOGY

NEURO-ONCOLOGY

NEUROPHYSIOLOGY & NEURAL PLASTICITY

POSTER SESSION 3 • October 11, 12:00-14:00

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

NEURODEGENERATION

NEUROINFLAMMATION

NEUROIMAGING & CLINICAL NEUROLOGY

NEURO-ONCOLOGY

NEUROPHYSIOLOGY & NEURAL PLASTICITY

COMMUNI ORAL CATIONS

Assessing wireless implantable microbots using high-resolution 3D imaging techniques

Teresa Giannattasio (1) - Michela Fratini (2) - Francesco Brun (3) - Luca Brombal (3) - Syed Bilal Nizami (1) - Valeria Palumbo (1) - Joao F. Ribeiro (4) - Flavia Franceschini (1) - Marco Micali (1) - Eugenia Guida (1) - Susanna Dolci (1) - Luca Berdondini (4) - Manuel Scimeca (1) - Alessandro Mauriello (1) - Allegra Conti (1) - Nicola Toschi (1)

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Brain pathologies exhibit high variability due to different brain regions affected and aberrant electrical activity in neural cells among individuals. The CROSSBRAIN EU project introduced wireless implantable devices called microbots (µBots, 100x100x100 μm^3) designed to allow highly precise modulation of brain regions with minimal invasiveness. These devices target specific spatiotemporal events to advance the neuromodulation field with tailored treatments. This study aims to compare various imaging techniques to identify the most accurate method for localizing µBots after implantation and studying their functionality. Experiments were conducted using non-functional *silicon (Si) dummy µBots*, which replicate the shape and size of actual µBots, implanted in ex-vivo adult wild-type mouse brains. To evaluate the positioning and integration of the dummies within the tissue, we compared standard microscopy, transmission electron microscopy (TEM), computed tomography (CT) and synchrotron radiation-based X-ray phase-contrast 3D virtual histology (XPCT). Our analysis revealed challenges due to the high density of dummies, which damaged tissue integrity during brain sectioning, thereby diminishing the efficacy of 2D-imaging methods such as standard microscopy and TEM. CT imaging effectively localized the dummies at the implantation sites but did not allow for analysis of the dummy's interaction with the brain tissue. XPCT provided detailed visualization of both the dummies and adjacent brain tissue, revealing differences in absorption indices and offering insights into the cellular and vascular structures of the brain. This study confirms that XPCT is a promising ex-vivo technique for precisely investigating the position and interactions of small brain implants within brain tissue, aiding the development of tailored neuromodulation therapies. This 3D technique is particularly useful for customized brain therapy technologies, especially for the latest neurodevices that are not compatible with MRI.

PULSAR: an in silico model to predict ultrasound treatments in preclinical settings

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Focused Ultrasound (FUS) combined with microbubbles (MBs) administration is an emerging non-invasive method for enhancing drug delivery across the blood-brain barrier (BBB). This technique is increasingly used in clinical trials for treating neurodegenerative diseases due to its safety and effectiveness. To deliver acoustic pressure (AP) in the brain and safely permeabilize the BBB, treatments are planned before FUS exposure using patients' computed tomography scans to correct cranial aberrations of the FUS beam. In animal experiments, the absorption of FUS by the skull varies among individuals, influenced by factors such as age, weight and specific skull regions. However, preclinical settings often lack optimization across various animals and brain regions before experiments start. In vivo experiments demonstrate varying FUS transmission across distinct skull regions, leading to non-uniform BBB permeabilization, variable molecular uptake and diverse tissue effects. We present PULSAR, an in-silico model designed to plan FUS experiments in rodents by predicting the AP transmitted through their skulls for different FUS settings. A simulated AP map was obtained using a skull model absorbing the FUS beam. Validation of the in-silico model included in vitro experiments using a FUS transducer and a hydrophone in water, sampling around the focal spot. Skull attenuation of the AP was assessed with a rat skull placed between the hydrophone and transducer. Our study confirms varying FUS attenuation across different rat skull regions, highlighting the need for a computational model to optimize FUS probe positioning and acoustic parameters. PULSAR can be used with a stereotactic system to adjust the probe's position to deliver the desired AP and safely treat the brain, thus enhancing FUS safety and improving drug delivery in the brain. This model represents an advancement in preclinical FUS research, aiding more accurate and effective treatments for neurodegenerative diseases.

Instance-level explanations in multiple sclerosis lesion segmentation: a novel localized saliency map

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Introduction. Explainability is expected to play a fundamental role in building trust between physicians and methods based on artificial intelligence. However, no methods can explain the segmentation of single instances, such as multiple sclerosis (MS) lesions.

Methods. We trained and tested a 3D U-Net for white matter lesion segmentation on 687 MS patients, with fluid attenuated inversion recovery (FLAIR) and magnetisation prepared rapid gradient-echo (MPRAGE) scans collected at the University Hospital of Basel, Switzerland. Pre-processing steps included co-registration, bias correction and normalization. We addressed instance-level explanations by aggregating saliency maps of voxels belonging to the lesion volume Ω. For a given lesion, the implementation consisted in: 1) injecting noise in the input to obtain 50 versions; 2) computing saliency maps corresponding to voxels in Ω and determining their voxel-wise maximum; 4) combining 50 versions to obtain a lesion-level explainable map. The relevance of perilesional tissue seen by the network was assessed on 3839 lesions, by: 1) masking out the input image to contain only the lesion 2) gradually including more surrounding voxels up to *25mm* from the lesion edges. At each iteration we observed the average and standard deviation across patients of the mean prediction score in Ω, and the number of segmented lesions.

Results. In explainable maps for FLAIR, positive values accumulate inside Ω, while negative values populate the perilesional tissue. For the MPRAGE, we observe a dual trend with negative values in Ω, and a lower magnitude. The final experiment shows that the prediction score for a lesion increases and plateaus after including perilesional tissue distant *12-15mm* from the lesion border.

Conclusion. We provided instance-level explanations in a semantic segmentation task. Experiments suggest that the network's predictions rely on lesion hyperintensity in FLAIR and, to a certain extent, the perilesional tissue.

Coenzyme A fueling with pantethine limits autoreactive T cell pathogenicity in experimental neuroinflammation

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Immune cell metabolism governs the outcome of immune responses and contributes to the development of autoimmunity by controlling lymphocyte pathogenic potential. In this study, we evaluated the metabolic profile of myelin-specific murine encephalitogenic T cells, to identify novel therapeutic targets for autoimmune neuroinflammation. By performing unbiased metabolomics analysis, we detected a potential break in the coenzyme A (CoA) synthesis pathway in actively-proliferating encephalitogenic T cells, compared to resting T cells. CoA fueling with the CoA precursor pantethine affected essential immune-related processes of autoreactive T cells, such as antigen-specific proliferation, cytokine production, and integrin-mediated cell adhesion, both *in vitro* and *in vivo*. Mechanistically, pantethine exerted its immunomodulatory effects in encephalitogenic T cells by linking metabolic reprogramming to alteration of intracellular signaling pathways. We then evaluated the impact of pantethine treatment on the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of human multiple sclerosis (MS). Our data show that pre-clinical treatment with pantethine inhibited EAE development in two different mouse strains. Importantly, pantethine also significantly ameliorated the disease course when administered after disease onset in a therapeutic setting. Finally, pantethine limited pro-inflammatory cytokine production by human T helper 1 (Th1) and Th17 cells *in vitro*, as well as by T cells from MS patients, confirming its translational potential. In conclusion, we demonstrated that CoA fueling with pantethine in pro-inflammatory and autoreactive T cells may represent a novel therapeutic approach for the treatment of autoimmune neuroinflammation.

Effects of anti-CD20 therapy on T lymphocyte-dependent synaptic excitotoxity in Multiple Sclerosis

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T and B lymphocytes play a crucial role in the pathogenesis of Multiple Sclerosis (MS). B lymphocytes activate autoreactive T-cells, driving cortical pathology and disease progression. Anti-CD20 monoclonal antibodies targeting B-cells have been approved for different MS phenotypes, but their beneficial effects remain unclear. Our recent study using a T-cell chimeric MS model demonstrated that peripheral T-cells from progressive MS (PMS) patients, probably through the involvement of the TNF, increase excitatory transmission in the striatum of control animals, replicating the synaptic damage observed in the MS mouse model. We hypothesize that anti-CD20 therapies, such as Ocrelizumab, could exert neuroprotective effects by modulating PMS T cell-driven synaptic abnormalities.
To test this, we collected human T-cells from healthy subjects (HS), PMS patients at diagnosis, and Ocrelizumab-treated PMS patients. These T-cells were incubated on murine cortico-striatal slices, and glutamatergic activity was recorded using the patch clamp technique. Transcranial magnetic stimulation (TMS) assessed intracortical excitatory and inhibitory transmission, and long-term potentiation (LTP)-like plasticity in HS and in PMS patients.
We found that, at least 2.5-years of treatment with Ocrelizumab reversed PMS T cell-induced glutamatergic alterations. Interestingly, after 5 years of Ocrelizumab treatment, PMS T cell-synaptotoxicity re-emerged, and was prevented by TNF antagonism. TMS evaluation showed an impairmed inhibitory intracortical transmission and no LTP-like plasticity in PMS patients at T0 compared to HS. Ocrelizumab-treated PMS patients significantly improved LTP-like plasticity.
Thus, Ocrelizumab, probably modulating the pro-synaptotoxic factor TNF, influences B and T lymphocyte interactions, counteracting T cell-mediated inflammatory synaptopathy, and reducing excitotoxic neurodegeneration in PMS.

Calorie restriction as a novel therapeutic tool to modulate immune system during multiple sclerosis

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There is a strong relationship between metabolic state and immune tolerance through a direct control exerted on immune cells by specific intracellular nutrient-energy sensors. An increased "metabolic work load" represents a novel issue linking metabolism with loss of self-immune tolerance. In this context, several dietary interventions have been shown to influence disease progression of experimental autoimmune encephalomyelitis (EAE), the experimental model of Multiple Sclerosis. Our approach aims at dissecting at the cellular level the mechanism of action of Caloric Restriction (CR) on disease progression, in relapsing remitting Multiple Sclerosis (RR-MS) subjects. Firstly, we evaluated whether CR impact on clinical progression (in terms of expanded disability status scale score (EDSS), number of relapses and lesions activity etc.) in RR-MS subjects. Moreover, we examine the impact of CR on nutritional status and on the immunophenotype of different circulating immune cells of RR-MS subjects. We also investigated the effect of different dietary regimens on the metabolic asset of conventional T (Tconv) cells (measurement of glycolysis and oxidative phosphorylation) and on the ability to induce tolerogenic regulatory T (Treg) cells from RR-MS subjects. We observed that CR positively impact on the clinical and nutritional status of RR-MS subjects and modulates the activation of different immune T cell subsets. Moreover, CR is able to reduce glycolytic capacity of pro-inflammatory T cells and promote peripheral conversion of inducible Treg cells compared to Free Diet (FD)-RR-MS subjects. Overall, these data suggest that modulation of metabolic state via calorie restriction is able to improve the outcome of RR-MS and efficacy of first line drug treatment.

Modulation of the Nerve Growth Factor signaling impacts on microglial phenotype

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The neurotrophin Nerve Growth Factor (NGF), known for its role in supporting neuronal survival and differentiation, exhibits pleiotropic effects on non-neuronal cells, particularly within the peripheral immune system. Related to this, our work identified potent immunomodulatory properties of NGF via Tropomyosin receptor kinase A (TrkA) on cultured microglia, as NGF directs these cells toward a neuroprotective phenotype. In this study, we present *in vivo* evidence supporting the neuroprotective role of NGF signaling in murine brain microglia. First, we explored the therapeutic potential of an NGF mutein, *human NGF painless* (hNGFp), in a neurodevelopmental disease, Rett syndrome (RTT). Using a non-invasive intranasal delivery in female MeCP2+/- mice, a model for RTT, we observed a reversal of microglial morphology deficits in treated animals and a restoration of altered expression of key neuroimmune-communication cytokines, such as Fractalkine, suggesting that hNGFp treatment can ameliorate symptoms in the MeCP2+/- model by exerting neuroprotection also through microglial modulation. Secondly, to directly assess the functional role of microglial NGF-TrkA signaling, we generated a novel inducible transgenic mouse [CX3CR1-CreERT/+:TrkA(fl/fl)] allowing specific TrkA deletion in microglia (cKO). Knocking out microglial NGF-TrkA signaling reduced microglial density and morphological complexity, and increased phagocytosis of excitatory synaptic puncta, selectively in the primary somatosensory cortex and hippocampus, also consequently altering memory behavior. Live imaging via two-photon microscopy revealed that branch motility in cKO microglia is increased in baseline (augmented surveillance) and not responsive to exogenous NGF. In conclusion, our data suggest that NGF-TrkA signaling can influence pivotal microglial activities, even in pathological conditions. Modulating the NGF-TrkA axis on microglia *in vivo* holds promise as a broad therapeutic neuroprotective strategy.

Epidermal Langerhans Cells promote Painful Diabetic Neuropathy through neuroimmune-mediated mechanisms

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The interplay between non-neuronal cells and nerve afferents in the epidermis has a prominent role in health and disease. Painful Diabetic Neuropathy (PDN), one of the most common and intractable complications of diabetes, is characterized by the remodeling of cutaneous innervation and neuropathic pain. Even though increasing evidence suggests an important role for epidermal non-neuronal cells, such as resident immune cells, in the development of PDN, the mechanisms underlying this neuropathy remain largely unknown. To investigate how epidermal cells communicate with cutaneous afferents and how this communication affects PDN, we have adopted an experimental approach that combines a mouse model of PDN and skin biopsies from PDN patients. We performed single-cell RNA sequencing (scRNA-seq) of the epidermis in mice fed a high-fat diet (HFD,42%fat) and a regular diet(RD), including both male and female subjects. Unsupervised clustering of scRNAseq data from HFD and RD mouse epidermis revealed several distinct clusters, including keratinocytes and Langerhans Cells (LCs), a population of resident antigen-presenting cells sharing a common ontogeny with macrophages and thus considered resident-macrophages. We observed a significant increase in LCs in the epidermis of HFD mice, suggesting their role in promoting neuronal excitability. We demonstrated that LCs are crucial in neuro-immune communication and may be involved in axonal degeneration/regeneration in PDN via semaphorin-plexin pathways. We have identified a panel of inflammatory molecules secreted by LCs and potentially linked to the onset and maintenance of PDN. We have also validated key targets through in-situ hybridization and immunohistochemistry. Our findings highlight the pivotal role of Langerhans cells in the development of PDN, revealing their functional association with sensory afferent neurons in the epidermis. The disrupted neuron-immune communication between LCs and cutaneous afferents may be responsible for the neuropathic pain in PDN and the remodeling of cutaneous innervation in both mice and PDN patients. Identifying LCs as disease-driving immune cells may pave the way for topical therapeutic treatment strategies for PDN, specifically targeting LCs in the epidermis, such as antigen-specific immunotherapies.

Microglia replacement as a therapeutic tool for neurological disorders

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Microglia are tissue-resident macrophages of the brain parenchyma. They are essential for regulating brain development and homeostasis and play a key role in regulating inflammation. Microglia are also implicated in many neurological disorders, making them interesting targets for therapeutic intervention. Microglia have an embryonal ontogeny and self-renew throughout life without any peripheral input. Replacing dysfunctional microglia with healthy counterparts or genetically engineered counterparts is a promising approach to treat brain disorders. Our aim is to develop an in vivo microglia-replacement approach as a foundation for future microglial-based cell therapies. We have created genetic and pharmacological mouse models in which we can deplete the endogenous microglia allowing for engraftment of progenitor cells. Additionally, we optimized multiple protocols to differentiate induced pluripotent stem cells (iPSC) into microglia progenitors. Our results show that microglia progenitors can successfully engraft as microglia-like cells in the brain after intracranial injection, however they remain distinguishable from endogenous embryonal microglia highlighting the importance of ontogeny. We continue to optimize iPSC differentiation protocols to generate microglia progenitors that after engraftment are more capable of resembling their embryonal counterparts and we further optimize transplantation strategies to enable more translatable and efficient engraftment. This work will lay the basis for a new treatment strategy in which microglia can be engineered and microglia replacement can be used to effectively treat neurological disorders.

Myelin content in major white matter tracts is associated with clinical disability and serum neurofilaments in patients with Multiple Sclerosis

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Myelin accelerates and enhances the electrical signal transmission in the central nervous system. Myelin Streamline Decomposition (MySD), a method that deconvolutes the myelin signal along white matter tracts using diffusion tractography, was extended to account for focal lesions (MySD-Lesion). We applied MySD-Lesion and selected two major fiber tracts, cortico-spinal tract (CST) and cingulum bundle (CB), to assess the relationship between their myelin content and both patients' disability and neuroaxonal damage in Multiple Sclerosis (MS) patients. We included 130 MS patients (77f, 52 pMS; 45.714.3 years; median disease duration: 5.82 [14.6] years, median EDSS: 2.5 [3]) that underwent 3D FLAIR, MP2RAGE, multi-shell diffusion and MT saturation imaging at 3T MRI. White matter lesions (WML) were segmented automatically, followed by manual correction. We applied MySD-Lesion to obtain the myelin signal contribution of CST and CB for both hemispheres. Patients underwent clinical assessment including the Expanded Disability Status Scale (EDSS) and serum neurofilament light chain (sNfL) sampling. sNfL z-scores were corrected for age, sex and body mass index. We used linear regression models to explore the association of myelin values in CST and CB (independent variable) with (i) sNfL z-score and (ii) EDSS. Covariates were WML volume, disease phenotype, treatment [for sNfL z-score], and age and sex [for EDSS]. Negative associations with EDSS were found for both the left (:-0.15, p:0.01) and right (:-0.13, p:0.004) CST. A negative relationship was measured between sNfL z-scores and the left (:-0.16, p:0.001) and right (: -0.13, p:0.001) CST and the left (:-0.02, p:0.002) CB. Associations remained significant after Holm correction ($p< 0.019$). Lower myelin values in the CST and CB were significantly associated with increased disability and neuroaxonal damage. Future work needs to assess the predictive value of myelin damage along major brain tracts longitudinally.

Detection of TDP-43 seeding activity in the olfactory mucosa from patients with frontotemporal dementia

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Aim: Frontotemporal dementia is a neurodegenerative disease with unavailable biomarkers for diagnosis. Autopsy studies showed aggregated TAR DNA-binding protein 43 (TDP-43) in the olfactory bulb of individuals with Alzheimer's disease and in the olfactory areas of amyotrophic lateral sclerosis patients. Thus, we explored TDP-43 seeding activity and aggregates detection in the olfactory mucosa (OM) of patients with frontotemporal lobar degeneration with TDP-43-immunoreactive pathology (FTLD-TDP) by TDP-43 seeding amplification assay (TDP43-SAA) and immunocytochemical analysis. **Materials and methods:** The TDP43-SAA was optimized using frontal cortex samples from 16 post-mortem cases with FTLD-TDP, FTLD with tau inclusions, and controls. Subsequently, samples of OM were collected from 17 patients with FTLD-TDP, 15 healthy controls, and three patients with *MAPT* variants. SAA and immunofluorescence were performed. **Results**: TDP43-SAA discriminated with 100% accuracy postmortem cases presenting or lacking TDP-43 neuropathology. TDP-43 seeding activity was detectable in the OM with 82.4% sensitivity and 86.7% specificity. In TDP43-SAA positive samples, cytoplasmatic deposits of phosphorylated TDP-43 in the olfactory neural cells were detected. **Discussion**: This is an exploratory pilot study. Increasing the number of patients and controls and a detailed case-by-case analysis to validate our results will be necessary. This is the first study that investigated the TDP-43 aggregation in OM of patients with FTLD-TDP and controls, and the detection of TDP-43 aggregates in OM opens up an enormous range of research possibilities, including the use of OM swabs to monitor the disease progression in clinically affected patients, in asymptomatic mutation carriers and for clinical trials. **Conclusion**: TDP-43 aggregates can be detectable in OM, suggesting that TDP43-SAA and immunofluorescence might help identify and monitor FTLD-TDP in living patients.

Injectable glucose-releasing materials to rescue cells from oxygen-glucose deprivation and its implication for neurotransplantation: a novel approach to an old problem?

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Despite decades of research in neurotransplantation showing its potential in halting or reversing neurodegenerative diseases such as Parkinson's Disease, the approx. 90% of graft death during the early weeks post-transplantation severely limits this strategy. Recently, findings have suggested that oxygen and glucose deprivation (OGD) at the grafting site is likely to be the cause of cell loss in the first 24-48 hours. Thus, we cultured human foetal ventral mesencephalic tissue as well as pluripotent stem cell-derived dopaminergic, medium spiny, and cortical neuron progenitors, and SH-SY5Y cells at different glucose concentrations (0-1 g/L) either in normoxia (pO2 21%) or near anoxia (pO2 0.1%); cell viability was assessed with a metabolic assay (PrestoBlue). While 1 mg/mL glucose was able to sustain cell viability for up to 3 days in anoxia, its paucity caused cell loss already after 24 hours in normoxia. For this reason, we developed injectable glucose-releasing materials (iGEMs) which could be co-injected during the transplantation. The first generation of iGEMs microparticles (80-120 and 120-180 µm series) was obtained by coating glucose crystals with poly-ethylene glycol diacrylate (PEGDA) by emulsion photo-polymerisation. These led to 24-48 hours of glucose release and were able to rescue our SH-SY5Y OGD model after 5 days of OGD incubation (>80% cell viability across the iGEMs groups vs approx. 10% and 70% in glucose-free particles and free glucose solutions matching the iGEMs groups, respectively). Moreover, by using a template-assisted photo-polymerisation approach to investigate several acrylate-ending monomers as glucose-containing cylinders (1.5 x 2 mm), we were able to release glucose for up to 14 days, while also rescuing SH-SY5Y in OGD (up to 80 ± 12% GEM vs 12 ± 3% empty cylinders at day 10, $p<0.0001$) thus proving that sustained glucose release is possible and could sustain cell viability in OGD.

Targeting the Enteric Nervous System to Halt the Progression of Parkinson's Disease

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Recent studies have highlighted that insoluble alpha-synuclein (αS) aggregates, the pathological hallmarks of Parkinson's Disease (PD), are found not only in the CNS but also in the enteric nervous system. To explore this, we developed a 3D primary enteric culture (EC) model to study αS aggregation in the intestine. ECs were isolated from the colon of adults Prp Human A53T αS transgenic mice, a PD model characterized by severe constipation and intestinal dysfunction. ECs remained viable in vitro for over a month. Their 3D cytoarchitecture is characterized by distinct layers that resemble the structure of the intestine *in vivo*, with smooth muscle cells functioning as physical support beneath glial cells and enteric neurons. The electrical activity of ECs was assessed with a lentiviral hSyn-driven GCaMP6s calcium sensor. Infected neurons exhibited KCl-induced-Ca^{2+} activity, confirming formation of action potentials. We tested whether αS could spread and propagate in a prion-like manner using αS Pre-Formed Fibrils (PFFs), alone or combined with CsgA, a bacterial amyloid protein suspected to promote toxic template conversion of prion-like proteins. IF analyses showed ECs neurons recapitulated aggregation and spreading of toxic αS species as it can be observed in CNS neurons. Moreover, αS aggregation led to a notable reduction in GFAP staining, indicating a loss of glial cells following PFFs treatment. To counteract αS aggregation, we administered therapeutic antisense oligonucleotides (ASOs) against αS expression for one month, which significantly reduced endogenous αS aggregation. Our findings suggest that ECs are a valuable model for modulating αS aggregation and serve as an effective tool for screening potential therapeutic agents aimed at halting or reducing the progression of PD pathology from the gut to the brain.

Stromal Vascular Fraction-laden hydrogel for the treatment of spinal cord injury

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Spinal cord injury (SCI) causes severe loss of sensation, movement, and control of bodily functions below the injury site. This study investigates stromal vascular fraction (SVF), a cell mixture from adipose tissue with regenerative potential. To improve SVF delivery and survival, biocompatible Agarose-Carbomer Polyethylene Glycol (AC PEG) hydrogels are used, mimicking the natural body environment and supporting nutrient, oxygen, and waste diffusion for optimal SVF function. In vitro, 25,000 SVF cells were encapsulated within AC-PEG hydrogels, and cell viability was monitored using CFSE/propidium iodide staining. In a mouse SCI model, SVF-loaded hydrogels were implanted at the injury site. Long-term cell viability and engraftment were evaluated using IVIS imaging. Hindlimb locomotor performance was assessed weekly using the Basso mouse scale, and fixed spinal cord sections were analyzed with ex-vivo immunohistochemistry to observe cellular and tissue changes. The study found that a pre-deposited 3D extracellular matrix significantly enhanced SVF cell viability compared to other formulations, maintaining high cell density in vitro for up to 12 days. IVIS imaging showed sustained fluorescence from the hydrogel and cells up to 9 days post-treatment, indicating gel retention and long-term cell survival. Functional recovery in treated mice was significantly improved, lasting up to 14 days post-injury. Neuropathological analysis revealed increased microglia reactivity and a rise in astrocyte numbers, suggesting their contribution to recovery, with microglia clearing debris and releasing neurotrophic factors, and astrocytes forming scar tissue and modulating inflammation to support healing. The findings suggest that SVF therapy enhances functional recovery primarily through paracrine effects, promoting an anti-inflammatory environment involving stem cells and macrophages.

Development of novel immunotherapeutic approaches for pediatric CNS tumors

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Pediatric malignant central nervous system (CNS) tumors represent 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. The aims of this study were to analyze the capacity of NKG2D-CAR T cells to eliminate high-grade pediatric CNS tumor cells. 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 cell-mediated lysis, with cytotoxicity ≥30% when 20:1 effector: target ratios of were used. Furthermore, NKG2D CAR T cells showed ability to penetrate and eliminate glioblastoma tumor-spheres. In an orthotopic murine model of human glioblastoma, an intracranial injection of NKG2D CAR T cells drastically reduced tumor growth. However, NKG2D CAR T cells showed no clinical benefit when they were administered intravenously (IV), and this could be related to the difficulty of NKG2D CAR T cells to trespass the blood brain barrier. However, the migration assays we performed showed NKG2D CAR T cells were capable of crossing human endothelial cells and pericytes without causing cell damage nor affecting intercellular interactions. Furthermore, a biodistribution experiment i*n vivo,* showed that NKG2D CAR T cells home the brain 24 hours after IV injection. Last, we have recently isolated Extracellular Vesicles (EVs) from NKG2D CAR T cells (Exo-NKG2D CAR). We have found Exo-NKG2D CAR maintain CAR expression and decrease tumor growth in glioblastoma tumor slices. 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.

BRAFV600E mutation and PTEN deletion in neural stem precursor cells give rise to glioma and neurofibromatosis

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The RAS/BRAF/MEK/ERK pathway is highly mutated in cancer, with mutations of the *BRAF* gene accounting for about 7% of cancers. The most frequently observed BRAF mutation is the V600E, that induces persistent activation of BRAF, inhibiting its inactivation and leading to a continuous pathway stimulation.
In the nervous system, BRAFV600E mutation has been found among low-grade glioma, pediatric oligodendroglioma-like tumors, pediatric glioblastoma, and adult epithelioid glioblastoma, as well as in peripheral nervous system tumors.
To understand if BRAFV600E mutation can drive nervous system tumor formation, we developed a mouse model in which BRAFV600E along with *Pten* mutations are driven by the Tamoxifen-inducible Sox2-CreER, a deleter specifically active in Neural Stem/Progenitor Cells (NSPC). We observed that Pten deleted and BRafV600E mutated central NSPCs are prone to transform into low grade gliomas while peripheral NSPCs transform into paraspinal plexiform neurofibromas and MPNSTs. To prove that NSCPs were the tumor cells of origin we specifically deleted *Sox2* in these cells by crossing BRaf/Pten mice with conditional $Sox2^{loxP/loxP}$ mice. None of the Sox2-deleted BRaf/Pten mice developed tumors compared to Sox2-wildtype BRaf/Pten mice.
In vitro analysis on BRaf/Pten mutated NSPCs revealed that these cells show increased proliferation and preferentially differentiate towards oligogendroglial-like fate.
Moreover, BRaf mutated NSPCs show increased Sox2 protein levels, compared to wildtype. RNA-seq and transcript analysis revealed that Sox2 mRNA levels, instead, are equal between mutated and wildtype NSPCs, suggesting that Sox2 increased protein levels are due to its stabilization in the BRaf mutated genotype. In support of this observation, in BRaf mutated NSPCs, Sox2 protein levels do not change following proteasome degradation and translation inhibition. In addition, we observed a biochemical interaction between Sox2 and BRafV600E, suggesting that BRafV600E mutation might be responsible for Sox2 protein stabilization, and this could be the mechanism through which BRaf mutate NSPCs drive tumor formation.

Modeling glioma progression in mouse and human neural organoids

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Early stages of glioma progression, following the first molecular lesion, are still largely unknown. Using in vivo murine models to study these aspects poses challenges due to limited access to early stages and difficulties in manipulating complex environments. Neural organoids (NO) emerge as an accessible, manipulable, and translatable in vitro alternative. Our aim is to model glioma progression using both mouse (mNO) and human (hNO) neural organoids. For mNO, murine embryonic stem cell line E14Tg2A is used to create embryoid bodies (EB) that are differentiated into unguided neural organoids. For hNO, EB are generated from human induced pluripotent stem cells ISFi001-A and patterned towards dorsal or ventral identity. They are then fused, obtaining human cerebral assembloids. For glioma generation, mouse high-grade glioma primary cells (mHGG) are co-cultured with mNO or transplanted in hNO. Both mNO and hNO display neural rosettes composed of neural progenitors (Sox2+), neurons (MAP2+ and NeuN+) and astrocytes (GFAP+). In addition, hNO feature microglia (Iba1+), oligodendrocyte lineage cells and interneurons. Tumor take rate is 95% for co-culture (103/108 mNO) and 100% for transplantation (5/5 hNO). After 30 days, 74.8±24.8% of mHGG are ki67+ and 80.6±8.2% are Sox2+, indicating that tumor cells proliferate and preserve stemness. In mNO, 30 days after the start of the co-culture there is a significant increase in infiltration, measured as the median distance of mHGG cells from the edge of organoids (10 days: 77.2±22.9 mm; 20 days 71.2±17.5 mm; 30 days: 107.0±26.6 mm; 40 days: 99.6±20.3 mm). Therefore, mHGG preserve their invasive properties in the organoid environment. These results suggest that mNO and hNO models support proliferation, stemness and invasive potential of mHGG cells. Thus, they offer promising in vitro platform for investigating glioma progression, early-stage tumor dynamics and potential therapeutic interventions in both murine and human contexts.

Preclinical testing of a novel therapeutic approach to counteract Glioblastoma Multiforme

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This abstract is not available online on request of the presenting author.

Quantifying muscle activation in Octopus vulgaris stereotypical motion

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Limb motion of skeletal animals is generated by the input of brain and spinal cord to antagonistic muscles following motion-specific activation patterns. The coordinated action of antagonistic muscle pairs allows the body part to move using muscle forces applied to the bones and tendons. Limb motion thus can have up to four degrees of freedom (DOF). The arm of an octopus does not contain any rigid counterparts to human bones and virtually has infinite DOF. This could impose a huge load on the motor control system. Yet, several of the octopus's arm motions, like withdrawal reflex and reaching are performed stereotypically using only few DOF and are rather autonomous from the brain. To fully evaluate the complexity of the motor programs at central and peripheral level, here we investigated the activation patterns of arm antagonistic muscles and their role in stereotypic actions with electromyography (EMG) recordings. Previous EMG recordings of the dorsal portion of an octopus arm showed the possibility of a simple control mechanism for bend propagation during reaching movements. However, the involvement of antagonistic muscles in different type of motions is yet to be disclosed. By using EMG, we investigated muscle activity in a whole arm *ex vivo* preparation during arm withdrawal reflex and reaching elicited through either mechanical or electrical stimuli. Whole arm movements were analysed off-line using DeepLabCut™ on the video footage to reconstruct the kinematic of the motion in two-dimensions. We showed synchronised EMG activity at the level of single muscle with local arm deformation. This data will help to understand the mechanisms of central and local control of muscle activation in soft-bodied animal model which can be crucial for software development in emerging fields like soft robotics.

Spinogenesis of cerebellar Purkinje cells is locally repressed in an activity-dependent way

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In cerebellar Purkinje cells (PCs) two types of dendritic branches can be identified based on spine density, diameter and excitatory input: the proximal branches have a low spine density, are thicker and contacted by a single climbing fiber (CF), while the distal ones have a high spine density, are thinner and innervated by parallel fibers. It was previously proposed that CFs activity suppresses spinogenesis based on the observation of increased spine density on PCs proximal dendrites following treatments affecting the whole circuit. These include the resection of cerebellar pedunculi, infusions of blockers of voltage-gated sodium channels or of AMPA glutamate receptors and partial degeneration of CFs-generating neurons. However, it is still unclear if CFs alone are responsible for this repression of spinogenesis or the process involves also other elements of the circuit, and whether it is exerted by single CFs specifically on the dendritic branches that they individually innervate. Here, through *ex vivo* morphological analysis of tissues derived from mice whose CFs were transduced *in vivo* by lentiviral vectors encoding shRNAs to silence NaV1.1/1.2 expression, we assessed if the electrical activity and/or the trophic state of single CFs repress PC spinogenesis. We observed that knocking-down NaV1.1/1.2 (responsible of action potential generation) in sparse CFs determines a significant increase in spine density specifically on the innervated dendritic territory, with no effects on distal dendrites or surrounding PCs. On the contrary, the atrophy of CFs by silencing GAP-43 results in decreased spine density. These data provide a direct demonstration that individual CFs repress spinogenesis specifically on the innervated PC dendritic domain via their electrical activity, while CF atrophy is not sufficient to de-repress spinogenesis. The results provide new insights in the understanding of activity-dependent structural plasticity in the cerebellar cortex.

Transcranial direct current stimulation (tDCS) promotes myelin repair and plasticity in the mouse motor cortex

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Myelin disruption and oligodendroglia dysfunction in the cortical gray matter are consistent features of a wide range of neurodegenerative and psychiatric disorders, in which executive and sensory functions are impaired. Myelin alteration, in fact, affects the integrity and the activity of neural circuitries by slowing down action potential conduction and predisposing axons to degeneration. Former studies have shown that enhancing neuronal activity positively impacts oligodendroglia proliferation, maturation and re-/myelination *in vivo*. Thus, in this frame, the use of non-invasive brain stimulation techniques might be a promising non-pharmacological strategy to promote myelin repair. In this study we investigated the effects of anodal transcranial direct current stimulation (A-tDCS) in a mouse model of unilateral lysolecithin-induced myelin lesion in the primary motor cortex (M1). In concomitance with spontaneous myelin repair initiation, mice received A-tDCS over the intact cortex while performing motor activity. Myelination and oligodendroglia dynamics along with functional outcomes have been studied at different time points, in both injured and intact cortices. In the lesioned cortex, A-tDCS sustains oligodendroglia survival and accelerates their maturation and remyelination, with respect to control mice. Also, myelin appears to be increased in the directly stimulated M1. This occurs despite a stable number of oligodendroglia, suggesting novel myelin formation or remodeling of the existing internodes in the intact cortex. We also investigated functional effects by measuring M1-M1 synchronization before and after myelin injury. Interhemispheric coherence analysis at 28 days revealed a partial recovery of the inter-cortical cross-talk in A-tDCS mice respect to the unstimulated lesioned mice. Altogether, our data suggests that A-tDCS sustains myelin repair in the mouse cortex, supporting its potential application in myelin disorders.

Ketogenic diet treatment in GLUT1-DS patients: identification of ion channel signaling deregulation related to both epigenetic changes and splicing events

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GLUT1 deficiency syndrome (GLUT1-DS; #606777) is a rare neurometabolic condition characterized by drug-resistant seizures. It is caused by mutations in the SLC2A1 gene, which impair glucose supply to the brain. Ketogenic diet (KD) treatment provides an alternative fuels for energy, whereas its underlying mechanisms remain elusive. After confirmation of metabolic status, we aimed to explore the transcriptional dysregulations of GLUT1-DS pediatric patients before and after KD and also to assess alterations in methylome profiles.
FIA-MS/MS was carried out for the analysis of amino acids and acyl carnitines. Bulk RNA-sequencing was performed using Illumina Total RNA kit and sequenced on NS500. Differential expression analysis was conducted using R Package DESeq.2 and GSEA was performed using ClusterProfiler. Analysis of alternative splicing was implemented using IsoformSwitchAnalyzeR. Methylation sequencing was performed on PromethION 2 Solo.
Metabolite analysis has confirmed the ketosis status in two patients in diet. Transcriptome data showed a strong epigenetic signature, a deregulation in ion channel signaling and an activation of immune response as effect of diet. 4 snRNAs resulted up-regulated before KD highlighting a significant change in splicing events between two conditions.
Among 325 isoforms detected, the P2X7 ion channel receptor resulted to interact with GLUT1. The non-coding P2X7 isoform is up-regulated after treatment, and its increased expression could alter the transcription/transduction of P2X7 coding isoform, leading to a down-regulation of ion channel signaling and subsequent reduction of interferon.
Methylation data showed different methylation regions in target genes: the P2RX1 gene, an interactor of P2X7 receptor, resulted hypermethylated after KD.
This preliminary study highlighted some pathways related to KD, which appears to have an effect on expression of ion channel signaling through the combination of epigenetic changes and splicing events.

Novel frontiers in Aicardi-Goutières Syndrome: association between a RNU7-1 variant and histone dysfunctions

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Aicardi-Goutières Syndrome (AGS) is a rare and genetically determined pediatric disorder currently associated with mutations in 9 genes. Mutations in *RNU7-1* (AGS9) lead to the least characterized form of the disease. This gene encodes for the U7 small nucleolar RNA (U7 snRNA), a member of the small nuclear ribonucleoprotein complex (U7 snRNP). U7 snRNP operates the cleavage of the Poly-A tail in replication-dependent histones (RDHs) pre-mRNAs. The aim of this work is to dissect the role of *RNU7-1* variations in AGS pathogenesis. We both investigated canonical AGS features such as the upregulation of IFN-α, interferon-stimulated genes (ISGs) and specific outcomes of a *RNU7-1* variant in primary fibroblasts obtained from an AGS9 patient and compared to a healthy matched control. Total RNA was extracted with TRIzoL™ reagent, and the gene expression was determined by Real-Time PCR. ELISA, Western Blot analysis and immunofluorescence were performed to assess protein expression. MTT assay was used to investigate cell viability. RNA immunoprecipitation (RIP) was carried out to assess the physical association between proteins and target RNAs. Transmission Electron Microscopy (TEM) was used to identify eventual alterations in subcellular compartments. Our results highlight the upregulation of ISGs and the common increase in IFN-α production. Lastly, we assessed the enrichment in Poly-A tail of RDH transcripts suggesting the lack of functionality of AGS9 U7snRNP in the cleavage of Poly-A tail. The aberrant form of U7snRNP was also investigated via RIP, and we determined the impairment of the U7 snRNA binding to ZFP100, a component of the U7snRNP complex. Lastly, TEM analysis revealed an important decrease in chromatin content and in nucleolus size in AGS9 fibroblasts. In conclusion, this work highlights novel molecular mechanisms related to AGS9 mutation which lead to the upregulation of ISGs, IFN-α overproduction, misprocessing of RDH mRNAs, and chromatin reduction.

A novel regulatory role of NBS1 at the primary cilium highlights impinges on cerebellar development and medulloblastoma insurgence

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Neurodevelopmental disorders (e.g., microcephaly and ataxia) and cancer predisposition are clinical features commonly associated with DNA Damage Response (DDR)-defective syndromes, including the Nijmegen Breakage Syndrome (NBS). NBS is caused by mutations in human NBS1 gene, which expresses for a member of the MRE11/RAD50/NBS1 (MRN) complex, an essential player of DDR. The primary cilium (PC) is a non-motile organelle that nucleates from the centrosome/basal body (BB) during interphase. PC acts as a sensory antenna for the regulation of several signaling pathways important in both development and cancer, including the SHH pathway. Defects in PC lead to diseases termed 'ciliopathies' among which some characterized by defects in brain development and mental retardation. We have recently demonstrated that NBS1-KO in cerebellar granule cell progenitors (GCP) leads to impaired cerebellar development, resulting in an hypoplastic cerebellum, reduced GCPs proliferation and premature differentiation due to the inhibition of the SHH pathway, which was confirmed in *in vivo*, *ex vivo* and *in vitro* models. Moreover, NBS1-KO targeted to the central nervous system or GCPs completely abrogates the insurgence of the SHH-subtype medulloblastoma (MB), the most common malignant pediatric brain tumor. Importantly, NBS1, localizes at the centrosomes and BB and its depletion leads to severe alterations of PC morphology and function, as detected in *in vivo*, *ex vivo* and *in vitro* frameworks, resulting in altered protein trafficking in the PC and downregulation of the SHH pathway. Similar PC alterations were observed in human fibroblasts harboring the 675Δ5 mutation, the most frequently observed in NBS patients. Altogether, our data indicate that NBS1 plays a pivotal role in cerebellar development and tumorigenesis through its previously undisclosed function at the PC and in the regulation of the SHH pathway.

POSTER SESSION

1

Addressing the potential impact of a gene therapy approach on CDD patient-derived brain organoids

EBN 01

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CDKL5 deficiency disorder (CDD) is a rare developmental epileptic encephalopathy caused by mutations in the *CDKL5 gene. There are currently no approved treatments that address the root cause of the disease.* Recently, we demonstrated that a new gene therapy approach based on a secretable TATk-CDKL5 protein is more effective than CDKL5 alone in compensating behavioral and neuroanatomical defects of the mouse model of CDD. These results underscore the possibility that this new gene therapy approach could represent a powerful tool for the treatment of CDD. However, the effectiveness of this approach in restoring correct neuronal activity in a context consisting of patient-derived neuronal cells has not yet been verified. Interestingly, brain organoids from human pluripotent stem cells derived from CDD patients show neuronal hyperexcitability that could underlie the seizures that characterize children with CDD. Here we utilized patient-derived brain organoids as a model to validate the efficacy of a gene therapy approach for CDD, and compared the effects of CDKL5 gene therapy with TATk-CDKL5 gene therapy. We found that CDD *brain organoids* exhibited reduced cell proliferation and increased neuronal cell death, in comparison with control *brain organoids*, confirming that CDKL5 plays a role in neuronal survival. Expression of both CDKL5 and TATk-CDKL5 improved defective cell proliferation in CDD brain organoids to control levels, while only TATk-CDKL5 restored neuronal survival. Similarly, while the CDKL5-expressing brain organoids showed no improvement compared to untreated CDD organoids, the organoids expressing TATk-CDKL5 showed a decrease in neuronal hyperexcitability, evaluated with micro electrode arrays (MEA) as number of bursts and synchrony index. In conclusion, these findings suggest that a cross-correction-based gene therapy approach is more efficient in improving neuronal survival and function of CDD brain organoids.

Impact of Synapsin II Silencing on Excitatory Transmission and Calcium Channel Function

EBN 02

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Synapsin II (Syn II), a pivotal member of the synapsin family, is a synaptic vesicle-associated phosphoprotein which plays critical roles in regulating neurotransmitter release at both excitatory and inhibitory synapses within the central nervous system. Synapsins contribute significantly to synaptic plasticity and maintain the delicate balance between excitatory and inhibitory synaptic transmission. Notably, mutations in synapsins genes have been identified in forms of epilepsy with or without ASD. Syn II has been shown to interact with the presynaptic calcium channel P/Q, but the functional consequences of this interaction need to be studied. This study investigates the effects of SynII silencing by RNA interference in primary hippocampal neurons, focusing on its impact on excitatory transmission, intrinsic excitability and calcium current density. Our findings reveal that the knockdown of Syn II leads to increased spontaneous and evoked excitatory transmission associated with an increase in the calcium current density of P/Q-type channels. Additionally, Syn II silencing results in decreased excitability at the single-neuron level, but increased overall excitability at the network level. Collectively, these findings shed light on the complex role of Syn II in synaptic function and its ensuing impact on neuronal excitability due to the functional interactions with the P/Q-type calcium channel. This study provides a foundational understanding for future research in this domain, offering potential insights into the mechanisms underlying neuronal function.

EBN 03

Ketogenic diet in the GluN2A(N615S) mouse model improves behavioral impairments in a sex-based manner, rescues audiogenic seizures and affects neuronal plasticity and neuroinflammation

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Background Developmental and epileptic encephalopathies (DEEs) are early-life onset syndromes in which drug-resistant epilepsy is associated with cognitive impairment. The GluN2A(N615S)-mutated mice carry a mutation in the *Grin2a* gene coding for the GluN2A subunit of the NMDA glutamate receptor and display symptoms similar to those described in DEE, thus representing a valuable model to study *GRIN*-related DEEs. **Methods** We investigated the effects of a ketogenic diet (KD) on the epileptic phenotype and behavior in the GluN2A(N615S) model. After behavioral and seizure testing, mice were sacrificed and several tissues were collected. Brains slices were stained to evaluate neuronal plasticity – by using WFA for perineuronal nets (PNNs) and parvalbumin (PV) for PV^+ inhibitory interneurons (PVIs) – and neuroinflammation with Iba1 to mark microglia. After slides preparation and microscopy acquisition, all images were aligned to the reference atlas to analyze the expression of the above mentioned markers together with anatomical information. **Results** Our data demonstrated for the first time in this model that KD reduces the susceptibility of mutant mice to audiogenic seizures (AGS) and slightly improves performance in some behavioral tests. Moreover, the use of both sexes pinpointed differences in behavioral performance and AGS susceptibility in response to KD based on sex. Preliminary HIC analysis on brain slices revealed in KD-fed mutated mice an increase of PNNs and PVIs, therefore leading us to hypothesize that beneficial effects of KD could be mediated by an enhancement of inhibitory GABAergic activity. As we observed reduced microglia expression in some brain areas of KD-fed mutant mice, our results suggest also an anti-inflammatory effect of KD. **Conclusion** Results of behavioral tests, together with *ex-vivo* analysis of the brains, are leading us to identify potential molecular and physiological mechanisms underlying the effects of KD.

Gene therapy as a potential disease-modifying approach in creatine transporter deficiency

EBN 04

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Creatine Transporter Deficiency (CTD) is a X-linked neurodevelopmental disorder characterized by cerebral creatine (Cr) deficiency, intellectual disability, seizures and autistic-like behavior. To investigate the potential for gene therapy as a disease-modifying treatment for CTD, we developed an adeno-associated 9 viral vector (AAV9) carrying a human *SLC6A8* transgene (encoding the creatine transporter, CrT) under the control of a ubiquitous promoter. The AAV9-*SLC6A8* vector was administered to male neonatal (postnatal day 1) CrT knockout (KO) mice and wild-type (WT) littermates via intracerebroventricular injection. Three weeks post-injection, western blot and immunofluorescence analysis revealed high expression and widespread distribution of the transgene into the brain, along with a significant increase in cerebral Cr levels measured by gas chromatography/mass spectrometry (GC/MS), thus confirming that the exogenous CrT was functional. The restoration of CrT led to the rescue of functional hypoconnectivity in KO animals and the improvement of autistic-like stereotyped behavior. However, this treatment did not improve cognitive function in KO mice and resulted in a deterioration of mnemonic performance in treated WT mice. Overall, our findings support the potential for gene therapy for the treatment of CTD, highlight the need for improved construct design to maximize therapeutic benefits and reduce safety risks. Ongoing efforts aim to develop cassettes that achieve more controlled levels and patterns of *SLC6A8* expression.

Unraveling the Role of Microglia in Fragile X Syndrome across development

EBN 05

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The Fragile X syndrome (FXS) is the most common neurodevelopmental disorder associated with intellectual disabilities. In FXS, an increasing repetition of the CGG triplet (more than 200) in the *fmr1* gene leads to hypermethylation of the 5'UTR, resulting in gene silencing and absence of the Fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein capable of regulating the expression of various types of RNA and is essential for synaptic formation and function. FMRP is expressed not only in neurons but also in astrocytes and microglia, the other key cells of the brain. Despite many studies focusing on the role of FMRP in neurons and locally at synapses, only a few pieces of evidence have reported the role of the protein in brain cell types. Preliminary results obtained through immunofluorescence revealed hyperactivation of microglia and a significant increase of the engulfment in *fmr1* knockout (KO) mice, as evidenced by high levels of PSD95 within CD68 in microglia at p20. Analyses conducted at various time points, from developmental age to adulthood, showed a decrease in engulfment in KO mice at p40, despite hyperactivity. These results indicate a crucial role of the FMRP protein in microglia-mediated neuronal remodeling in a specific time window. Additionally, they suggest a potential lack of communication between microglia and neurons in KO mice, compromising the efficiency of the remodeling process and influencing neural development. This study aims to examine in detail the cellular and molecular changes of microglia in FXS, highlighting their impact on neuronal circuits and behavior.

EBN 06

Impact of Prenatal and Early Postnatal Exposure to Short-Chain PFASs on Cognitive Function in Adult Rats

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Per- and polyfluoroalkyl substances (PFAS) are widespread environmental pollutants known to disrupt metabolism and endocrine function. The increasing concern over these contaminants highlights the urgent need to understand their full impact on health. While long-chain PFAS pose health risks, data on potential adverse effects of their short-chain replacements are limited. This study investigated the impact of prenatal and postnatal exposure to two short-chain PFASs (PFBA and GenX) on neurodevelopment and adult behavior in rats. Pregnant rats were exposed to PFBA- and GenX-contaminated feed (0.5 and 5 mg/kg pc/die) starting 30 days before mating, throughout the gestation period and until weaning. Pups underwent neurological testing from post-natal day 7 until day 21. In adulthood, animals were assessed for motor coordination and balance with the rotarod, and for spatial learning and memory with the Morris Water Maze (MWM). The results indicate that prenatal exposure to PFBA and GenX, while initially showing no impact on early neurodevelopment, resulted in significant behavioral changes in adulthood. Specifically, 5mg/kg pc/die doses of PFBA and GenX impaired performance in the MWM probe trial for both male and female rats, with more pronounced deficits observed in females (one-wayANOVA: malePFBA vs maleCTRL, $p= 0.0018$; femalePFBA vs femaleCTRL, $p= 0.002$; maleGenX vs maleCTRL, $p=0.0035$; femaleGenX vs femaleCTRL, $p<0.0001$). Additionally, females showed significant alterations even at 0,5 mg/kg dose PFAS exposure (one-wayANOVA: femalePFBA vs femaleCTRL, $p= 0.0058$; femaleGenX vs femaleCTRL, $p<0.0001$). These findings suggest that prenatal exposure may have long-term consequences for adult cognitive function possibly acting directly or indirectly on the development of related brain pathways.

Supported by Italian Ministry of Health, "PFASs": Investigating health hazard of short chain PFASs: toxicology, food contamination and effects of prenatal exposure on neurodevelopment (RF-2019-12370332).

EBN 07

Unveiling the role of TREM2-Na+/K+ ATPase Axis in microglia to neuron crosstalk during development

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Defects in communication between microglia cells, the immune resident cells of the brain, and neurons underly the pathophysiology of many neurodevelopmental disorders. The Triggering Receptor Expressed on Myeloid cells 2 (TREM2), a phagocytic receptor exclusively expressed by microglia in the brain, plays a critical role in hippocampus maturation by controlling the bioenergetics of developing neurons and the process of synapse elimination. However, how TREM2 signalling controls these mechanisms is still an unresolved question.

By performing a pull-down assay on purified synaptosomes using the extracellular domain of TREM2, we recently identified the neuronal Na^+/K^+ ATPase α3 (ATP1A3) as a putative novel neuronal interactor of the TREM2 receptor.

To determine if TREM2-ATP1A3 interaction is important in mediating microglia to neuron crosstalk, we characterized ATP1A3 signalling in brain slices and primary neuronal cultures. Interestingly, ATP1A3 expression appeared reduced in the hippocampi of TREM2 knockout (KO) mice both at postnatal day 1 and 18 and in primary culture at 4 and 14 days in vitro.

By combining confocal microscopy, live imaging, and metabolic assays, we observed that acute blockage of ATP1A3 impairs neuronal mitochondrial metabolism and alter neuronal morphology, resembling the phenotype previously observed in TREM2 KO neurons. Interestingly, 3 days co- culture of TREM2 KO neurons and WT microglia rescued both ATP1A3 levels and mitochondrial metabolism. By performing glutamate synaptic imaging in mature primary cultures co-cultured with WT or TREM2 KO microglia, we observed that WT and TREM2 KO microglia showed a different modulatory effect on synapses, suggesting that a dynamic communication between microglia and neurons requires TREM2. Altogether, our preliminary results indicates that TREM2-ATP1A3 axis is important player in controlling neuronal development.

EBN 08

DNA damage response does affect neither the localization of the Nijmegen Breakage Syndrome 1 protein at the centrosome/basal body nor the primary ciliogenesis

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The preservation of DNA and centrosome integrity is essential for cell homeostasis, and it is insured by the DNA damage response (DDR) and centrosome damage response, respectively. Increasing evidence suggests a functional crosstalk between DDR and centrosome-regulating pathways that converge in the handling of the cell cycle and seem particularly important in the nervous system. Indeed, alterations in both DDR and centrosomal genes may affect the neuronal precursor pool, causing microcephaly. Accordingly, mutations in *NBS1*, a subunit of the MRE11/RAD50/NBS1 (MRN) complex that plays a crucial role in the DDR, leads to the DDR-defective Nijmegen Breakage Syndrome, characterized by microcephaly. Of note, NBS1 localizes at the centrosome and regulates their replication and function. We recently demonstrated that NBS1 also localizes at the basal body (BB), regulates primary ciliogenesis and impairs the proliferation driven by primary cilia (PC)-dependent mitogenic pathways in neuronal precursors. Since NBS1 moves into the nucleus in response to DNA damage and its depletion induces accumulation of DNA damage, we hypothesized that this latter may influence ciliogenesis and/or the localization of NBS1 at the centrosome/BB. Our findings indicate that the accumulation of DNA damage, induced by different clastogenic drugs, is not sufficient to induce alterations in primary cilia (PC) frequency, length and morphology and does not alter NBS1 localization at the centrosome/BB. These results indicate that NBS1 localizes at the centrosome/BB independent from the activation of the DDR pathway and that the DNA damage is not sufficient to alter PC. They also suggest that NBS1 may regulate centrosome/BB and PC homeostasis, independently from its canonical role in the DDR.

EBN 09

Closed-loop stimulation of the ventral subiculum in the rat pilocarpine model of temporal lobe epilepsy: two case reports

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Temporal lobe epilepsy (TLE) represents the most prevalent form of adult focal epilepsy, often refractory to anti-seizure medications. Alternative treatments include deep brain stimulation characterized by continuous long-lasting stimulation of a specific brain region. Since epileptic seizures are unpredictable and occur with different frequency among patients, or over time in the same patient, a continuous stimulation is not necessary and might lead to adverse effects. To overcome this limitation, we investigated the effect of a closed-loop stimulation in the rat pilocarpine model of TLE and we present here two case reports.

Sprague Dawley rats received pilocarpine intraperitoneally to induce status epilepticus, terminated after 30 minutes. Two weeks later, they underwent surgery for electrodes implantation. A neuromorphic computing system driven by artificial intelligence was developed and integrated with the EEG-recording system to deliver a closed-loop stimulation in the ventral subiculum. In epileptic rats, closed-loop (monophasic square, 100 µs, 1 Hz, amplitude based on input/output curve) was activated for 7 days (9:30-18:30), after a 2-week baseline period and followed by a 2-week washout.

Closed-loop stimulation induced a reduction in seizure severity, which persisted over the washout period. However, this occurred along with an increase in the seizure frequency, while data on seizure duration were inconsistent between the two rats treated with the closed-loop protocol. Interestingly, a preliminary analysis of seizure onset zone indicated that closed-loop stimulation likely activated new epileptic *foci* not observed in the baseline period.

Despite the unpromising data on seizure frequency and onset, the reduction in seizure severity is a noteworthy finding, especially from a clinical point of view, since the closed-loop protocol in the ventral subiculum would reduce the number of debilitating seizures with a general improvement of the patient's quality of life.

Investigating early microglia activation and neuronal Ca2+ excitability in PS2APP mice

ND 01

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Alzheimer's disease (AD) is the primary cause of dementia, characterized by irreversible neurodegeneration. Growing evidence suggests that neuroinflammation plays a pivotal role in AD, with microglia and extracellular ATP (eATP) being pivotal players in this process. Our project aims at investigating whether eATP influences microglia activation, AD-associated neuroinflammation, and neuronal Ca^{2+} excitability in an AD mouse model (B6.152H) expressing human PS2-N141I and APP Swedish mutations. We carried out experiments at various disease stages: pre- and post-Aβ plaque deposition, at 2 and 6 months of age, and during overt cognitive deficits at 9 months.

Our morphological analyses demonstrated early microglia activation before Aβ plaque deposition, accompanied by an increased expression of the inflammasome NLRP3, as well as elevated levels of the inflammatory cytokines IL-1β, IL-6 and TNF-α.

Additionally, we are interested in determining whether microglial activation is associated with neuronal hyperexcitability, a feature that emerges at the initial stages of AD. For this purpose, we investigated both spontaneous and evoked neuronal Ca^{2+} activity, by means of GCaMP6f Ca^{2+} indicator, combining 2-photon Ca^{2+} imaging and electrical stimulation in somatosensory cortex (SSCx) of mouse brain slices. Preliminary results revealed neuronal hyperactivity in 2-month-old AD mice, emphasizing an altered phenotype in this mouse model already at the early stages of the disease.

Different experimental strategies will be employed to modulate neuroinflammation and neuronal excitability, eventually defining new potential therapeutic targets to delay/halt AD neurodegeneration.

ND 02

Characterization of the molecular mechanisms leading to ELAVL4/HuD's altered levels in oxidative stress conditions with possible implications for sporadic ALS

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ELAVL4/HuD is an RNA Binding Protein expressed in the nervous system, that plays a key role in neuronal development promoting growth in neurites. Previous works showed the involvement of HuD in sporadic ALS and in the context of oxidative stress. Moreover, it has been documented the existence of different first exons in the transcript of HuD potentially leading to the expression of different isoforms potentially expressed in different cellular conditions, such as oxidative stress.
However, the molecular mechanisms leading to the altered levels of HuD in these contexts, and the regulatory mechanisms of the isoforms are still unclear, therefore, this study aims to characterize the mechanisms leading to HuD upregulation in oxidative stress conditions throughout acute and chronic stress treatments performed on iPSC-derived Motor Neurons (MNs) and Co-cultures of Skeletal Muscle Cells (SkM) and MNs via Sodium Arsenite administration. These models allow the study of the oxidative stress response in MNs alone and in co-culture with SkM. Through qRT-PCR analysis we observed a variation in the expression specific isoforms in chronic oxidative stress conditions.
In acute oxidative stress conditions, bioinformatic analysis revealed a decrease in the levels of the first intron. This was also observed both in iPSC-derived MNs and Neuroblastoma Cells (SK-N-BE). This led us to hypothesize the existence of two different regulatory mechanisms: one acting on the splicing of the first intron under acute oxidative stress, and a second one acting on the transcription of HuD's isoforms under chronic oxidative stress.
We are currently working on the identification of the molecular mechanisms leading to the upregulation of HuD in sporadic ALS.

A novel Parkinson's disease mouse model to study structural and functional plasticity in dopaminergic neurons related to levodopa-induced dyskinesia

ND 03

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Levodopa Induced Dyskinesia (LID) is a pathological condition caused by prolonged treatment of Parkinson's Disease (PD) with L-DOPA. Thanks for its good efficacy L-DOPA is up to now the most used drug for PD treatment. LID is characterized by the emergence of stereotypical involuntary movements after few years of treatment. This transition is leading to a progressive loss of L-DOPA therapeutic effect and it is unfortunately irreversible. In preclinical models, LID progression was associated with a prominent astrocytic response, and a moderate microglial cell reaction restricted to this striatal area correlated with increased levels of two pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β). For this study we used a mouse model of LID based on the 6-hydroxydopamine (6-OHDA) model of PD. In this model the dopaminergic neurons of the substantia nigra pars compacta innervating the striatum and the cortex are unilaterally damaged using 6-OHDA. To understand the temporal and spatial dynamics of the L-DOPA effect during LID we implemented *in vivo* imaging of Dopamine with two-photon microscopy. Our data show that after the administration of L-DOPA, dopamine is increasing in both brain hemispheres, but the increase is more visible in the lesioned side suggesting the lack of regulation of the dopamine reuptake in the lesioned side. To understand how LID progression is associated with the number of dopaminergic neurons and the availability of dopamine we are developing a novel PD model based on a CRE inducible shRNA targeting DOPA decarboxylase in order mimic the progressive decrease of DA availability typical of the progression of PD. Finally, to understand the interplay between the progression of LID and neuroinflammation we will evaluate astrocytes and microglial cells activation in striatal tissue from our PD model with immunostaining, western immunoblotting combined with ELISA to detect inflammatory cytokines (TNF-α, IL-1β).

ND 04

Sex affects resting-state electroencephalographic rhythms in patients with dementia due to Parkinson's and Lewy body diseases

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Parkinson's disease with dementia (PDD) and dementia with Lewy bodies (DLB) are more prevalent in males than females. Furthermore, they typically showed abnormally high delta (< 4 Hz) and low alpha (8-10 Hz) rhythms from resting-state electroencephalographic (rsEEG) activity. Here, we hypothesized that those abnormalities may depend on the patient's sex. An international database provided clinical-demographic-rsEEG datasets for cognitively unimpaired older (Healthy; N = 49; 24 females), PDD (N =39; 13 females), and DLB (N =38; 15 females) participants. Each group was stratified into matched female and male subgroups. The rsEEG rhythms were investigated across the individual rsEEG delta, theta, and alpha frequency bands based on the individual alpha frequency peak. The eLORETA freeware was used to estimate cortical rsEEG sources. In the Healthy group, widespread rsEEG alpha source activities were greater in the females than in the males. In the PDD group, widespread rsEEG delta source activities were lower and widespread rsEEG alpha source activities were greater in the females than in the males. In the DLB group, central-parietal rsEEG delta source activities were lower, and posterior rsEEG alpha source activities were greater in the females than in the males. These results suggest sex-dependent hormonal modulation of neuroprotective-compensatory neurophysiological mechanisms in PDD and DLB patients underlying the generation of rsEEG delta and alpha rhythms.

ND 05

The activation of ADAM10 modulates adult hippocampal neurogenesis in a murine model of Alzheimer's disease

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In Alzheimer's disease (AD), adult hippocampal neurogenesis (AHN) is altered and linked to learning and memory deficits. Post-mortem studies on AD human brains showed a remarkable reduction in the number and maturation of hippocampal immature neurons (DCX^+ cells). In AD murine models, immature neurons are actively recruited into the engram following a hippocampaldependent behavioral task. However, this is severely compromised because immature neurons exhibit reduced spine density and altered transcriptional profile. Promoting AHN restored new neurons and improved memory performance in AD mice.
ADAM10 (A-disintegrin and metalloproteinase 10), is an enzyme involved in non-amyloidogenic processing of the Amyloid precursor protein (APP) and one of the highly expressed genes in the engram. Recent studies also demonstrated that subchronic administration of a cell-permeable peptide (PEP3) able to inhibit ADAM10 endocytosis resulted in a rescue of cognitive impairment and increased synaptic dysfunction in APP/PS1 mice at early AD stages.
Herein we report the results of studies investigating the effects of PEP3 on AHN both *in vitro* and *in vivo*. *In vitro* studies, we observed that PEP3 significantly increased neuronal differentiation both in WT and APP/PS1 adult hippocampal neural progenitor cells (ahNPC) cultures, with no effect on astrogliogenesis. *In vivo* studies, our data showed a significant increase both in the percentage of immature neurons and in their morphological complexity in PEP3-treated compared to inPEP3treated mice. No effect of peptide treatment was observed on the morphological complexity of hippocampal astrocytes.
Altogether these data suggest that in early AD stages, subchronic PEP3 treatment may increase adult neurogenesis in the dorsal hippocampus of APP/PS1 mice. In the future, it will be relevant to identify ADAM10 targets that may be implicated in the modulation of AHN.

Funding: This work was supported by PRIN 2017 (2017MYJ5TH_003)

miRNA-mRNA network investigation in Alzheimer's and Frontotemporal Dementia patients'

ND 06

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Alzheimer's disease (AD) and Frontotemporal Dementia (FTD) often show an overlapping clinical picture, which may lead to misdiagnosis. The investigation of miRNAs carried by extracellular vesicles (EVs) isolated from patients' plasma could provide a new diagnostic tool. This work aimed at identifying deregulated miRNAs, shared between the two pathologies but that presented a different trend. Furthermore, the mRNA targets of a subgroup of the validated miRNAs were identified and studied with the idea of understanding their involvement in dementia pathogenesis. Plasma samples were collected from 20 healthy controls (HC), 20 AD and 13 FTD patients and small EVs (SEVs) were isolated from by differential centrifugation. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples of 20 HC, 20 AD and 10 FTD. MiRNA libraries were generated and sequenced. Bioinformatic analyses were performed to obtain the list of deregulated miRNAs. A subgroup of deregulated miRNAs was selected for validations and then miRNAs-mRNAs interaction prediction was carried out for 3 of the validated miRNAs. We found a total of 339 and 291 differentially expressed miRNAs in FTD and AD respectively. Interestingly, 74 miRNAs were commonly deregulated: 38 showing the same trend while 36 with the opposite trend. 7 miRNAs were selected for technical validation in SEVs. A further validation step was performed on PBMCs, only on the 5 miRNAs with opposite trend between AD and FTD. Targets were identified only for those miRNAs giving the best results and validated. Notably, miR-638 resulted downregulated in AD in both SEVs and PBMCs while two of its targets, CDK2 and SP2, were observed to be upregulated. In conclusion, our data highlight the importance of miRNAs cargo examination in EVs of FTD and AD patients. Indeed, their potential is exploitable both for biomarkers discovery and for gene expression alterations' study in dementia pathogenesis.

Unveiling MAP1B as a new possible phenotypic determinant for sporadic bulbar ALS patients

ND 07

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Amyotrophic Lateral Sclerosis (ALS) is a complex disease with strong variability. Bulbar ALS (bALS) is considered the most severe due to rapid deterioration, short survival time, and worse quality of life. Our aim was to perform two RNA-seq studies on total RNAs and miRNAs in peripheral blood mononuclear cells (PBMCs) from 40 spinal ALS patients (sALS), 10 bALS patients and 19 healthy controls (HC) for total RNA-seq and 19 sALS patients, 6 bALS patients and 11 HCs for miRNA-seq. From total RNA-seq we found 38 deregulated (DE) genes in sALS patients and 373 DE gene in bALS. From miRNA-seq we found 17 DE miRNAs in sALS patients and 10 DE miRNAs in bALS patients. We performed computational research for DE miRNAs' target and crossed results with DE genes from RNA-seq. While in sALS patients, we detected only 1 DE miRNA that had a DE gene as target; in bALS group we identified 3 DE miRNAs, downregulated, which had their target among the DE genes emerged. By cross-referencing targets of identified miRNAs to find shared genes, we evidenced a single target gene (*MAP1B*), upregulated in RNA-seq of bALS, common to the 3 miRNAs, largely involved in neuronal maturation, differentiation and survival. Only targets of mir-92a-3p revealed involvement in muscle contraction. The same miRNA targets TDP-43, largely involved in ALS pathogenesis for its tendency to aggregate in neurons. MAP1B is a component of Lewy Bodies, identified in bALS and capable of interacting with neurofibrillary tangles. Deregulation of mir-92a-3p and MAP1B may cause ectopic protein accumulation in motor neurons (MNs), leading to faster MNs degeneration in bALS phenotype. We validated our results by RT-qPCR in PBMCs and overexpressed mir-92a-3p in SH-SY5Y cells, and looked for MAP1B decrease. RT-qPCR demonstrated the downregulation of MAP1B following miRNA overexpression. MAP1B could represent a future therapeutic target for modulating and improving the poor prognosis of bALS patients.

Set up of "Quadripartite Synapse", a new in vitro model to study synaptic function

ND 08

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The human brain is the most complex organ in biology. This complexity is due to the number and intricate connections of brain cells that have so far limited the development of in vitro models for brain research. To this aim, we set up a new in vitro model of quadripartite synapse formed by neurons, astrocytes, and microglia, taking advantage of the Nichoid technology, a 3D scaffold microfabricated. For this purpose, we first created a model of hippocampal neurons and astrocytes co-cultured in the 3D scaffold to generate brain micro-tissues of 30 µm thickness. After 21 days in-vitro, we observed that astrocytes as well as neurons had become well-differentiated and colonized the entire volume of the Nichoid. Interestingly, in the Nichoid, astrocytes displayed a more physiological morphology, closer to the in-vivo condition, appearing starrier compared to 2D cultures. Moreover, 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. Therefore, our results showed that the Nichioid can be used as a 3D tool for studying brain-resident cells. To advance towards establishing a novel 3D in vitro model of the quadripartite synapse, we decided to add the last cell type involved in synaptic function, microglia, defining the best conditions for the primary co-culture of neurons, astrocytes and microglia in 2D. These results show that the Nichoid is a new 3D device to investigate the structure and morphology of synapses in vitro as well as to study the study of complex cell-cell interactions, the identification of key intracellular pathways involved in synaptic function/dysfunction. Lastly, this 3D scaffol can potentially be further used for drug screening in different brain diseases. The next step will be to move co-culture from 2D to 3D.

Underlying mechanisms for altered reactivity of posterior cortical electroencephalographic alpha rhythms in DLB and PDD

ND 09

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The amplitude of resting-state electroencephalographic (rsEEG) rhythms is a promising biomarker to investigate the abnormalities of oscillatory neurophysiological thalamocortical mechanisms related to the cortical arousal and vigilance in wakefulness in patients with dementia due to neurodegenerative diseases as Alzheimer's (ADD), Parkinson's (PDD) and Lewy Body (DLB) diseases. We tested the hypothesis that the reactivity of posterior rsEEG alpha (about 8-12 Hz) rhythms during the transition from eyes-closed to -open condition may be lower in PDD than in DLB patients. A Eurasian database provided clinical-demographic-rsEEG datasets in 35 ADD, 65 PDD, and 30 DLB patients and 25 matched cognitively unimpaired (Healthy) persons. The rsEEG rhythms were investigated at individual delta, theta, and alpha frequency bands, as well as fixed beta (14-30 Hz) and gamma (30-40 Hz) bands. The eLORETA freeware was used to estimate cortical rsEEG sources. Results showed substantial (i.e., > -10%) reduction (reactivity) in the posterior alpha source activities from the eyes-closed to the eyes-open condition in 88% of the Healthy seniors, 53% of ADD patients, 53% of the DLB patients, and only 37% of the PDD patients. In these alpha-reactive participants, there was lower reactivity in the posterior alpha source activities in the three neurodegenerative groups than in the Healthy group. Furthermore, the reduction of the occipital alpha reactivity was stronger in the PDD and DLB groups than in the ADD group. Finally, the reduction of the parietal alpha reactivity was stronger in the PDD group than in the ADD and DLB groups. These results suggest that those patients may be characterized by very poor reactivity in the posterior cortical mechanisms desynchronizing rsEEG alpha rhythms in relation to increased vigilance levels as an interesting neurophysiological biomarker that may be used as a primary endpoint for interventions to improve vigilance and quality of life in those patients.

Neural and Cardiac Dysfunctions in a Parkinson's Mouse Model

ND 10

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Parkinson's disease (PD) is the second most common neurodegenerative disease in the population aged over 60. It is characterized by severe motor dysfunctions including tremor, rigidity, bradykinesia, and postural abnormalities. Non-motor symptoms such as neuropsychological disturbances and autonomic dysfunction are also common, causing dysphagia, orthostatic hypotension, and thermoregulation issues. PD represents a complex syndrome affecting both the central and peripheral nervous systems, including the noradrenergic system, with impacts on cardiac and vascular functions. In this project we used a 6-hydroxydopamine (6-OHDA) induced PD mouse model to investigate the remapping of cortical processing and to evaluate potential direct effects of dopaminergic alterations on cardiac function. In vivo wide-field imaging in awake animals allowed us to analyze the cortical activity, while transthoracic echocardiography and electrocardiogram (ECG) investigations were employed to assess cardiac dysfunction. Our preliminary results showed a hypoconnected asymmetrical network of PV neurons spanning somato-motor-visual cortical areas in PD mice. Cardiac analyses indicated alterations and a reduced ejection fraction in PD mice compared to controls, and histological examinations suggest an imbalance in cortical excitatory/inhibitory activity. These findings provide significant insights into the impact of dopaminergic depletion on cortical activity in a PD mouse model, highlighting the critical importance of understanding the interrelation between brain and heart in PD. This understanding could lead to comprehensive therapeutic approaches targeting both the neurodegenerative and cardiovascular aspects of the disease.

ND 11

Disentangling the relationship between social cognition, executive functions, and behaviour changes in Amyotrophic Lateral Sclerosis

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Background Social Cognition (SC) deficits are included in the ALS-FTDS revised diagnostic criteria. However, the impact of SC assessment on cognitive classification and the cognitive-behavioural correlates of SC remain unclear. This cross-sectional study aimed to assess the impact of SC assessment on ALS-FTDS categorization, and explore the relationship of SC with executive functions (EF) and behaviour changes in a cohort of ALS patients.
Methods 121 patients and 56 healthy controls from the Turin ALS Center underwent cognitive/behavioural testing, included the SC subdomains of facial emotion recognition (FER), and cognitive and affective theory of mind (ToM).
Results Patients performed significantly worse than controls in all SC explored domains, and 45% of patients exhibited a deficit in at least one SC test, dissociated from the presence of EF deficits. In 13% of cases, the SC deficit was isolated and subclinical. SC assessment contributed to the attribution of cognitive impairment in 10% of patients. Through a statistical clustering approach we found that ToM only partially overlaps with EF, while behaviour changes are associated with emotional disorders (anxiety and depression).
Conclusions SC is overall independent from EF in ALS, with ToM only partially associated with specific EF measures, and behaviour changes associating which emotional disorders. The influence of SC on cognitive categorization and the frequent identification of a subclinical SC impairment have implications in a clinical setting, considering the significant impact of cognitive and behavioural impairment on disease burden and therapeutic choices. In the research setting, these findings may help to disentangle the cognitive anche behavioural manifestation of the ALS-FTD spectrum and encourage exploration of SC impairment as an early marker of cognitive or behavioural dysfunction.

ND 12

Increased expression of transferrin receptor 1 in the brain cortex of mouse model of familial Alzheimer's disease is associated with activation of HIF-1 signalling pathway

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Alzheimer's disease (AD) is a progressive incurable neurodegenerative disease characterized by accumulated amyloid-β (Aβ) peptide aggregates and intracellular neuronal aggregates of hyper-phosphorylated τ. Imbalance of iron is also implicated in AD pathogenesis. Iron levels have been found to increase in AD brain during the disease progression. Moreover, studies in AD patients revealed association between iron accumulation in the parietal cortex and hippocampus with Aβ and τ pathologies as well as oxidative stress, ferroptosis and neuronal loss.
There is growing evidence that transferrin receptor 1 (TfR1) expression is altered in AD brains, which can contribute to iron dyshomeostasis. Studies showed that TfR gene transcription is regulated by hypoxia and that HIF-1A, the best characterised transcriptional activator of hypoxia-sensitive genes, is the key player in this process. However, the involvement of HIF-1 signalling pathway in the regulation of TfR1 expression in the AD brain has not been studied.
Considering a significant role of TfR1 in brain iron transport and CNS drug delivery, this study provides important information on changes in TfR1 expression in brain cortex and isolated brain microvessels from 5xFAD mice. In addition, we investigated if Aβ peptides can affect expression of TfR1 in brain endothelial cells *in vitro*. We revealed an increase in TfR1 protein levels in the brain cortex of 5xFAD mice, associated with activation of the HIF-1 signalling pathway as well as accompanied by oxidative stress and inflammation. Also, no changes in TfR1 expression were detected in isolated microvessels and hCMEC/D3 cells treated with Aβ peptides, indicating that the Aβ pathology alone was not able to induce TfR1 upregulation in the endothelial cells of the brain vasculature.
Overall, the findings of the study demonstrated that modulating TfR1 expression via targeting HIF-1 signalling pathway may be a novel pharmacological intervention for the treatment of AD.

ND 13

Biophotonics-based analysis of multifunctional liposomes as drug delivery systems for brain diseases

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Drug delivery to the brain is challenging because of the blood–brain barrier, which limits drugs' access to the brain. Liposomes (LPs) have been proposed as drug-delivery systems that often enhance therapeutics' delivery into the brain, thanks to their versatile properties. However, LPs' characterization represents a crucial step in achieving a comprehensive overview of their physical, chemical, and biological features, and in evaluating their efficacy and safety in biological systems.

Herein, we propose Raman Spectroscopy (RS) and Surface Plasmon Resonance imaging (SPRi) for the characterization of multi-functionalized LPs as putative agents against neuroinflammation and microglial dysfunctions in Glioblastoma multiforme and Alzheimer's disease; in particular, RS and SPRi were used to test their reproducibility, stability and binding affinity with selected receptors. LPs were functionalized with mApoE and with a metallo-protease sensitive lipopeptide, to guarantee the targeted delivery and release of an encapsulated drug in diseased areas.

RS analysis allowed to statistically discriminate LPs with different functionalization patterns, showing that each molecular component has an influence on the Raman spectrum for a LP formulation. Moreover, Raman data highlighted good synthetic reproducibility for tested formulations, and confirmed their stability within one month from the synthesis.

Moreover, SPRi analysis showed that mApoE-LPs mainly interacted with brain receptors, whereas they did not interact significantly with peripheral receptors expressed on monocytes and lymphocytes. SPRi results confirmed not only the presence of mApoE on LP surfaces, but also its binding affinity, thanks to its specific interaction with selected receptors.

In conclusion, our results confirmed that RS and SPRi represent valuable tools for the biochemical characterization of multi-functional LPs, and to evaluate the efficacy and reproducibility of their synthesis.

Identification of prodromal biomarkers of Alzheimer's disease in 5XFAD mice: focus on brain-derived extracellular vesicle content

ND 14

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Extracellular vesicles (EVs) are nanosized membranous particles secreted by cells into the extracellular space containing a variety of functional cargoes, including lipids, proteins, mRNAs and miRNAs. EVs represent a physiological mechanism of intercellular communication but also a key player in the development of several neurodegenerative disorders, such as Alzheimer's Disease (AD). Recently, EVs have been efficiently isolated from cerebrospinal fluid and blood of AD patients and transgenic AD mouse models demonstrating their capability to transport pathological cargoes, such as amyloid β (Aβ), pro-inflammatory cytokines and miRNA. Furthermore, a correlation between specific miRNAs found in EVs and the severity of AD has been proposed. However, the underlying pathological mechanisms remain unclear. We aimed to investigate the composition of EVs isolated from 5XFAD, a transgenic model of AD, mouse brains unveiling their pathological function during prodromal and early phases of AD. In order to isolate EVs directly from mouse brain tissue, a new methodological approach was standardized. Cortical- and hippocampal-isolated EVs were analyzed through the Nanoparticle Tracking Analysis system and the average size was about 100 nm, as expected. Subsequently, the expression of specific membranous EV-antigens was measured by western blot analysis; in addition, untargeted proteomic approach was used in order to further characterize these samples. Total RNA was extracted from EVs and the expression of brain-enriched miRNA was detected through qPCR approach. Obtained results validated a new methodological approach for *ex-vivo* EVs isolation from cortical and hippocampal dissected brain areas and for their content characterization during different phases of AD progression.

ND 15

The cognitive decline associated with Alzheimer's Disease is paralleled by serotonergic dysfunctions and altered PFC-DRN communication in the 3xTg-AD mouse model

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Alzheimer's disease (AD) is characterized by a slowly progressive cognitive decline, involving the dysfunction of various neurotransmitter pathways, including the serotonergic (5-HT) system. Indeed, treatment with selective serotonin reuptake inhibitors reported positive effects on AD onset, progression, and cognition. A crucial region for 5-HT production is the dorsal raphe nucleus (DRN), which provides most of the 5-HT innervation to various brain areas. One of the most important, the prefrontal cortex (PFC), is highly interconnected with the DRN and forms a bidirectional circuit with a role in numerous functions, comprising cognitive processes. Our study aimed at investigating the involvement of the 5-HT system and the PFC-DRN circuit in cognitive decline in AD, studying the 3xTg-AD mouse model at 4 and 8 months of age, namely before and after disease onset. Immunohistochemical analysis of the DRN for tryptophan hydroxylase-2 (TPH-2) revealed a decrease in 5-HT neurons in 8-month-old 3xTg-AD mice compared to controls, and a trend toward reduced TPH-2+ neurons in young mice, despite no differences were observed in the total number of neurons at either time point. Moreover, *in-vivo* single-unit extracellular recordings in the DRN showed reduced 5-HT neuronal activity at 8 but not at 4 months in the 3xTg-AD compared to controls. Finally, the novel object recognition (NOR) test confirmed cognitive impairment in 8-month-old 3xTg-AD mice, and local field potential (LFP) analysis of the DRN-PFC circuit during the novel object exploration phase showed decreased coherence between these two areas in the alpha frequency range at 8 months in 3xTg-AD mice. In this preclinical model, our results indicate that the progression of AD is associated with an impairment of the 5-HT system and the dysfunction of the PFC-DRN circuit, in parallel with cognitive decline.

ND 16

The small-molecule PERK inhibitor LDN-0060609 rescues human retinal astrocytes from ER stress-mediated cell death, thereby targeting primary open-angle glaucoma

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Introduction: Human retinal astrocytes (HRA) promote neuronal survival, and their loss precedes optic nerve (ON) degeneration in the pathogenesis of primary open-angle glaucoma (POAG). Endoplasmic reticulum (ER) stress and PERK kinase activation lead to HRA death in vitro and in vivo, compromising their neuroprotective effects upon the ON. Thus, inhibition of the PERK pathway may protect HRA and prevent POAG development.
Aim of the study: The study aimed to evaluate the properties of the small-molecule PERK inhibitor LDN0060609 (LDN) in HRA upon ER stress in vitro.
Materials and methods: Experiments were conducted on the primary HRA cell line. To induce ER stress, HRA were exposed to 500nM thapsigargin. LDN efficacy was assessed by measuring phosphorylation of the major PERK substrate – eIF2α, by Western Blot. Expression of ER stress-related proapoptotic genes (ATF4, DDIT3, BAX, Bcl-2) was determined by qRT-PCR analysis. Cytotoxicity and genotoxicity of LDN0060609 were evaluated using the XTT assay and alkaline comet assay, respectively. Apoptosis was assessed by caspase-3 assay, and cell cycle analysis via flow cytometry with propidium iodide staining.
Results: LDN demonstrated its highest efficacy in ER-stressed HRA cells at 25 µM, significantly inhibiting eIF2α phosphorylation. At the same concentration, it notably decreased the expression of proapoptotic ER stress-related genes (ATF4, DDIT3, BAX, Bcl-2) in ER-stressed HRAs. LDN exhibited no significant cytotoxicity or genotoxicity towards HRA cells at any used concentration. Whereas LDN-0060609 treatment improved cell viability, reduced DNA damage, decreased caspase-3 levels, and restored cell cycle distribution in ER-stressed HRA cells.
Conclusions: LDN showed a protective effect towards HRA by alleviating the negative effects of ER stress, hinting at its prospective role in POAG treatment. This work was supported by National Science Centre, Poland (grant no. 2016/21/B/NZ5/01411).

Characterization and modulation of OPN-MMP-9 axis in Amyotrophic Lateral Sclerosis organoids

ND 17

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by loss of upper and lower motorneurons (MNs). The patient undergoes progressive muscle paralysis that leads to death within 2-5 years from diagnosis. Current challenges comprise the introduction of efficient cellular models that mimic ALS features *in vitro* and the discovery of disease-modifying drugs. Osteopontin (OPN) is a multifunctional glycophosphoprotein that takes part in several physiological and pathological conditions. In the context of ALS, OPN was observed to have a double-edged role: a neuroprotective action on astrocytes through CD44 receptor and a neurodegenerative effect on neurons when binding αvβ3-integrin. We are currently working on the generation and characterization of MN organoids that recapitulate genetic ALS in particular *TARDBP*, *SOD1* and *C9ORF72* mutated forms. We reprogrammed patient-derived peripheral blood mononuclear cells into induced pluripotent stem cells (iPSCs). We are now differentiating iPSCs into MN organoids in order to perform morphological and cellular characterization with microscopy and immunofluorescence. We will then perform RT- qPCR and Western Blot to characterize OPN-MMP-9 axis in the organoids. Once known the main joints of the pathway, inhibition of the lead actors will be performed in order to understand the role of OPN-MMP-9 modulation in ALS. This research will produce new 3D organoid models for the study of genetic ALS and it will clarify the impact of OPN on the disease.

Neutrophil crosstalk with leptomeningeal macrophages contributes to the development of experimental autoimmune encephalomyelitis

NI 01

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Meningeal inflammation is a detrimental process fueling central nervous system (CNS) dysfunctions that has been recently included among the key pathological features of multiple sclerosis (MS). Moreover, recent pioneer research has linked neutrophil accumulation within the leptomeninges of MS patients with a more aggressive disease phenotype. In this context, our project used an MS mouse model, the experimental autoimmune encephalomyelitis (EAE), to identify a previously unrecognized crosstalk between neutrophil extravasating into the leptomeninges and local macrophages. This myeloid interaction occurred at the early stages of the disease, potentially fueling its worsening and progression. Particularly, our single-cell RNA sequencing data suggested that neutrophils displayed a migratory and activated profile, prone to cell-cell interaction during the acute phase of EAE. These transcriptomic results were confirmed by advanced *in vivo* two-photon microscopy experiments. With this approach, we demonstrated the pivotal role of integrin-dependent neutrophil-macrophage interactions in controlling neutrophil dynamics and localization within the inflamed leptomeninges. Moreover, we collected preliminary data suggesting that the depletion of circulating neutrophils before EAE onset impairs the activation of leptomeningeal macrophages. Remarkably, the local administration of anti-integrin antibodies disrupted the leptomeningeal neutrophil-macrophage axis, inducing a progressive neutrophil decompartmentalization. Interestingly, intrathecal anti-integrin therapy also ameliorated the EAE clinical course, impairing the activation of CNS macrophages. Globally, our results suggest that infiltrating neutrophils and leptomeningeal macrophages represent a proinflammatory axis favoring disease development and progression, and interfering with this leptomeningeal myeloid crosstalk may be effective in attenuating neuroinflammatory conditions.

NI 02

Meningeal lymphoid structure as secreted hideout of EBV-induced proliferating cells in multiple sclerosis

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Chronic inflammation compartmentalized in central nervous system niches, such as meningeal tertiary lymphoid-like structures (TLS), is suggested to play a key role in progressive multiple sclerosis (pMS) and to represent the main intracerebral hideouts where Epstein-Bar virus (EBV) persists and stimulate chronic inflammation.
To further characterize the meningeal B cell phenotypes and features potentially linked to EBV-induced immune response.
Formalin-Fixed Paraffin-Embedded (FFPE) and fixed frozen sections from 60 post-mortem pMS cases (40 MS from UK MS Society Tissue Bank and 20 MS from WUSTL Tissue Bank) and 10 healthy donors (CTRL) were immunostained to obtain semi-quantitative cell counts of meningeal T- and B-cells and macrophages. Combined immunohistochemistry and immunofluorescence detection of EBV, both latent (EBNA2, LMP1, LMP2) and lytic (BRLF1) cycle antigens, was performed in the same MS cases.
The presence of B cells expressing the Epstein-Barr virus nuclear antigen 2 (EBNA2) and the proliferating marker Ki67, were detected in the meninges of the MS subgroup characterized indeed by a significant highest number of B cells compared to both macrophages ($p=6.218x10-13$) and T cells ($p=7.000x10-4$). In particular, most of the detected B cells, in presence of EBNA2 and other latent cycle markers, such as the latent membrane protein 1 and 2A (LMP1 and LMP2A), were characterized by the expression of the germinal center marker CD81 and by the elevated immune-reactivity for K-light immunoglobulin chains and immunoglobulin Ig-M. These features were detected in the subgroup of MS cases with shorter time from progression to wheelchair-use and early age at death.
Summarizing, sustained MS intrathecal expansion and accumulation of B cells occur in the meningeal compartment and is associated with active anti-viral (EBV) immune response.

Persistence of Epstein-Barr virus infection in Multiple Sclerosis brain: altered cytotoxic immune response and immune evasion strategies

NI 03

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Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system resulting from complex interactions between known susceptibility genes, lifestyles and environmental factors. Among the latter, Epstein-Barr virus (EBV) infection shows the strongest association with MS, and has been demonstrated to be a prerequisite for disease development. The effectiveness of drugs targeting B-cells, the latent EBV reservoir, strengthens the view that reducing EBV load, and therefore the cellular immune response to EBV, has beneficial effects on people with MS. Despite these evidences, the pathological mechanisms linking EBV to MS pathology, and favouring the persistence of the virus in the MS brain, are still unclear. To explore the effectiveness of the EBV immune control in MS, we performed careful neuropathological studies of the presence and functional characteristics of tissue resident memory (Trm) T cells, and of the involvement of the viral immune evasion mechanism based on the PD-1/PD-L1 axis, in post-mortem brain tissue donated by persons with secondary progressive disease. We found: presence of EBV-infected B cells and EBV-specific CD8 T cells in the inflammatory infiltrates; alterations in the cytotoxic functionality of the Trm cells; expression of molecules related to the viral immune evasion mechanisms on B and T cells invading MS brain. These evidences suggest that EBV could use the PD-1/PD-L1 axis to establish a persistent infection in the MS brain, which induces a continuous, inefficient and highly detrimental, intracerebral antiviral immune response. Our data support a model of MS as “a rare neurological complication of a very common viral infection” in which EBV is the main driver of the disease.

This research was supported by FISM - Fondazione Italiana Sclerosi Multipla - cod. 2022/R-Single /006 and financed or co-financed with the ‘5 per mille’ public funding, and by European Union within BEHIND-MS project.

The role of microglia in the neuroinflammatory response following perinatal stroke

NI 04

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An ischemic event occurring during the early stages of development, i.e. the perinatal period, may overlap critical time windows for proper brain development. Although the stroke-induced inflammation arises centrally in the nervous system, compelling evidence reports an acute and chronic widespread peripheral inflammatory reaction orchestrated by key inflammatory mediators (mainly cytokines and chemokines), linked to ischemic brain injury outcomes. Microglia cells, as the intrinsic immune cell population, are decisive in the damage response and brain development. Once activated following stroke, they are involved in the pathological processes with a dual role as participants to brain pathology and contributors in the repair attempts. Growing evidence reveals the significance of inflammation as a risk factor for lifelong neurodevelopmental consequences, yet the complete characterization of this complex interplay between the systemic inflammation, the microglia and behavioral outcomes is still an unmet need. To tackle this challenge, we used the middle cerebral artery occlusion (MCAO) stroke model in juvenile mice (P14) to induce the cortical lesion and we assessed the motor and cognitive functions by a battery of behavioral tasks, in both acute and chronic. Next, we analyzed the peripheral blood samples collected at different time points to unveil the levels of principal factors that might correlate with motor behavioral outcomes. Finally, to investigate the role of microglia in perinatal stroke, after 7-days microglia depletion with a CSFR-1 inhibitor, we expect to reveal a functional recovery compared to vehicle animals. This study will provide novel insights into the neuroinflammatory response, following perinatal stroke.

Myricetin loading into microglial cells' extracellular vesicles as alternative therapeutic strategy for neuroinflammation

NI 05

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Microglia cells are resident macrophages of the central nervous system. They represent the first active defence mechanism against pathogens and non-infectious injuries such as hypoxia and endogenous misfolded proteins. However, continuous exposure to inflammatory stimuli leads microglial cells toward the M1 phenotype and consequent release of inflammatory and neurotoxic factors contributing to neuroinflammation and brain damage. In our previous studies, we reported that myricetin, a natural antioxidant and anti-inflammatory flavone, suppresses inflammatory pathways and keeps microglia in resting state, protecting neurons from death in an *in vitro* model of neurotoxicity (Boriero, D et al. (2021) FEBS J 288, 2347-2359). Nevertheless, its high hydrophobicity and low bioavailability limit its use in *in vivo* applications. To overcome myricetin's drawbacks, we propose the use of small Extracellular Vesicles (EVs) as a drug delivery system for myricetin in the context of microglia activation. EVs are cell membrane-derived nanovesicles that recently emerged as potential drug delivery systems thanks to their non-immunogenicity, nanometric size and ability to cross the blood-brain barrier. In this work, EVs have been isolated from a cell-conditioned medium (CCM) of the murine microglia BV2 cell line through differential ultracentrifugation and filtration steps. They have been characterised for size and quantity through nanoparticle tracking analysis (NTA) and western blot for the expression of vesicle markers. Once isolated, the EVs have been encapsulated with myricetin through passive diffusion in presence of saponin and purified by ultrafiltration. High-performance liquid chromatography has been used to evaluate the encapsulation efficiency of EVs.

NI 06

Nanoparticle-mediated delivery of a new TSPO ligand suppresses inflammation in LPS-stimulated microglia and in a mouse model of Alzheimer's Disease

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The translocator protein 18kDa (TSPO) is a conserved outer mitochondrial membrane protein, implicated in inflammation, cell survival and proliferation. In the central nervous system (CNS), the expression of TSPO is markedly upregulated in activated microglia, a condition common to several neurological disorders including Alzheimer's Disease.
Over the years, TSPO emerged as an important theranostic agent, being provided with diagnostic and therapeutic properties. Indeed, TSPO expression represents a diagnostic feature of neuroinflammation and TSPO ligands may act as putative therapeutic agents for those neurological disorders characterized by an important neuroinflammatory component.
In the present study, we synthesized a new TSPO ligand, nanoincapsulated in self assembling nanoparticle (SANP), that we named TEMNAP an hybrid between temazepam and naproxen. The effectiveness of TEMNAP in mitigating inflammatory processes was investigated in lipopolysaccharide (LPS)-activated BV2 microglial cells, while the effect on cognitive behavior and *in vivo* inflammation was explored in Tg2576, a genetic mouse model of Alzheimer's disease.
SANP-TEMNAP significantly reduced *in vitro* and *in vivo* the expression of iNOS and CD86, two pro-inflammatory markers of hyperactived microglial cells. In addition, when peripherally administered *in vivo*, transferrin (TF)-conjugated SANP-TEMNAP (SANP-TF-TEMNAP) ameliorated cognitive functions in Tg2576 mice by preventing the hippocampal hyperactivation of microglial cells.
These results emphasize the role of transferrin-conjugated self-assembling nanoparticles as cargo for brain delivering of TSPO ligands as therapeutic agents useful in preventing neuroinflammation associated to cognitive decline.

NI 07

Experimental midbrain lesion drives hippocampal monoamine drop causing NLRP3-mediated neuroinflammation and Alzheimer's disease-like deficits

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Dopamine (DA) and serotonin (5HT) monoamines are neurotransmitters known to tune cognitive and non-cognitive functions progressively altered in neurodegenerative disorders as Alzheimer's disease (AD). Recent preclinical and clinical works highlighted midbrain alterations and deficits of monoaminergic ascending pathways in midbrain-target areas, as the hippocampus, since the AD prodromal phase. Although neuroinflammation was historically linked to amyloid beta (Aβ) plaques and tau pathology, nowadays it is considered responsible for exacerbation of AD pathology. However, a direct connection between the observed midbrain deficits and insurgence of neuroinflammation in AD is missing. We hypothesized that an early midbrain degeneration can *per se* drive hippocampal neuroinflammation, in absence of Aβ pathology. To prove this, we developed ventral midbrain-lesioned mice through a unique viral approach allowing the overexpression of the pro-apoptotic active *Caspase-3* (Casp-3) under the Tyrosine Hydroxylase promoter expressed in DA midbrain neurons and in neighboring 5HT cells of the interpeduncular nucleus (IPN). Upon midbrain lesion, we found that low levels of hippocampal monoamines were strictly related to microglia-mediated inflammation. Molecular analysis revealed that this inflammatory process depends on NLRP3-inflammasome pathway. Furthermore, pharmacological treatments boosting DA and 5HT tone blunted NLRP3-mediated hippocampal neuroinflammation in Casp3-injected mice. The same midbrain lesion applied to pre-plaque stage-Tg2576 mice, a validated AD mouse model, caused hippocampal hyper-inflammation and increased intracellular Aβ load, thus accelerating the AD phenotype. Overall, our results demonstrate that precocious midbrain damage triggers neuroinflammatory events, in turn worsening AD pathology.

Inflammatory mediators and kynurenine pathway in children and adolescents with bipolar disorder

NI 08

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Bipolar disorder (BD) is a mood disorder characterized by recurrent alternating episodes of depression, mania or hypomania, and euthymia. Early onset of the disorder (childhood-juvenile BD) is associated with a more severe course, worse prognosis and higher suicide rate. Stress may cause inflammatory mechanisms involved in the pathophysiology of BD and, in addition, a proinflammatory environment may modify tryptophan metabolism towards the kynurenine pathway. This work is an observational study in which 51 patients with childhood and juvenile BD were followed for one year of follow-up, and 37 matched controls. The levels of inflammatory mediators and metabolites of the kynurenine pathway were determined in plasma and peripheral blood mononuclear cells. A correlation analysis was also performed between both biochemicals variables, and their correlation with the child global assessment scale (c-GAS). This study shows a significant increase in proinflammatory mediators and neurotoxic thyptophan-derived metabolites at baseline and during the follow-up of patients with childhood-juvenile BD, as well as associations between them and with the c-GAS clinical scale. The results suggest that plasma levels of tumoral necrosis factor α, interleukin 1β, nuclear factor of transcription κB, indoleamine 2,3-dioxygenase and quinolinic acid may be risk factors of the disorder, while plasma levels of serotonine and acid kynurenic may function as protective. These findings may represent an improvement in the treatment and evolution of childhood-juvenile BD, if moved towards personalized medicine due to the clinical heterogeneity presented by these patients.

Novel bioactive compounds inhibiting microglial activation for the treatment of neuroinflammatory conditions

NI 09

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Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Amyloid-β plaques (Ab) and neurofibrillary tangles, which lead to neuronal loss and impaired cognitive functions. Neuroinflammation is another hallmark of the disease, and microglial cells are the primary players among the innate immune cells promoting the inflammatory responses into the brain. The increasing AD incidence demands the urgent development of effective therapeutic strategies. In recent years, a wide range of natural compounds with pharmacological activities provided some beneficial effects for AD treatment. Hence, in this work, we aimed to identify novel plant extracts with anti-inflammatory properties to reduce neuroinflammation, mitigating AD pathogenesis. Initially, we performed *in vitro* MTT assay to assess the potential toxicity of twenty-three plant-derived extracts. Then we investigated the anti-inflammatory properties of safe extracts measuring lipopolysaccharides (LPS)-induced nitric oxide (NO) production and TNF-a pro-inflammatory cytokine levels on both SIM-A9 microglial cell line and primary microglia. Our findings showed that Althea Officinalis, Adenophora Lilifolia, Dianthus Superbus, and Succisa Pratensis do not exhibit toxic cellular effects. Additionally, these plant extracts significantly reduce NO production and the release of TNF-a in LPS-treated microglial cells. Taken together, our data show that the potential anti-inflammatory properties of plant extracts may be promising in attenuating neuroinflammatory response and reducing symptoms of AD.

Effects of environmental enrichment on neuroinflammation, synaptic and behavioral impairments in a mouse model of autism spectrum disorder

NI 10

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R451C-NLGN3 mice represent a mouse model of Autism Spectrum Disorder (ASD) with construct and face validity. Our previous work reported an impairment of synaptic plasticity in the dorsal striatum of R451C-NLGN3 mice. These observations are particularly relevant given the central role of the striatum in the pathophysiology of ASD, which is supported by clinical, genetic, and experimental evidence.
Recent studies suggest that neuroinflammation can drive changes in the development and function of the Central Nervous System (CNS). When activated, this process is actively counterbalanced by specific anti-inflammatory/pro-resolving signaling pathways. Based on this scenario, we hypothesized that an ongoing neuroinflammatory process, together with defects in the pro-resolution pathway might be involved in the manifestation of the synaptic and behavioral alterations observed in the R451C-NLGN3 mouse model of ASD. Therefore, in the present project we investigated whether the ASD-linked R451C mutation in the synaptic adhesion protein NLGN3 might instigate a neuroinflammatory process in the striatum, triggering/worsening the synaptic dysfunction and the ensuing behavioral alterations observed in the mouse model. Our results show that, indeed, in the dorsal striatum of R451C-NLGN3 mice both microglia and astrocytes express higher levels of pro-inflammatory markers, increased levels of pro-inflammatory cytokines and reduced level of specialized pro-resolving lipid mediators, indicating the occurrence of a neuroinflammatory process.
Notably, different studies have reported that combined sensory/motor/cognitive stimulation induced by environmental enrichment may blunt neuroinflammation in various pathological conditions. We therefore analyzed the effect of a 8-week long environmental enrichment, and found that it was able to revert the neuroinflammatory, synaptic and behavioral impairments of the R451C-NLGN3 mouse model of ASD.

Corticosterone treatment induces a mild stress condition on hippocampal murine astrocytes

NI 11

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Major Depressive Disorder (MDD) is the most common mental health disorder, affecting 280 million people worldwide. The pathophysiology of MDD is complex, and recent evidence indicates that inflammation can drive negative outcomes. To better define the mechanisms linking inflammation and depression, we set up a preclinical in vitro murine model using primary hippocampal astrocytes treated with subtoxic doses of corticosterone for 24 and 48 hours.
This study aimed to characterize the effects of corticosterone on primary hippocampal astrocytes from a biochemical point of view. We tested corticosterone on astrocytes in a dose-response concentration for 24 and 48h hours, to define a subtoxic-dose that induce cellular stress without cell death. Once established the subtoxic dose (25 uM), we performed biochemical analysis to study the stress pathways. In particular, we studied JNK, GSK and AKT kinases signalling, that are known to be involved in different stress responses. Concerning the P-JNK/JNK ratio, we observed a significant decrease at 24 hours, while the ratio returned to the control level after 48 hours of treatment, suggesting that it is not implicated in the corticosterone response. GSK3 is involved in the regulation of inflammatory responses in astrocytes, P-GSK3/GSK3 ratio did not present any significant changes. The P-AKT/AKT ratio showed a significant increase after 24 hours of treatment, while at 48 hours it significantly decreased.
Finally, we studied GFAP and ALDH1L1, two specific markers commonly used to assess the proliferative and reactive state of astrocytes, respectively. No significant changes were detected in the levels of GFAP and ALDH1L1 in corticosterone-treated compared to control. Our results suggest that in our in vitro model corticosterone induced a mild stress condition, characterized by an AKT activation without a reactive gliosis response.

Sex-specific activation of brain barrier resident macrophage-like cells in APP/PS1 mice

NI 12

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The choroid plexus (ChP), an intraventricular structure composed primarily of specialized epithelial cells in contact with fenestrated capillaries, establishes the blood-cerebrospinal fluid (CSF) barrier. The ChP is well known for actively producing CSF and transporting solutes to and from the brain, thus playing a crucial role in maintaining cerebral homeostasis. In healthy aging, the ChP undergoes structural and functional modifications, also exhibiting higher levels of inflammatory markers and increased infiltration of immune cells. Interestingly, some of these architectural and functional disturbances are thought to accelerate brain aging and contribute to the progression of neurodegenerative disorders, such as Alzheimer's disease (AD). However, the involvement of the ChP in AD onset and progression, the timing of its disruption and the specific cell types involved in the process are still poorly understood. Here, we characterized the ChP of an AD mouse model (i.e., APP/PS1 mice) at different stages of the disease. We detected neither major structural modifications nor amyloid plaque formation in epithelial cells of male and female APP/PS1 mice. However, we found that the density of resident and ChP-specific macrophage-like cells was significantly increased in APP/PS1 mice compared to littermate controls. Both cell types displayed a clear phagocytic activated state, suggesting an active inflammatory response. Notably, this phenotype was present only in APP/PS1 male mice, indicating a sex-specific ChP response in AD mice. These observations suggest a potential role of ChP inflammatory responses in AD pathogenesis, challenging the traditional neuron-centric view.

Mitochondrial itaconate drives anti-inflammatory effect in reactive microglia

NI 13

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Friedreich's Ataxia (FA) is an inherited autosomal neurodegenerative disorder caused by a mutation in the gene encoding for the mitochondrial protein frataxin (FXN) leading to the decrease of its content. To better investigate the molecular mechanism beyond this pathology, 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 level. Metabolomic analysis reveals that LPS treatment leads to an increase of glucose catabolism, more evident in FXN- microglia cells. Itaconate, a mitochondrial metabolite, is produced by macrophages in response to inflammatory stimuli. Consistent with this evidence, we found that itaconate production is increased after inflammatory stimulus in FXN- cells. In line with this result, the enzyme responsible for itaconate synthesis, i.e. Irg1, is upregulated in microglia cells of FA cerebellum. Treatment of BV2 FXN- cells with itaconate protects against inflammatory stimuli through a NRF2-mediated antioxidant mechanism. These findings point to itaconate as a novel therapeutical option to improve FA-related inflammatory symptoms.

BTK inhibitors modulate remyelination in Multiple Sclerosis

NI 14

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Introduction: Mechanisms for repairing myelin in multiple sclerosis (MS) are disrupted, leaving axons vulnerable to damage, particularly in progressive MS. Nowadays, remyelination is a crucial but unmet therapeutic need in MS. Bruton's tyrosine kinase inhibitor (BTKi), like ibrutinib, showed promise in delaying MS progression. However, remyelination studies have utilized prenatal models. Here, we propose a novel in vitro model using cells from postnatal mice to assess the effects of ibrutinib on remyelination, aiming to quantify remyelination while minimizing animal use.

Aims: 1. Validate an in vitro model of de- and re-myelination using postnatal mice; 2. Investigate ibrutinib's effects on remyelination.

Materials and Methods: Mouse pup spinal cord cultures were demyelinated with lysophosphatidylcholine (LPC) and treated with or without ibrutinib (1 µM) for 7 days. Cultures were then analyzed for myelination using immunofluorescence staining and image analysis.

Results: Cultures comprised 45% OPCs/Oligodendrocytes, 25% neurons, 20% microglia, and 10% astrocytes. Myelination reached 59%. After LPC-mediated demyelination, myelination improved (49% vs. 59%), with ibrutinib-treated cultures showing superior remyelination (60%) compared to untreated cultures (49%), approaching levels in undemyelinated controls (59%).

Discussion: This study demonstrates ibrutinib's positive effects on remyelination in a new *in vitro* animal model. These effects likely involve interactions between BTK-expressing cells and OPCs. Although we observed no differences in extracellular vesicles (EVs) release from microglial cells, we speculate that ibrutinib may modify EVs content, influencing OPCs/microglia interaction.

Conclusions: • Postnatal mouse cultures exhibit remyelination after LPC-mediated demyelination.• Ibrutinib enhances remyelination in a mouse model of de- and re-myelination. • Further studies are needed to assess ibrutinib's effects on EVs content and remyelination in human cell-based models.

Combined pharmacological and cellular therapies to regulate glial cells in spinal cord injury

NI 15

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Spinal cord injury (SCI) is a devastating neurological disorder that currently lacks an effective treatment. Despite promising results from preclinical studies, none of these therapies translated into successful clinical outcomes. This necessitates for the development of novel therapeutic strategies.The aim is to combine two therapeutic treatments that target different aspects of secondary injury to maximize the therapeutic effects: selective nanoparticles to target astrocytes and microglia/macrophages and the Stromal Vascular Fraction (SVF) with paracrine effects, aiming to modulate the inflammatory response, promote angiogenesis, and enhance regeneration. **Methods:** (i) SVF was harvested and processed under GMP conditions and were fluidically loaded into lyophilized hydrogels (HG). Cell survival, adhesion, and density were evaluated over time in vitro and in vivo. (ii) A new functionalized nanogel-based nanovector (NG) addressed to microglia and astrocytes was tested in vitro and in vivo. (iii) Rolipram loaded NG and SVF+HG was administered at an acute stage in a preclinical model of SCI, and behavioral and neuropathological analysis were carried out using an innovative count system based on machine learning (AI). **Results:** (i) We evaluated the therapeutic efficacy of NG-Roli both in vitro and in vivo. (ii) We characterized and optimized the composition of the HG and we evaluated the therapeutic efficacy of SVF-loaded HG both in vitro and in vivo. (iii) Finally, we combined both NG-RhB/Rolipram and SVF-loaded HG therapies to combine their effects and enhance their therapeutic potential. **Conclusion:** Co-administration of NG-Roli and SVF-HG demonstrated an additive therapeutic effect, significantly exceeding the efficacy of individual treatments. This finding highlights the importance of a multimodal approach that simultaneously targets multiple pathological mechanisms in SCI.

Monitoring muscle and nerve damage in ALS and CMT1A animal models using in vivo imaging

NIM 01

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Biological markers are a valid tool for understanding neurological diseases, including neuromuscular disorders. The application of imaging biomarkers, specifically for the *in vivo* investigation of diseases, proved to be a fundamental resource for identifying early pathological changes, monitoring disease progression, and evaluating therapeutic interventions. Charcot-Marie-Tooth (CMT) and Amyotrophic Lateral Sclerosis (ALS) are neurological diseases characterized by progressive muscle weakness and atrophy. CMT encompasses a heterogeneous group of inherited neuropathies, mostly characterized by muscle atrophy due to nerve damage, while ALS is a terminal neurodegenerative disorder causing muscle weakness and paralysis secondary to progressive loss of both upper and lower motor neurons. In this study, we explored the usefulness of a deep imaging protocol to assess nerve and muscle pathology in animal models of ALS and CMT diseases, namely PMP*22* transgenic rats and SOD1*G93A* mice. The protocol includes 7 Tesla Magnetic Resonance Imaging (MRI), Ultrasonography (US), Photoacoustic (PA) and micro-PET imaging. MRI scanning includes morphological and fat suppression sequences to evaluate nerve integrity and muscle fat infiltration. US was used to analyze the muscle texture, while PA imaging allowed us to combine anatomical information with molecular measurements, such as the assessment of collagen and lipid content. Finally, we used micro-PET imaging to appreciate metabolic differences in muscle and brain. Overall, we defined an original protocol for live monitoring of muscle and nerve pathology using cutting-edge technologies. We are confident that this study will provide effective and valuable means for the stratification of patients based on anatomical, functional, and molecular measurements.

NIM 02

Microstructural Changes and Damage in ARSACS Corticospinal Tracts: PROSPAX Cohort Insights

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Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) is a rare recessive form of spastic ataxia, characterized by the development of a significant spasticity as a possible reflection of a corticospinal tract (CST) involvement. This study aims to test the hypothesis of a damage gradient in the CST using part of the data from the PROSPAX consortium (37 ARSACS, F=16;33.4±12.4 years; and 29 healthy controls F=16;42.1±17.2 years).

The microstructural integrity of the CST was examined and the spatial distribution of damage along the tract was tested using profilometry analysis. Additionally, a potential thickening of pontine fibers was also analyzed. Possible correlations between CST microstructure and clinical severity, evaluated via the Spastic Paraplegia Rating Scale (SPRS) and the Scale for the Assessment and Rating of Ataxia (SARA), were probed.

Compared to controls, ARSACS patients showed significant bilateral changes in CST microstructure, with reduced fractional anisotropy (FA) and increased mean diffusivity (MD) and radial diffusivity (RD) ($p<0.001$). Profilometry analysis revealed a gradient of damage, with the most pronounced FA reduction in the midbrain, cerebral peduncles, and posterior limb of the internal capsule. Furthermore, patients showed an increased pontine volume ($p<0.001$), mainly due to the middle cerebellar peduncles with a reduction in transverse pontine fibers, supporting a neurodevelopmental alteration hypothesis. A bilateral negative correlation ($p=0.02$) between CST integrity above the pons and SPRS emerged, along with a significant correlation with SARA that was found for the left CST ($p=0.002$).

These findings suggest the possible use of this metrics as potential biomarkers for disease progression and therapeutic interventions in this condition, despite the cross-sectional design of the study and dMRI limitations. Future research should include spinal cord evaluations to further understand the extent of CST damage.

Impact of Conventional and FLASH Radiotherapy on glioma murine model

NO 01

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Glioblastoma multiforme (GBM) is the most common primary brain cancer in adults with poor prognosis. Despite the current state of knowledge on its genetic characteristics, and technological advanced in surgery and radiotherapy relatively little progress has been made to improve patient's outcome. Radiotherapy (RT) is a crucial treatment for GBM following surgical resection to improve both local control and survival. Unfortunately, radiotherapy resistance is frequently observed in GBM patients, which is the major reason for the high mortality rate.
In such context, the development of ultra-high dose-rate irradiation (≥ 40Gy/s), termed FLASH radiotherapy, has been shown experimentally, on animal brain cancer models to allow a remarkable sparing of health tissue in comparison with conventional dose rates, opening a new perspective in the clinical management of GBM.
As a young neuroscientist, first year PhD student at Scuola Normale Superiore, I am deeply involved in the track-down of the biological basis of the FLASH effect in glioblastoma animal model induced in motor or visual cortex. Specifically, after assessing tumor growth by MRI and conducting behavioral assays, I evaluate the brain connectome following radiotherapy treatment using the CLARITY approach.
These results suggest that flash radiotherapy is effective in reducing the growth of GBM *in vivo.* Moreover, we are investigating the sparing effect of FLASH through motor tests.

A patient-derived Glioblastoma organoid model to ensure 3R principle in pre-clinical research

NO 02

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Glioblastoma multiforme (GBM) is the most prevalent primary malignant brain tumor in adults characterized by high invasiveness, heterogeneity and recurrence rate. GBM poor prognosis requires to target biomedical research towards the discovery of effective treatment strategies. In this scenario, 3D cultures are emerging as representative *in vitro* models to study tumor biology. In this work, we aimed to describe a robust and reproducible protocol for the generation and the maintenance of a patient-derived GBM organoid model, also defined tumoroid, that could accurately recapitulate original tumor phenotypical characteristics and marker expression. Tumoroids were responsive to temozolomide (TMZ) treatment, a chemotherapeutic drug used as standard of care therapy, proving to be suitable models to study personalized therapies. As a part of our effort, we focused on developing animal-free protocols for 2D and 3D cell cultures, in order to further ensure 3R Principle in preclinical research. Given this, our model allows us to investigate pathophysiological mechanisms underlying GBM: in particular, we are focusing on the role of autophagy, a lysosomal-mediated degradation pathway regulating cellular homeostasis and associated with human cancer, in GBM. To date, autophagy involvement in GBM is still controversial but recent studies suggest an impairment of autophagy efficiency, mostly involving the autophagy initiator ULK1. We will hopefully evaluate the involvement of autophagy actors in GBM using a reliable patient-derived GBM organoid model.

The role of β-Hydroxy-β-methyl butyrate (HMB) in glioma growth suppression

NO 03

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Glioblastoma (GBM) represents one of the most aggressive malignancies of the central nervous system (CNS), characterized by limited therapeutic options and high recurrence rate. One of the factors contributing to the poor prognosis of glioblastoma is its highly invasive nature, which enables glioblastoma cells to migrate and infiltrate adjacent healthy brain tissues. This diffuse infiltration complicates efforts to achieve complete surgical resection, thereby limiting the effectiveness of surgical interventions and contributing to the tumor's recurrence and resistance to treatment. This invasive behavior is driven by a complex interplay of genetic, molecular, and environmental factors that regulate cytoskeletal dynamics, cell adhesion, and extracellular matrix degradation. GBM cells secrete proteolytic enzymes, including serine proteases, cathepsins, and matrix metalloproteinases (MMPs), which degrade ECM proteins. Previous studies reveal that the gut microbiota plays a significant role in glioblastoma progression through the modulation of neurotransmitters and immune responses, and also gut microbiome can influence the growth of gliomas by altering the composition of microbes and metabolites, impacting tumor development. Previously we have shown that gut microbiota plays an important role in the gut-brain axis both in physiological and glioma conditions. Here we show that in the presence of glioma, fecal metabolite β-hydroxy-β-methylbutyrate (HMB) was significantly reduced compared to the same mice before tumor cell inoculation. Of note, HMB administration reduces tumor growth and proliferation in carcinoma cells. To understand whether HMB could be effective against glioma cells, we first demonstrated in vitro that HMB directly inhibits proliferation in both mouse and human glioblastoma cell lines and inhibits glioma cell basal cell migration. In in vivo studies, oral gavage of HMB to glioma-bearing mice resulted in a significant reduction in tumour volume compared to control mice, suggesting that this leucine metabolite, which is produced in the gut, may have an anti-tumour role in glioma.

Keywords: Glioblastoma, Gut-Brain Axis, Metabolites, HMB, Proliferation, Migration

Molecular study of Ferroptosis and Chaperone-mediated autophagy crosstalk: involvement in GBM resistance

NO 04

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Glioblastoma multiforme (GBM) are the most frequently astrocyte-derived brain tumors in adults, characterized by high aggressiveness and thus lead to a poor prognosis. The gold standard of treatment is Temozolomide (TMZ) associated with radiotherapy (Stupp Protocol). The primary effect of TMZ is to methylate the guanine residues O6 position and induce the block of gene transcription, increasing GBM sensitivity to radiation and inducing pro-autophagic and apoptotic processes. TMZ also acts on activating the Chaperone-Mediated Autophagy (CMA) mechanism, a process triggered by ROS release into the cell. However, TMZ can induce non-responsive behavior in some GBM cells, causing treatment resistance. Indeed, only in TMZ-responsive cells, CMA can impair the Hypoxia-inducible factor (HIF-1α) activity and its downstream processes. Moreover, Ferroptosis, an iron-dependent cell death mechanism, has been described as effective in GBM, increasing oxidative stress, and enhancing cell death.
This study aims to reconstruct the molecular mechanism in which ferroptosis and CMA are involved in GBM resistance.
To validate the critical role of ROS in this pathway, viability assays after combined treatment with the mitochondrial scavenger MitoTEMPO were performed showing that the reduction of mitochondrial ROS impairs Erastin or TMZ reduction of cell viability in sensitive cells. This result suggests a role for mitochondrial ROS in the drug cytotoxic effect. However, it allows us to hypothesize that mitochondrial ROS release is not the only mechanism involved in ferroptosis induction after Erastin treatment. Indeed, in TMZ-resistant cell lines, the concomitant CMA blocking suggests that it is crucial for responsiveness to TMZ but is not always required for ferroptosis-mediated toxicity. Our results demonstrated that CMA promotes the degradation of GPX4 thus activating ferroptosis. On the contrary, if CMA is inhibited, GPX4 can be steadied, blocking the ferroptosis mechanism.

NO 05

Modulation of oxidative stress mediated by miRNA675-5p inhibition as a therapeutic approach to counteract resistance in glioma

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Glioma is currently the most aggressive central nervous system tumor, with a poor prognosis mainly attributable to the development of resistance to both chemotherapy and radiation. Hypoxia is one of the driving forces for tumor genesis pathways such as proliferation, migration into surrounding tissue, and resistance. Among the factors primarly involved on hypoxia, HIF-1α is the major player with a strong correlation with the oxidative stress equilibrium. miRNA675-5p is a hypoxic miRNA involved in promoting and maintaining the HIF-1α process. On the contrary, its inhibition effectively reduces not only HIF-1α but all pathways related to it, including tumor growth. However, the molecular mechanism through which miRNA inhibition is effective also in resistant glioma cell lines has not yet been fully elucidated. The aims of this project are: i) to study the therapeutic efficacy of the miRNA675-5p inhibitor in a panel of resistant glioma lines; ii) to assess the cellular, molecular, and biochemical rearrangement of the cells after treatment; iii) to explain the involvement of the oxidative stress imbalance. Results showed that in all the cells analyzed, miRNA675-5p inhibitor can have a therapeutic efficacy on its own in resistant cell lines, by firstly reducing HIF-1α and its related pathways, an effect in which TMZ instead fails. The mechanism through which this occurs is the induction of oxidative stress. Indeed, its impairment reverses the cytotoxic effect of the miRNA. Cells treated with the inhibitor also acquire metabolic characteristics clearly distinct from cells treated with the scramble. Further studies are going to be conducted to design a protocol for visualizing miRNA675 inhibitor in cells through confocal microscopy. In conclusion, these findings provide a molecular and biochemical foundation for proposing miRNA675-5p inhibitor as a novel therapeutic strategy in glioma, particularly in overcoming resistance to existing therapies.

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

NO 06

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Glioblastoma (GBM) interaction with the surrounding microenvironment and brain resident cells has gained recently increasing relevance. The communication between neurons and glioma cells plays a role in tumor growth and invasion. However, the specific mechanisms involved and how different cell states and mutational background impact on this interaction are still to be defined. To this end, we developed an in vitro model displaying 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. After 7 days of coculture, neurons boost glioblastoma cells proliferation and this supportive effect occurred even without physical contact suggesting a putative role of soluble factors. Our results also indicated that the enhanced proliferation was tightly dependent on neuronal activity, with higher and lower proliferative rates associated to enhanced or reduced firing, respectively. To address GBM heterogeneity, a panel of twelve cell lines heterogeneous in transcriptional subtype and in genetic aberrations, was evaluated. Results showed that the vast majority of the cell lines (75%) showed a higher proliferative rate when cultured with neurons ($p < 0.05$), with the exception of three cell lines that appears to be insensitive. Our functional data were confirmed also by bulk RNA-seq: Ingenuity Pathway analysis revealed the specific enrichment of proliferation and cell division related processes, while neuronal-insensitive lines resulted significantly upregulated in apoptosis and cell death pathways (Z score > 1.5).

These results pinpoint the central role of the neuro-tumoral unit in glioblastoma progression. Indeed, neuronal activity boost cancer cells proliferation through mechanisms that might require paracrine signaling. Further ongoing analysis of neuronal-induced pathways could elucidate the molecular mechanism underpinning neurons-to-glioblastoma communication.

Study on sex-based myeloid cell differences in a mesenchymal glioblastoma mouse model

NO 07

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Glioblastoma (GBM) is the most common malignant primary tumor of the central nervous system with an extremely poor prognosis. Sex differences in GBM incidence, outcome and clinical data are well recognized, however the biological mechanisms which driven this discrepancy are not fully understood. The impact of gender on GBM immunosuppressive tumor microenvironment, is still among the unmet clinical needs. To investigate sex differences in the tumor progression and its implication on the immune landscape we exploited the GBM mouse model obtained injecting orthotopically the SB28 glioma cells. We FACS characterized this novel preclinical model and we observed that it recapitulates the poorly immunogenic characteristics of human GBM. Male/Female differences in terms of engraftment rate and tumor growth were analyzed by in vivo imaging analysis and comparing the tumor size in slices at different time-point. Despite SB28 cells are not likely to trigger a strong immune response according to their immunogenic features, flow cytometry and immunofluorescence analysis showed high infiltration of myeloid population in tumor core and border in both sexes. Investigating the sex-specific phenotype of microglia and macrophages during tumor growth and their interaction with glioma cells and TME will permit to better understand the sex-based myeloid cell behavior, suggesting sex-specific approaches that could lead to improve the therapeutic efficacy of immunotherapy in GBM.

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

NP 01

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

NP 02

Secure attachment protects against depression symptoms vulnerability in women and prevents depression-like behavior and Ih potentiation in DBA/2J females

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The attachment bond between infant and caregiver affects the development of brain areas connectivity. In a growing number of studies, an insecure attachment has been associated with increased vulnerability to development of psychopathologies, such as depression, in adult life. Early attachment is a behavioral system preserved across evolution and suitable for cross-species translational studies. Indeed, recent researches using rodent models suggest that disrupting the attachment bond with the primary caregiver is a major risk factor for developing dysfunctional phenotypes later in life. In this scenario, the Repeated Cross Fostering (RCF) is an early-life protocol recapitulating the interference with the attachment bond. Furthermore, studies on humans suggest that the presence of an alternative stable caregiving figure may prevent the negative consequences of an insecure attachment bond. With a translational approach, we first investigated the link between attachment style and vulnerability to depressive symptoms in a human non-clinical cohort and found that secure attachment protects against depression symptoms vulnerability in women only. Then, by exploiting our well-established RCF model, we investigated the protective effects of a Stable Alternative Caregiver (SAC) proposing the first rodent model of "earned-attachment" to promote secure attachment in mice. We found that DBA/2J females exposed to RCF develop a depression-like behavior in adult life paralleled by the potentiation of the I_h current in dopaminergic neurons (DA) of the Ventral Tegmental Area (VTA); and both depression-like behavior and I_h potentiation were prevented by the presence of a SAC. Overall, this study demonstrates that a SAC can prevent the long-term adverse effects of insecure attachment focusing on the neurobiological pathways involved.

NP 03

In vitro modeling of the human neuromuscular junction in a microfluidic device for the study of facioscapulohumeral dystopy

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The neuromuscular junction (NMJ) is a chemical synapse that forms between the axon of a spinal motor neuron and a skeletal muscle fibre. While the complexity of this highly specialized structure makes in vitro modelling a very challenging task, NMJ co-culture systems have been developed over the past two decades to address the problems encountered in animal models. A further improvement has been provided by microfluidic chip which, unlike the classical co-culture, allows the spatial and temporal control of different microenvironments allowing to independently manipulate neuronal and muscle cells. This allows to study the mechanisms involved in the formation and maintenance of NMJ. Therefore, by exploiting an organ on-a-chip approach, our aim is to obtain a reliable and predictive human NMJ in vitro model in both physiological and pathological conditions in order to unravel the interplay between muscle and motor neuron leading to synapse damage and to neuromuscular diseases. In this work, we mainly focus on facioscapulohumeral dystrophy (FSHD) as it is identified as one of the most common forms of muscular dystrophy, affecting 1 in 8000 people. Despite its frequency, the mechanisms that leads to the development and progression of the disease are still not fully understood as the involvement of NMJ given the neural impairment. For this purpose, motor neurons deriving from human induced Pluripotent Stem Cells (hiPSCs) and myogenic progenitors from healthy and FSHD patient could be seeded in two separate chambers of a microfluidic device. The two cell populations are separated by microchannels that allow axonal growth but not cell bodies migration, allowing the compartmentalization of the two populations without interrupting cell-cell communication. Hence employing this approach, we can study the cellular and molecular mechanisms underlying the FSHD pathology and the way in which the muscular and motor neuron components influence each other. The configuration is versatile enough to accommodate patient-specific cells and perform functional and molecular analysis.

Medial anterior prefrontal cortex stimulation down-regulates implicit reactions to threats and prevents the return of fear

NP 04

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Down-regulating emotional overreactions toward threats is fundamental for developing treatments for anxiety and post-traumatic disorders. The prefrontal cortex (PFC) is critical for top-down modulatory processes, and despite previous studies adopting repetitive Transcranial Magnetic Stimulation (rTMS) over this region provided encouraging results in enhancing extinction, no studies have hitherto explored the effects of stimulating the medial anterior PFC (aPFC, encompassing the Brodmann area 10) on threat memory and generalization. In this study, we aimed to test an rTMS procedure over the aPFC to long-term down-regulate the defensive responses toward threat-predictive stimuli in humans. Participants underwent a threat learning procedure. A week later, one group was stimulated over the aPFC, while the other conditions were stimulated over the occipital cortex (OC), the dorsolateral PFC (dlPFC), or sham-stimulated. Immediately afterward, participants underwent implicit or explicit recognition tests. A further week later, participants were re-tested in a follow-up session for the potential endurance of rTMS effects over time. We found that rTMS over the aPFC immediately decreased implicit reactions to learned and new stimuli. These effects enduringly persisted one week later in the absence of rTMS. No effects were detected on explicit recognition. Critically, rTMS over the aPFC resulted in a more pronounced reduction of defensive responses compared to rTMS targeting the dlPFC. These findings reveal a previously unexplored prefrontal region, the modulation of which can efficiently and durably inhibit implicit reactions to learned threats. This represents a significant advancement towards the long-term deactivation of exaggerated responses to threats.

Role of Neuregulin 1 in the early-life immune activation mouse model of Autism Spectrum Disorders

NP 05

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Neuregulin 1 (Nrg1) is a key developmental neurotrophin with neuroprotective and anti-inflammatory properties. Studies in Nrg1 haploinsufficient mice reveal that interaction between Nrg1 and environmental factors may lead to pathophysiological processes typical of Autism Spectrum Disorders (ASD), a heterogeneous neurodevelopmental disorder for which neuroinflammation, genetic susceptibility and immune responses have been described. To investigate the role of Nrg1 in the pathophysiology of ASD, we used trans-membrane domain Nrg1 mutant mice (Nrg1 TM HET) exposed to early life immune activation (EIA), consisting in prenatal and early postnatal immune activations. To highlight any alterations related to the genotype and the EIA condition in the adult offspring, we evaluated the expression of a set of inflammatory, brain homeostatic and plasticity genes, together with their behavioural profile. Hippocampal expression of BDNF mRNA was lower in Nrg1 TM HET mice. Nrg1 TM HET mice showed also higher levels of TNF-α, lower levels of the microglial phagocytic receptor TREM2 and the presynaptic protein synaptophysin (SYP1), both in the hippocampus and prefrontal cortex. These changes were associated to higher level of locomotor activity and a disinhibitory/anxiolytic profile observed in Nrg1 TM HET mice. Moreover, we found a significant interaction between EIA and genotype for TREM2 and SYP1, both decreased only in EIA WT mice, and a main downregulation induced by EIA of BDNF and its receptor TrkB in both genotypes. These data indicate that Nrg1 haploinsufficiency impacts on the expression of genes relevant for brain homeostasis and neuronal function, and highlight a different pattern of long-term effects in Nrg1 TM HET mice in response to EIA, which deserves to be further explored. Nrg1 TM HET mouse colony founders were kindly provided by Prof. Tim Karl, Western Sydney University.

Shear stress and neuronal pathologies: Organs-on-a-chip model to study neurovascular interaction

NP 06

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Cerebrovascular diseases are disorders that affect blood flow and blood supply to the brain, causing around 9 million deaths per year. Variations in cerebral circulation are found in many neurological disorders, such as Parkinson's disease, Alzheimer's disease, and dementia. Recent studies suggest that abnormal flow patterns (e.g., shear stress) play an important role in jeopardising vascular endothelium morphology and function, triggering neuronal dysfunction and neurodegeneration. Yet, the mechanisms involved in the vascular-neuronal relationship and the effective treatment are poorly understood. The neurovascular unit (NVU) is a highly specialised structure composed of multiple cell types: the brain vasculature (the endothelium), perivasculature (pericytes and astrocytes), and parenchyma (astrocytes and neurons) with unique architecture. Despite decades of research, the link between blood flow alterations and brain dysfunctions remains elusive. This is mainly due to a scarcity of adequate models that recapitulate brain physiology in health and disease. To understand the interplay between flow, neurons, and the vasculature, it is necessary to study cell-cell interactions within the NVU. Understanding these links may provide insight into the disease process. Here we describe how brain flow affects vascular interaction and alters calcium activity through a human-relevant microfluidic platform known as Organs-on-a-Chip (OoC). The OoC we developed allows a more accurate simulation of the impact of blood flow alterations on vasculature and provides an exceptional opportunity to investigate cell-cell interactions. Overall, this project represents a comprehensive approach to unveiling the complexities of neuro-vascular interaction and their impact on neurological function, providing valuable insights into disease mechanisms and identifying potential avenues for therapeutic intervention.

Anxiolytic- and procognitive-like effects of a 30-day chronic treatment with a low non-psychedelic dose of psilocybin in C57BL/6J mice

NP 07

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A resurgence in using psychedelics to treat psychiatric disorders is underway, though current knowledge, especially regarding microdosing, is limited. In this study we have thus tested in mice if a chronic treatment with a non-psychedelic dose of psilocybin is well-tolerated and may affect anxiety- and depression-like behaviors, social behavior or memory performance. Adult male C57BL/6J mice were treated for 30 days with an intraperitoneal injection of vehicle or 0.05 mg/Kg psilocybin, monitoring their weight every 3 days. Mice were then tested in the Open Field (OFT), Light/dark box Test (LDBT), Forced Swim test (FST), T-Maze and Elevated Plus Maze (EPMT) tests, Three Chamber Sociability test, and Cued Morris Water Maze test. No difference between psilocybin- and vehicle-treated mice on the body weight over the 30 days was observed. We found an anxiolytic-like effect of psilocybin measured as increased time spent in the light zone of the LDBT, a lower latency to choose the first arm in the T-Maze test, and a reduced duration of grooming in the OFT. No effects of treatment were seen in the EPMT, FST and Three Chamber Sociability test. Finally, in the Cued Water Morris Test, the mice treated with psilocybin showed a reduced time to locate the submerged platform and a higher success rate in reaching the platform compared to mice treated with vehicle. This is the first study in rodents reporting the behavioral effects of repeated treatment with a low non-psychedelic dose of psilocybin. The treatment was safe and well tolerated. Interestingly, we found significant anxiolytic-like effects in some of the behavioral paradigms of anxiety, consistent with the effects observed in trials of psychedelic doses in humans and reported by subjects practicing psilocybin microdosing in the form of mushrooms. The other finding, needing confirmation in future studies, is the possible enhancement of spatial memory and learning observed in mice treated with psilocybin.

Union is strenght: towards the neurophysiological basis of social facilitation process in mice

NP 08

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Action observation among conspecifics can lead to the repetition of the same action. This process, called social facilitation, seems to be at the basis of social interaction and communication within the members of the same group or species. It has been demonstrated that this process occurs mainly in species that live in groups such as birds, primates, and rodents.
In the present study, we implemented a novel behavioural paradigm to investigate the SF process in mice, studying how the presence of a drinking conspecific can modulate motivation and behaviour in the observer. We demonstrated that the observation of a drinking mouse strongly facilitated drinking behaviour in a head-fixed observer, regardless of whether the actor had free access to water or performed a foraging task. Our data, obtained in the murine model, provide information about the possibility for the existence of a system involved in understanding behaviours with important social meaning. To support our hypothesis, we are currently investigating neurophysiological mechanisms (e.g., pupil diameter, electrophysiological activity) underlying social facilitation in our murine model. Additionally, we are focusing on the role of the anterior cingulate cortex (ACC) in SF process, considering its role as information hub for the social network. Preliminary results suggest that chemogenetic inhibition of this area profoundly impacts on SF.
Furthermore, we are exploring whether early-life experiences may affect sociability in adulthood. Our findings revealed that juvenile social isolation between postnatal days 21 and 35 significantly impairs the SF process when compared to environmental enriched (EE) mice. Additionally, chemogenetic inhibition of the ACC in EE mice seems to have a detrimental effect on SF. By elucidating the mechanisms underlying SF and the role of the ACC, we contribute to the understanding of how disruptions of these neural circuits may contribute to social deficits. Indeed, within the complex landscape of psychiatric disorders, social deficits emerge as a particularly prominent feature in neurodevelopmental conditions such as autism spectrum disorder and schizophrenia. Additional investigations focusing on the links between early-life experiences, neural circuits and social behaviour holds the potential to inspire innovative strategies to ameliorate these symptoms.

Astrocytes as novel targets for the pharmacological effects of Acetyl-L-Carnitine in chronic stress models

NP 09

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Acetyl-L-Carnitine (ALC) is a naturally occurring molecule as a donor of acetyl groups to proteins, including NF-κB p65, which has been shown to have antidepressant effects.
Our previous research showed that ALC promotes new neuron formation in adult mouse hippocampi.
In vitro this effect is linked to NF-κB pathway activation, via p65 protein acetylation.
In vivo ALC increases mGlu2 receptor levels, which also promotes neurogenesis. In a mouse model of depression elicited by unpredictable chronic mild stress (UCMS), 4 weeks of ALC treatment reversed depressive-like behavior, increased mGlu2 levels and new neurons in hippocampi. These findings suggest ALC antidepressant-like effects may be due to its ability to promote neurogenesis and upregulate mGlu2 receptors via p65 acetylation.
Astrocytes play a crucial role in maintaining brain function and have been recently implicated in the pathophysiology of depression. We demonstrated that UCMS induces region-specific alterations in astrocyte morphology, leading to atrophy in the hippocampus and prefrontal cortex and, conversely, increased complexity in the hypothalamus. Remarkably, ALC treatment reversed this atrophy, restoring astrocyte size and branching to control levels. Additionally, ALC treatment further enhanced astrocyte complexity in the hypothalamus.
Currently, we are investigating the mechanisms by which ALC reverses stress-induced astrocyte morphofunctional changes and depressive-like behaviour. We employed an *in vitro* model of chronic stress using immortalized astrocyte cultures exposed to chronic dexamethasone (DEX) treatment. We assessed the effects of DEX in absence or presence of ALC on astrocyte morphology (cell size, branching, complexity) as well as calcium signalling, ER stress, mitochondrial dysfunction, and ROS production.
By unravelling the mechanisms by which ALC counteracts the effects of chronic stress *in vitro*, this research may potentially shed light on future therapeutic strategy for depression.

Keywords: Acetyl-L-Carnitine, astrocytes, depression, chronic stress, dexamethasone

Mechanism of octopus arm muscle contraction: central control and local regulation

NP 10

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Octopuses are animals capable of a large variety of behaviors. Their nervous system has a peculiar organization, with two-thirds of the neurons distributed in the peripheral nervous system running all along the eight arms. Complex motions are produced through the integration of central motor commands and sensory feedback coming from the arms. However, octopus arms can also autonomously produce very fast stereotypical and reflex motions whose execution does not require a brain-centred feedback control mechanism. In this study, we asked how central brain commands are translated into stereotypical motion using arm-embedded strategies. We performed biomechanical experiments of step stretch protocol using *in vitro* preparation of isolated muscles while recording from the arm nervous system. We demonstrate that octopus arm muscles manifest stretch activation, a delayed increase in force following a rapid muscle length increase after rapid step stretches. This phenomenon is rate-sensitive, depending on the magnitude of the stretch applied, and is aborted in Ca^{2+}-free solution. To further elucidate the molecular mechanism underlying stretch activation, we performed a proteomic investigation and identified at least two relevant proteins involved in cell mechanical interaction: a Piezo-type mechanosensitive ion channel component and an Innexin (unc-9 – like) channel, massively localized at the muscle cell membrane level. Taken together, we showed the presence of a mechanism of intrinsic autonomous regulation of muscle contraction which is an integral part of both the arm muscular and neural network. This may represent a further simplification strategy in the central brain control of a limb with virtually infinite degrees of freedom and provide a new reference for studying the importance of brain-to-body interaction in motor control.

NP 11

Do we all learn in the same way? Neurocognitive mechanisms underlying sign-tracking and goal-tracking behaviour in evaluative learning paradigms

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Sensory stimuli, used as conditioned stimuli (CS), can be associated with an intrinsically appetitive stimulus (US), i.e. a reward, through associative learning. Interestingly, there is considerable inter-individual variability in how learning is manifested in animals (and humans). In some cases, CS are endowed with incentive salience, i.e. they become strongly attractive/desired, and drive automatic attention and approach, as typical of *sign-tracking*; in others, CS are just memorized as informative reward predictors, as typical of *goal-tracking*. Our aim was to probe major neurocognitive mechanisms underlying evaluative learning via a systematic assessment of varying behavioural phenotypes in the rat model and a characterization of related brain activity.
A total of 95 rats were used in a Pavlovian Conditioning Approach (PCA) paradigm, wherein a retractable lever and a cue light (or a tone), used as CS, were repeatedly paired (30 trials/session) with a high-palatable sucrose pellet (US). The protocol, including a training, a consolidation and an extinction phase, comprised 12 sessions. Rats were perfused either at the end of the consolidation or of the extinction phase to perform brain slicing and processing by immunohistochemistry (IHC) for the brain expression of the immediate early gene c-Fos.
Based on conventional measures currently taken as reference in the field, we quantified any bias in the speed and frequency of the contacts the rats had with the CS and US, respectively, and classified animals showing pure sign-tracker (12.5%) or goal-tracker (48%) phenotypes, and rats with mixed behaviours (39.5%). For a comparable number of pure sign- and goal-trackers, IHC results were assessed as differences in activation within specific cortical areas, comprising frontal and sensory regions, and subcortical nuclei, including within the dopaminergic system, revealing critical hubs hosting the neurocognitive signatures of the two opposing behavioural phenotypes.

NP 12

Activity-based anorexia disrupts the hippocampal membrane-bound glucocorticoid receptor signaling and impairs structural plasticity and spatial memory

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Anorexia Nervosa (AN) is a devastating psychiatric disorder marked by self-imposed food restriction and constant fear of gaining weight that, combined with excessive physical activity, results in massive weight loss. The etiology of AN, which predominantly affects adolescent females, is still under-researched. Evidence in anorexic patients suggests a hyperactivation of the hypothalamic-pituitary-adrenal (HPA) axis, the system responsible for stress response.
Our study aimed at investigating the stress-related mechanisms and structural changes induced by the anorexic phenotype in the hippocampus, a brain area involved in the negative feedback of the HPA axis. Thus, adolescent female rats were exposed to the combination of food restriction and wheel access, i.e., the activity-based anorexia (ABA) protocol, and sacrificed in the acute phase of the pathology (postnatal day [P]42) or after a 7-day recovery period (P49).
ABA rats exhibited significant weight loss, increased wheel activity and food anticipatory activity, a measure of enhanced motivation for running. In ABA rats, corticosterone plasma levels were elevated at P42 while decreased at P49. Molecular analysis of the hippocampal crude membrane fraction revealed reduced glucocorticoid receptor levels as well as decreased markers of cytoskeletal stability, such as caldesmon, n-cadherin and neuroligin-1. These molecular changes were accompanied by structural impairments, including reduced spine density and mushroom-shaped active spines. ABA rats exhibited cognitive deficits in the spatial order object recognition (SOOR) test. These molecular, morphological, structural, and cognitive deficits persisted even after 7 days of recovery.
Overall, our findings suggest that the AN induction alters the non-genomic response and induces a hippocampal reorganization that could explain the observed cognitive deficits, providing novel neurobiological basis for AN-induced long-lasting vulnerability.

Sponsored by MIUR PRIN (grant number: P2022E4MLS)

Effects of NK cell depletion on sleep/wake cycle in mice

NP 13

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Natural killer (NK) cells are the predominant innate lymphocyte subsets that mediate anti-tumor and anti-viral responses. NK cells play an important role in the immune response by producing interferon-gamma (IFN-γ). Previous studies with the direct application of IFN-γ in the brain produced an acute effect on the excitability of cortical pyramidal neurons in male Wistar rats and an increase in non-rapid eye movement sleep (NREM) duration related to high electroencephalographic (EEG) slow wave activity in male rabbits.
However, the role of NK cells in relation to IFN-γ signals on NREM sleep in physiological conditions has been little investigated in animal models. For this reason, we tested the hypothesis that, in a standard experimental dark-light 24-hour cycle, an abnormal sleep-wake cycle and NREM sleep EEG delta power density may be observed in 10 NK depleted mice compared to 10 matched control (CTRL) wild type mice.
We performed the behavioral analysis over 7 hours of light (12 p.m. to 7 p.m.) and 7 hours of darkness (12 a.m. to 7 a.m.), using video-EEG–EMG analysis to classify the behavior of each mouse into Movement, Passive wakefulness, NREM sleep, and REM sleep. For the EEG we used a parietal electrode, a frontal electrode, and a dorsal electrode (used as an electromyographic) to each mouse. Then, we extracted the NREM sleep periods and calculated the individual delta frequency (IDF), defined as the frequency bin showing the highest amplitude of the EEG power between 1 and 4 Hz. The results showed that, compared to the CTRL mice, the NK mice were characterized by less sleep during the “resting” light condition and less NREM sleep EEG delta power density during the last two hours of the dark condition.
These results complement previous evidence showing that the experimental cerebral administration of IFN-γ induced an increase in animal NREM sleep and a modulation of the excitability of cortical pyramidal neurons generating the EEG signal.

Neuronal markers linked to motor function recovery in a mouse model of stroke

NP 14

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Ischemic stroke, a leading cause of adult disability, occurs due to the obstruction of blood flow to the brain, resulting in neurological deficits. Following a stroke, neural circuits in the peri-infarct zone undergo plastic changes, facilitating spontaneous recovery of neurological function. However, the degree of spontaneous recovery varies significantly among patients, highlighting the need for neurophysiological markers to predict recovery outcomes and guide therapeutic strategies.

Using a well-established ischemic stroke model in mice, the distal middle cerebral artery occlusion (dMCAO), we examined the relationship between motor deficits and cortical changes in the peri-infarct area post-stroke. Behavioral assessments (rotarod, grip strength, and gridwalk) were performed at various intervals post-stroke (D02, D09, D30) to track motor performance evolution, classifying subjects as good or poor recoverers.

To investigate potential correlations between behavioral impairments and neuronal changes, we conducted both spontaneous and evoked in vivo local field potential (LFP) recordings in anesthetized Thy1-ChR2-YFP transgenic mice at D09 and D30, representing the sub-acute and chronic phases of stroke, respectively. Stroke-affected mice exhibited a significant reduction in total frequency power compared to controls, and those with poor recovery outcomes demonstrated decreased excitability in response to ipsilesional premotor cortex stimulation, contrasted by enhanced responses to contralateral primary motor cortex stimulation. Notably, we observed that in the ipsilesional premotor cortex, the reduction of the periodic offset was correlated with behavioral performance. These findings provide insight into the mechanisms underlying 'good' and 'poor' motor function recovery outcomes, opening new avenues for further preclinical research.

POSTER SESSION

2

POSTER SESSION

EBN 11

Neonatal treatment with astaxanthin-loaded stealth lipid nanoparticles fosters hippocampal neurogenesis in a mouse model of Down syndrome

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Down syndrome (DS) is characterized by a precocious increased oxidative stress (OS) that contributes to neurogenesis impairment, a crucial determinant of intellectual disability (ID) in DS. Neurogenesis defects are recapitulated in the Ts65Dn mouse model of DS at early life stages. No therapies currently exist for ID in DS. Astaxanthin (AST) is a carotenoid that exhibits powerful antioxidant activity by directly scavenging free radicals and modulating several pathways. One crucial target of AST is the NRF2 pathway, which is activated in response to OS and was found impaired in DS. Unfortunately, AST therapeutical use is limited due to its chemical instability and poor bioavailability. To overcome these obstacles, a new formulation of stealth solid lipid nanoparticles loaded with AST (AST-SSLNs) has been recently developed to prolong the systemic residence time and increase AST concentration in the brain.

The goal of our study was to establish whether neonatal treatment with AST-SSLNs is able to modify neurogenesis in the hippocampal dentate gyrus (DG), a region critically involved in long-term memory.

Ts65Dn and euploid mice received a subcutaneous injection with AST-SSLNs (10 mg/kg) or with not-loaded SSLNs from postnatal (P) day 3 to P15. On P15 pups were sacrificed and the brains of these mice were processed to evaluate the number of proliferating cells in the DG and the volume of the granule cell layer of the DG.

Ts65Dn mice treated with AST-SSLNs exhibited a large increase in the number of proliferating cells and in the DG volume. In addition, treatment had a positive impact on the NRF2 pathway that was found already impaired in neonate Ts65Dn mice.

These results suggest that the beneficial effects of this innovative treatment on hippocampal neurogenesis may be mediated by the improvement of the NRF2 pathway. The administration of this innovative nanoformulation may represent a promising therapeutic strategy for the amelioration of brain development in DS.

EBN 12

Investigating Purkinje cells role in seizures through optogenetic manipulation in zebrafish

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Purkinje cells (PCs) are the sole output of cerebellum, these cells are noted to be involved in motor control and lately also in seizures. Seizures occur when a disturbance disrupts the normal balance between excitatory and inhibitory impulses in the central nervous system, leading to hyperexcitability. In order to manipulate motor and epileptogenic circuits with the ultimate objective of studying the role of PCs in the onset and development of seizure's like events, we used established UAS/Gal4 zebrafish lines and applied optogenetic tools, employing genetically expressed photosensitive ion channels and pumps, as channelrhodopsin (ChR2) and anion channelrhodopsin-2 (ACR2), prompting activation or inhibition of PCs activity, respectively. This method could allow us to modulate two of the major phenotypes (motor impairment and seizure) shared by several neurodegenerative diseases. Our hypothesis is that optogenetic manipulation of PCs in zebrafish larvae will better comprehend the consequences at physiological level. We used double zebrafish transgenic GAL4 and UAS lines for opsins to drive expression of opsin in the PCs. We first used high-throughput behavioural assay to compare effects of opsin excitation or inhibition on motor behaviour of zebrafish larvae at 5 days post fertilization. Then we analysed the effect of stimulation or inhibition of PCs on epileptic seizure-like events frequency in zebrafish by measuring the local field potentials through brain electrophysiology recordings. Our preliminary data showed an effect on locomotion upon PCs firing modulation. More importantly inhibition of PCs causes an increased duration and power during light stimulus of seizures. While their stimulation caused a decreased of the power and duration of seizures upon treatment with a proconvulsant drug. Overall, our double transgenic optogenetic lines will be useful to entrepreneur the role of PCs in seizures and locomotion in both physiological and disease condition.

The cerebellum development regulator RENKCDT11 as a new gene involved in autism spectrum disorder

EBN 13

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Autism spectrum disorder (ASD) is a neurodevelopmental disease characterized by restricted and repetitive behavior, deficits in social communication, and motor difficulties. Several factors are involved in ASD etiopathology mainly determining brain dysfunctions. However, morphological and functional abnormalities of cerebellum have been recently found in ASD, although their role in autism pathogenesis is still limited. Our research group discovered a novel gene *REN* ^{KCTD11} (REN) as a key regulator of cerebellum differentiation. Interestingly, REN belongs to KCTD family, whose members have been found related to neurodevelopment disorders, including autism.
Here, we found that the loss of *REN* in a knock-out mouse model generated in our lab (REN KO) determines a deficit in the behavioral profile compared to wild-type mice (REN^{-}WT) showing an autistic-like behavior. These aspects correlated with significant morphological alterations of REN-KO cerebella, which appear bigger than their wild-type counterpart. Moreover, the molecular analysis of REN-KO cerebella *vs* REN^{-}WT revealed a strong reduction of expression levels of risk genes related to autism. Notably, the expression of many of these genes is specifically altered in REN-KO cerebella and not in the rest of REN-KO brain, supporting a primary role of cerebellum in the observed deficits of this mouse model. These data have been further supported by RNASeq analysis of REN-KO *vs* REN-WT cerebella, which highlighted an alteration of fundamental pathways involved in neural plasticity, calcium/potassium voltage channel activity, and synaptic neurotransmission. Overall, our findings strongly suggest that the loss of REN causes structural and functional alterations of the cerebellum, which impact neural plasticity and neurotransmission thus predisposing to ASD.

EBN 14

A seizure onset zone analysis using high-density stereo-EEG in the pilocarpine model of temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) represents one of the most common forms of acquired drug-resistant epilepsy in adults and is characterized by spontaneous recurrent seizures originating from temporal lobe structures. The current study aims at investigating seizure onset zone in an animal model of TLE using high-density stereo-EEG.

Sprague Dawley male rats received pilocarpine intraperitoneally to induce status epilepticus (SE) terminated 30 minutes later by ketamine/diazepam. Two weeks later rats (n=33) underwent surgery for implantation of epidural electrodes in the frontal cortex and depth electrodes in several temporal lobe structures in variable combinations to have 3 different montages. Video-EEG recordings have been performed up to week 15 post-SE. Analysis was performed offline. A modified Racine scale was used to score seizure severity. Frequency domain analysis was carried out using Python. Experiments were performed in accordance with EU Directive 2010/63 and authorized by the Italian Ministry of Health.

We identified 4 types of focal seizures belonging to 2 families: (i) non-convulsive seizures included non-spreading seizures (0.9%), seizures spreading monolaterally (4.3%) and seizure with contralateral homologous evolution (27.9%), (ii) focal to bilateral tonic-clonic seizures spreading to the whole brain (66.9%). Power spectrum density analysis revealed that non-convulsive seizures were characterized by alpha and delta rhythms, while delta and beta bands prevailed in tonic-clonic seizures. The most frequent seizure onset zones were located in the ventral hippocampus and in the temporal cortex; 27.3% of the rats had a single epileptic focus, while 72.7% presented with multifocal epilepsy.

Our data show that high-density stereo-EEG provides a full characterization of the epileptic syndrome suggesting that montages with lower number of electrodes lead to undetected seizures and epileptic conditions, and provide less precise analysis of seizure onset zones.

Nanotools to monitor and study Angelman syndrome neurodevelopmental disorder

EBN 15

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Angelman syndrome (AS) is a neurodevelopmental disorder (NDD) caused by mutations or deletions of the maternally inherited Ube3a gene, which encodes for the ubiquitin ligase E3A (UBE3A) in neurons. AS is characterized by severe intellectual disability, speech impairment, ataxia, epilepsy, and behavioural abnormalities. UBE3A plays a key role in neurodevelopment but still little is known about its role in the pathogenesis of AS.
Monitoring AS progression and the effects of therapies is often complex and requires invasive procedures or behavioural observations (highly prone to subjective bias). Nowadays there is a high need for reliable and quantitative biomarkers of brain function in NDDs.
Moreover, the contribution of peripheral nervous system (PNS) neurons to the sensory deficits in NDDs and AS is still not clear. The reason is the difficulty of studying sensory neurons for their sparse distribution *in vivo*, their inaccessibility to localized experimental mechanical stimulation, and the loss of their original polarization once extracted and in vitro.
For these reasons, we are approaching AS from two different perspectives:
(I) We present a clinical assay based on the selective selection and enrichment of neuronal small Extracellular Vesicles (sEVs) from biological fluids, to identify molecular biomarkers deregulated or defective in AS. sEVs are crucial in several biological processes, and importantly sEVs of neural origin can cross the blood-brain barrier: they represent a potential window into brain pathological processes.
(II) We exploit micro-gratings (GRs) to study neuronal guidance processes in vitro, in UBE3A-deficient neurons. GRs can induce specific directional stimuli to neuronal cells, resembling in vivo extracellular cues. We develop GR substrates by solvent casting to allow the characteristic polarized growth of CNS and PNS neurons from different human and murine AS models, and to study their mechanosensing and mobility.

EBN 16

Transcriptional profiling and functional characterization of 3 patient-derived skin fibroblasts affected by Allan-Herndon-Dudley syndrome

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Genetics variants in *SLC16A2* gene encoding for the monocarboxylate transporter 8 (MCT8) cause a severe X-linked intellectual deficit known as Allan-Herndon-Dudley syndrome (AHDS). MCT8 promotes cellular uptake and efflux of thyroid hormones. 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 *SLC16A2* variations on the pathogenetic mechanisms of AHDS.
Fibroblasts were obtained from skin biopsies of 3 AHDS mutated patients and matched controls. RNA was extracted with TRIzoL™. Total RNA sequencing was performed with the CORALL Total RNA-Seq Library Prep Kit using Illumina NextSeq 500 Sequencer. Protein expression was valuated via western blot and immunofluorescence. MTT assay was used to compare cell viability. Live/dead assay discriminated live and dead populations. Lipids were detected via Oil Red O staining. A strong dysregulation in AHDS patients was highlighted by transcriptomic profiling, when comparing AHDS patients to controls. Moreover, MTT and Live/Dead assays demonstrated a reduced cell viability in AHDS_1 with a splicing variant [c.1690G>A (p.Gly564Arg)] and in AHDS_2 with a missense variant [NM:006517-5: c.623G>A; p.Gly208Asp]. The C-terminal missense variant [c.1690G>A (p.Gly564Arg)] did not affect fibroblasts viability, challenging a personalized *in vitro* fibroblast phenotype. Target genes expression resulted upregulated in both patients (AHDS_1 and AHDS_2). Furthermore, myelin related genes were significantly reduced in all investigated patients. The lipid staining revealed an increasing presence of lipid droplets in AHDS fibroblasts. Our preliminary data emphasize a mutation-specific impairment in patients' specific primary fibroblasts, that can be used as pre-clinical experimental model of this rare disease.

Angelman Syndrome: a study on neuronal cultures derived from patients' ips cells

EBN 17

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Angelman Syndrome (AS) is a rare neurodevelopmental disorder characterized by developmental delay, intellectual impairment, ataxia, paroxysmal laughter, and spontaneous seizure, caused by a deficiency in the ubiquitin ligase E3A (UBE3A) gene product. Although ubiquitously expressed in neuronal and non-neuronal cells, the UBE3A gene is paternally imprinted in mature neurons. Consequently, deletions, uniparental disomy, imprinting defects and gene mutations of the maternal chromosome 15q11-13 lead to an accumulation of UBE3A targets, specifically in neurons. The current understanding of the pathophysiology of AS relies mostly on studies conducted on the murine model of the disease, therefore alternative models based on patient-derived stem cells are required to better understand the molecular basis of the pathology in humans. To this aim, five different hiPSC lines, derived from children with various AS- associated genotypes, were provided by the FAST ITALIA biobank. After confirming their stemness, proliferation analysis was conducted, revealing no differences between patient and control iPSC lines. These iPSCs were then differentiated into Neural Progenitor Cells (NPCs) to further analyse their proliferation and differentiation capability. Subsequently, these NPCs were further differentiated into cortical neurons, which are being analysed for their morphology, action potential transmission and autophagic flow, since a block of autophagy, leading to cognitive impairment, has been observed in the brain of AS mouse model. Moreover, UBE3A expression is being evaluated at each stage of differentiation. No existing therapies effectively address all the deficits associated with AS; hence, there is an urgent need to identify new treatments. In this perspective, insulin-like Growth Factor II (IGF-II) receptor is emerging as a potential therapeutic target for AS due to its role in regulating protein metabolism. Our goal is to establish a reliable human cellular model for testing the activation of the IGF- II receptor as therapeutic approach to counteract the pathology.

Impact of Genotype on Maternal Care in a mouse model of Fragile X Syndrome

EBN 18

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Maternal care is crucial for pup survival and involves a complex plethora of behaviours influenced by various environmental and genetic factors. Previous studies using Fragile X mouse models revealed that WT males (Fmr1 +/+) born to heterozygous mothers (Fmr1 +/-) exhibited hyperactivity like Fmr1KO mice (Fmr1 -/y) born to either heterozygous or homozygous mothers (Fmr1 +/- or Fmr1 -/-). Despite these intriguing findings, information regarding the maternal behaviour of the different dams was not provided.

To enhance our understanding of the correlation between phenotype and genotype associated with Fragile X syndrome (FXS) and the potential differences between carrier and FXS dams, we investigated the maternal behaviour of Fmr1 +/- and Fmr1 -/- KO mothers and their strain-matched wild-type controls (C57BL/6J). Observations were conducted during the final hour of darkness in the light-dark cycle. Our analysis revealed that both Fmr1 +/- and Fmr1 -/- KO mothers exhibited poorer maternal behaviour, spending less time on pup-related activities compared to C57BL/6J-WT dams. Additionally, differences were observed between the two mutant groups: Fmr1 +/- mothers spent less time in the nest and displayed increased activity compared to Fmr1 -/- mothers.

In conclusion, these findings indicate that female Fmr1KO mice (line B6.129P2-Fmr1tm1Cgr/J) and their WT controls (strain C57BL/6J) exhibit distinct maternal care behaviours towards their litters, with significant differences related to the heterozygosity and homozygosity of the syndrome. For future research, these aspects should be carefully considered when designing experiments to analyse the phenotype of the offspring.

ND 18

SPEED TALK

Plasma p-tau and Amyloid biomarkers discrimination accuracy of biologically-defined Alzheimer's disease in a memory clinic setting: a head-to-head study

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Blood-based biomarkers offer a non-invasive and cost-effective means for Alzheimer's disease (AD) detection. We performed a direct comparison of these novel biomarkers in a memory clinic population to facilitate their implementation into clinical practice. We included 197 patients with cognitive complaints from the BIODEGMAR cohort at Hospital del Mar (Barcelona). CSF was used as standard-of-truth and patients were categorized as having an AD-CSF profile with two approaches: Lumipulse CSF Aβ42/p-tau181<10.25 (N=208) or Elecsys p-tau181/Aβ42>0.022 (N=157). The following biomarkers were measured in paired plasma and CSF: Aβ42, p-tau181, p-tau217 (Lumipulse, Fujirebio), Aβ42 and p-tau181 (NeuroToolKit, NTK, [Roche Diagnostics International Ltd]), p-tau181, p-tau217, p-tau231 and MAP-T (NULISA, Alamar) and p-tau217 (AlzPath). *P*-value and effect size of the group comparison (AD *vs* non-AD CSF profiles) were calculated using a Mann-Whitney *U* test. ROC curve analysis evaluated plasma biomarkers accuracy to discriminate AD from non-AD CSF profiles. Spearman test assessed the correlation between plasma and CSF biomarkers. All plasma biomarkers were significantly different between the AD and non-AD CSF groups, but the effect size varied among them. NULISA p-tau217, Lumipulse p-tau217 and NTK p-tau181 reported the greatest effect sizes for both the thresholds. ROC curve analysis revealed that the p-tau217 assays performed best and consistently over the two thresholds. The highest discrimination accuracy (AUC) values were for Lumipulse p-tau217 (AUC=0.96), NULISA p-tau217 (AUC=0.96), and AlzPath p-tau217 (AUC=0.93). In comparison to the p-tau assays, Aβ42 reported modest effect size and AUCs. Lumipulse p-tau217 and NULISA p-tau217 had the highest significant plasma-CSF correlation (ρ=0.79 and ρ=0.72). In a memory clinic population, various plasma p-tau plasma biomarkers, demonstrated high performance in distinguishing patients with biologically defined AD from those without.

ND 19

Blood glucocerebrosidase activity and total alpha-synuclein profile of Parkinson's Disease patients with and without GBA1 mutations

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Parkinson's Disease (PD) is a neurological condition characterized by accumulation of toxic forms of alpha-synuclein (asyn) protein. Mutations in GBA1 gene, encoding for glucocerebrosidase (GCase) enzyme, are the most important genetic risk factor for PD. In this study we measured the GCase activity and the levels of total asyn in Peripheral Blood Mononuclear Cells (PBMCs) from PD patients with and without GBA1 mutations (GBA-PD and nonGBA-PD respectively) compared to a group of non-mutated healthy subjects (HC). We measured the GCase enzymatic activity fluorometrically and the total asyn levels by ELISA assay in a cohort of PD patients (n=136) and HC (n=46) matched for age. First, GBA-PD showed lower GCase activity levels compared to both HC and nonGBA-PD patients. The total asyn levels were comparable among the three groups. Moreover, we investigated whether the combination of total asyn and GCase levels was able to better characterize the PD population. The z-score of total asyn/GCase activity ratio was significantly higher in GBA-PD patients compared to HC and nonGBA-PD. Finally, Receiver operating characteristic (ROC) curve analysis was performed for the two biochemical parameters. We found that asyn/GCase ratio significantly discriminated GBA-PD from nonGBA-PD (AUC=0.72) and HC (AUC=0.67). Of note, GCase activity alone emerged as the strongest discriminating factor for GBA-PD compared to HC (AUC=0.78) and nonGBA-PD (AUC=0.88). We confirmed the crucial role of GCase activity in discriminating GBA-PD from both nonGBA-PD and HC. Despite the combination of asyn and GCase was significantly different among patients and controls, we observed a low accuracy of ELISA blood asyn measure to identify pathological cohorts. Measures of pathologic forms of α-syn with other techniques with higher accuracy are critically needed.

Spinal cord organoids generation for the study of amyotrophic lateral sclerosis

ND 20

SPEED TALK

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disease, which causes motor neurons death. Unfortunately, only symptomatic treatments are available probably due to the absence of realistic models. Organoids are pluripotent stem cell-derived self-organizing structures, which allow the generation of tissues *in vitro*. We developed a new method for the generation of spinal cord organoids (SCOs) and we characterized them for morphology, transcriptomic and epigenetic profiles, comparing them to 2D cell cultures. By immunofluorescence analysis, we found that sporadic ALS (sALS) SCOs have a thicker glial layer compared to healthy controls (CTRL) SCOs, whereas CTRL SCOs showed longer neurites. By RNAseq, we found a strong deregulation in sALS SCOs compared to CTRL SCOs, especially in genes involved in extracellular matrix organization and a high similarity with pathological human spinal cord. Because of this major deregulation in SCOs transcriptomic profile, we performed an epigenetic characterization on SCOs and 2D cultures at each differentiation step. We investigated DNA methylation and we found a decreased methylation status in sALS SCOs, suggesting that the major deregulation is due to a minor methylated DNA and, consequently, to the possibility of a higher transcription rate. Moreover, we tested the gene expression of the principal DNA methyl-transferases (*DNMT1*, *DNMT3A*, *DNMT3B*) by RT-qPCR and the protein expression of DNMT1 and DNMT3A by western blot analysis. DNA methylation differences are mainly due to changes in DNMT1 protein expression, and not to changes in DNA methyl-transferases genes expression. In conclusion, our data suggest that SCOs are a promising tool for the study of ALS, inasmuch they recapitulate many ALS characteristics mimicking the environment in which the cells physiologically grow.

Establish a cell culture of human olfactory neuroepithelium collected by nasal swab

ND 21

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The olfactory neuroepithelium (ON) is a region close to the brain that has gained increased interest as a research tool for the study of neurodegenerative diseases (NDs). It comprises olfactory sensory neurons and glial-like cells such as supporting cells, microvillar cells, and stem cells. Some pathological proteins involved in NDs have been found in olfactory neurons and supporting glial-like cells derived from nasal swabs of affected patients, suggesting complex mechanisms of protein misfolding in the ON. The study aims to isolate and characterize cells derived from the ON of healthy subjects collected by nasal swabs.

The ON was obtained by nasal swabs performed at the medium and upper turbinate levels and were manually disaggregated and seeded in a 25 cm^2 flask or P24 wells coverslips and placed in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 culture medium, added by 20% fetal bovine serum, 1% penicillin/streptomycin and B27 supplement to support neuronal survival. Characterization of cells with immunocytochemistry was carried out on cells at different days of cultures until 10 days. Antibodies against NeuN, neurofilament, b3- tubulin, and PGP 9.5 were used for staining neuronal cells, and PAN was used for sustentacular cells at different days of cultures.

Immunofluorescence staining showed the presence of a mixed population of cells, including olfactory neurons and glial-like sustentacular cells, characterized for immunophenotypic markers and morphology. The positivity against NeuN, neurofilament, and β3-tubulin antibodies confirmed the presence of mature and immature neuronal cells. Moreover, we confirmed the nuclear localization of phosphorylated TAR-DNA binding protein-43 (pTDP-43) in supporting cells derived from healthy controls but not in their cytoplasm.

The complexity of the olfactory neuroepithelium provides a unique cellular resource for investigating the expression of pathological proteins associated with NDs in the ON.

ND 22

Exploring the gut-brain axis following traumatic brain injury: correlations between gut dysfunction metrics and neurological deficits

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Emerging preclinical and clinical data suggest that traumatic brain injury (TBI) is linked to gut inflammation, increased permeability, and changes in microbiota, potentially leading to neurological outcomes. This study explores whether TBI induces chronic gut alterations and their correlation with neurological impairments. Adult male C57BL/6J mice were subjected to sham surgery or severe TBI via controlled cortical impact (n=10/group), and assessed for sensorimotor function using SNAP and Neuroscore (NS) up to 2 months post-injury. Gut morphological changes were evaluated through hematoxylin and eosin staining.

Two months after TBI, a significant reduction in gut length was observed (sham: 46.8±1.8 mm; TBI: 45.0±1.1 mm, $p<0.05$), notably affecting the small intestine ($p<0.05$). Histomorphometric analyses revealed in TBI mice: a 21% decrease in villus height ($p<0.001$), a 7% increase in crypt depth ($p<0.05$), a 26% reduction in the villus height/crypt depth ratio ($p<0.001$), and a 35% decrease in Goblet cells per villus ($p<0.001$). Correlating gut changes with neurological outcomes, we found: i) a direct correlation between shortened small intestine and worsened sensorimotor performance at 1 week (SNAP, $p<0.01$, $r=0.9$) and 5 weeks (SNAP, $p<0.05$, $r=0.7$); ii) an inverse correlation between Goblet cells/villus and sensorimotor recovery rate (SNAP 7 weeks-1 week, $p<0.05$; NS 7 weeks-1 week, $p<0.05$).

The study indicates a significant relationship between TBI and gut morphological changes, in which gut length reduction and Goblet cell decrease were linked to worse TBI outcomes. These data will help in the design of gut-based interventions aimed at mitigating gut dysfunction with possible beneficial effects on traumatic brain injury-associated neuropathology.

ND 23

SPEED TALK

Multi-omics approach in Parkinson's disease: a comprehensive study of TMEM175 mutations effect on lipid and metabolic pathway in PD patients at cellular and circulating level

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Recently *TMEM175* gene, regulating lysosomal functioning, was found to be associated to Parkinson's disease (PD) insurgence. Our previous studies demonstrated that mutations in TMEM175 affected 6% of sporadic PD patients.
The study applied an untargeted lipidomic, metabolomic and proteomic analysis in plasma and dermal fibroblasts to assess the presence of a specific metabolic signature in PD patients. The study cohort included two groups of patients that differ for the presence of *TMEM175* mutations, and a group of matched controls.
We found that patients carrying *TMEM175* mutations displayed increased levels of Fatty Acyls, Glicerophospholipids and Sphingolipids both at circulating and cellular level.
Proteomic analysis revealed that PD patients carrying T*MEM175* mutations exhibited a wide deregulation of proteins related to lysosome, autophagy, mitochondrial and bioenergetic pathways, supporting a relevant role of this channel in regulating these cellular processes. To better explore a possible relationship between proteins and lipids altered in *TMEM175* PD patients we applied an integration of multi-omics data which evidenced the correlation between 12 key enzymes involved in metabolic pathway with Phosphatidylinositol, Phosphatidylcholine and Ceramides suggesting potential altered biosynthetic pathways in these patients. On the other hand, the plasma metabolomic analysis displayed a perturbation of amino acids metabolism with a strong alteration of L-glutamic acid in PD patients mutated in TMEM175. Strikingly, we found that Phosphatidylinositol 34:1 and L-Glutamic acid correlated with age at onset of the disease and with the severity of motor and non-motor symptoms specifically in patients carrying TMEM175 mutations.
Our results suggested that TMEM175 mutations might impact on metabolic pathways related to Endoplasmisc reticulum, lysosome and mitochondria functioning, and highlights new potential targets for PD disease.

Nrf2 and neuroinflammation in Parkinon's Disease patients at different clinical stages

ND 24

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Multiple evidence suggest that neuroinflammation plays a pivotal role in the pathogenesis of Parkinson's disease (PD) and has been considered a potential target for therapeutic intervention.
In this study, we explored the role of the transcription factor Nrf2, a master regulator of cell protection against inflammation and oxidative stress, as potential index of PD pathophysiology and biomarker of disease progression.
Therefore, we evaluated the expression of Nrf2 and Nrf2-related antioxidant enzymes in peripheral blood mononuclear cells (PBMCs) from PD patients at different stages of the disease (early, moderate and advanced stage according to Hoen & Yahr scale) and healthy controls (HC). Changes in the expression of these proteins were correlated with alpha-synuclein levels in PBMCs and plasmatic levels of inflammatory (i.e., IL-6, IL-1β, TNFα, IFNγ, CX3CL1) and neurodegenerative (neurofilament light chain, Nfl) biomarkers.
Nrf2 and antioxidant protein levels were assessed by Western Blot, while alpha-synuclein levels were evaluated by ELISA. ELLA™ technology was used to quantify plasma levels of inflammatory and neurodegenerative markers.
Preliminary results show a slight increase in Nrf2 and Superoxide Dismutase (SOD1) protein levels with advancing disease, accompanied by a decrease in pro-inflammatory TNFα in plasma. Plasma amount of Nfl is higher in PD patients than in controls, supporting the ongoing neurodegenerative process. Inflammatory cytokines and alpha-synuclein levels showed no differences between HC and PD patients.
The recruitment of patients is still ongoing, thus we expect that the increase of sample size will allow us to better define the role of Nrf2 in PD progression. Moreover, correlations with gender will allow us to potentially understand the contribution of sex hormones in PD, a disease that predominantly affects men.

This work has been supported by Fondazione Regionale per la Ricerca Biomedica (Regione Lombardia), project ID 3433068 - Acronym: LINKING PARK

Targeting the RNA-binding protein HuD to control ALS disease

ND 25

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HuD is an RNA-binding protein encoded by the ELAVL4 gene, which is expressed in the nervous system and plays a crucial role during nervous system development. However, recent evidence suggests its potential involvement in neurodegenerative processes, including Amyotrophic Lateral. Sclerosis (ALS), a neurodegenerative disease characterized by death of motoneurons (MNs). Notably, upregulation of both mRNA and protein levels of HuD has been observed in MNs derived from FUS P525L human induced pluripotent stem cells (hiPSCs) and in a Fus-Δ14 knock-in mouse model, impacting on MNs' transcriptome and phenotype. HuD's gain-of-function effect also demonstrated to exacerbate cell-autonomous effects of the FUS P525L variant, leading to defects in Neuromuscular Junctions' establishment and apoptosis phenomena, evident in co-cultures of hiPSC-derived MNs and skeletal muscle cells, with a rescue of both phenotypes after siRNA against HuD treatment. Moreover, RNA interference against the HuD-related gene elav in Drosophila model demonstrated to completely rescue the disfunctional motor phenotype induced by FUS P525L overexpression. Recent evidence also indicates a potential role for HuD in sporadic ALS. Specifically, HuD levels rise in response to oxidative stress in MNs derived from hiPSCs, and this phenomenon is also observed in sporadic ALS patients who exhibit an oxidative stress signature. Based on these findings we designed RNA-based teurapeutic molecules as siRNAs, miRNA mimics and Antisense Oligonucleotides gapmers that will be tested in FUS P525L MNs and MNs-SKMs co-cultures with the aim of reducing HuD's levels obtaining a phenotypic rescue.

ND 26

Mitochondrial alterations, oxidative stress and alpha-synuclein levels in iRBD as predictive biomarkers of Parkinson's Disease

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Idiopathic REM sleep behavior disorder (iRBD) is a key prodromal symptom for Parkinson's Disease (PD). However, there are no accessible biomarkers that can help predicting PD onset in iRBD patients yet. Hence, we focused our attention on pathological mechanisms shared by sleep disorders and PD in order to identify potential predictive markers.
Both PD and sleep disorder models have shown the presence of mitochondrial alterations and oxidative stress. Additionally, an increase in small extracellular vesicles (sEVs) release and α-synuclein (αsyn) levels have been observed.
Our study involved 20 controls, 11 iRBD subjects, and 11 PD patients with RBD (PD-RBD). We assessed the expression levels of mitochondrial complexes, and mitochondrial proteins TOM20, PGC1-α, and SOD2 in peripheral blood mononuclear cells using western blotting. Plasma oxidative stress was evaluated by assessing TBARs and catalase activity. Plasma sEVs were isolated through differential centrifugation and quantified by Nanoparticles tracking analysis. αsyn levels were evaluated using ELISA assays.
Our results showed higher concentration of plasma sEVs and sEV-associated αsyn in both PD-RBD and iRBD compared to controls. Interestingly, a significant increase of free circulating αsyn was observed in iRBD plasma compared with controls. Preliminary analysis showed lower levels of mitochondrial complexes I, II, and IV in PD-RBD in comparison with controls, while a smaller reduction of complex IV alone was observed in the iRBD group. PGC1-α levels were decreased in both PD-RBD and iRBD patients. TBARs assays showed an increased lipid peroxidation in iRBD and PD-RBD. Catalase activity appeared slightly higher in both groups.

This study offers preliminary insights into αsyn distribution, mitochondrial alterations, and oxidative stress in iRBD patients, paving the way for identifying potential predictive biomarkers of conversion risk to PD and novel targets for potential neuroprotective interventions.

APache: a novel neuronal autophagic marker regulating autophagosome retrograde trafficking

ND 27

SPEED TALK

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Neurons are particularly dependent on efficient quality control pathways to maintain cellular homeostasis and functionality due to their polarization and extended lifetime. Autophagy is an endo-lysosomal degradation pathway that recycles damaged or aged proteins and organelles. In neurons, autophagosomes form at synapses and are retrogradely trafficked to the soma to fuse with lysosomes for cargo degradation. Several presynaptic endocytic proteins have been found to regulate both synaptic vesicle (SV) recycling and autophagy, and defects in the autophagic flux have been linked to neurodevelopmental abnormalities and neurodegeneration in mouse and humans.

In 2017 Piccini and colleagues characterized the previously unknown protein APache (*Kiaa1107*) as a neuronal-specific protein that interacts with the clathrin adaptor AP-2 and found that it is an essential player in the regulation of neuronal development and SV cycle *in vitro* and *in vivo*.

In our study we combined electron, fluorescence and live imaging microscopy with biochemical analysis on primary neuronal cultures to define APache role in neuronal autophagy. We show that APache acts as a crucial player in the autophagy process, regulating the retrograde transport of autophagosomes to the soma. APache colocalizes with autophagosomes in primary cortical neurons and the induction of autophagy increases APache expression level at synaptic boutons. APache silencing causes a blockade of autophagic flux, leading to a severe accumulation of autophagosomes and amphisomes at synaptic terminals and along neurites due to defective retrograde transport of autophagosomes along the axons.

Interestingly, APache expression is significantly reduced in the brain of sporadic Alzheimer's disease patients. Together, our data identify APache as a key regulator of the neuronal autophagy, and hypothesize that its dysfunctions may contribute to the early cellular alterations and synaptic dysfunctions observed in neurodegenerative diseases.

Identification and characterization of plasma and CSF biomarkers for stratification of cerebral amyloid angiopathy patients

ND 28

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Cerebral amyloid angiopathy (CAA) is one of the main types of cerebral small vessel disease and a major cause of spontaneous intracerebral haemorrhages. The pathogenesis is unknown, although the disease is probably due to an abnormal production of amyloid beta protein (Aβ). The diagnosis of CAA is difficult due the overlapping of symptoms and neuroradiological characteristics with other neurological diseases; integrating cerebrospinal fluid (CSF) and plasma biomarkers into diagnostic workup of patients may support correct distiction and early identification of CAA phenotypes.

Eighty patients affected by probable or possible CAA according to established criteria (Boston 2.0) were recruited at Fondazione IRCCS Carlo Besta (Milan, Italy). Based on neuroradiological evaluation, patients were classified as CAA and Deep perforator arteryopathy (DPA).We evaluated Aβ and tau biomarkers in plasma and CSF through an ultrasensitive single molecule array method (SiMoA, SR-X), as compared to age/sex matched control subjects [healthy donors, HD (n=32)] and subjects with unrelated diseases [UNR (n=19)].

We found a peculiar protein profile in CSF of CAA patients: Aβ40 concentration (3630 pg/ml) was decreased (P=0,0264) compared to DPA (6097 pg/ml); a more marked decrease (P<0,0012) was found in Aβ42 level (124,3 pg/ml) compared to DPA (339,3 pg/ml) and UNR (352,3 pg/ml).Same analyses in plasma revealed a similar pattern in CAA patients: Aβ40 concentration (208,1 pg/ml) was decreased without statistical significance compared to DPA (254,7 pg/ml); there was a more marked decrease (P<0,05) of Aβ42 level in CAA patients (7,195 pg/ml) in comparison with both HD (8,88 pg/ml) and DPA (10,45 pg/ml).We demonstrated a distinctive and peculiar CSF and plasma protein profile in patients with CAA.A further validation of these preliminary findings in larger cohort is needed with the final aim of integrating circulating biomarkers into the clinical practice for a better CAA patient stratification.

Transcriptomic Profile of Skeletal Muscle Biopsies from Duchenne and Becker Muscular Dystrophy Patients

ND 29

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The complexity of RNA metabolism has become crucial in neuromuscular diseases, especially for Duchenne Muscular Dystrophy (DMD) and Becker Muscular Dystrophy (BMD). DMD is associated with mutations in Dystrophin gene that disrupt the protein reading frame causing premature stop codons. In contrast, patients with BMD usually have in-frame deletions that maintain the correct reading frame. Our goal is to search for possible pathways that differs between the two diseases, in which DMD develop a severe phenotype compared to BMD. Here we aimed to evaluate the transcriptomic profile in muscle biopsy of DMD and BMD patients. We collected RNA obtained from muscle biopsy of pediatric DMD patients (n=12) and BMD patients (n=6). Through RNA sequencing, the differentially expressed (DE) genes of DMD patients versus BMD patients were analyzed. Through principal component analysis (PCA), we were able to identify a clear difference between the two groups based on gene expression pattern. DMD patients compared to BMD patients showed a particular activation of genes involved in collagen synthesis, extracellular matrix organization, and oncostatin M-dependent pathways, which plays an important role in the fibrosis process. This suggests that a more severe phenotype in DMD than BMD patients may be due to greater deregulation of these pathways, reflecting the clinical picture of patients observed. Furthermore mRNAs expression levels evaluated by RT-qPCR confirmed RNA-seq data. Enrichment pathways analysis revealed the strong alteration in collagen synthesis and extracellular matrix organization, suggesting different severity degree in the establishment of fibrotic processes. All the collagen genes validated by RT-qPCR were strongly upregulated in DMD patients. This study provides preliminary insights into the difference in gene expression between the two groups and lays the basis for the identification of possible mechanism that differentiate between the two diseases.

Targeting the small GTPase RIT2 as a therapeutic strategy in Parkinson's Disease

ND 30

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Parkinson's Disease (PD) is a neurodegenerative disorder characterized by the accumulation of phosphorylated α-Synuclein (pS129-αSyn) in surviving *Substantia Nigra* dopaminergic neurons. The major unmet clinical need in PD is the lack of disease-modifying treatments. Genome-wide association studies identified the small GTPase RIT2 as a PD risk factor. *RIT2* mRNA is down-regulated in brains of idiopathic PD patients. Increasing RIT2 levels in PD cellular and murine models, overexpressing LRRK2 G2019S or αSyn-A53T, attenuates lysosomal dysfunction, limits pS1292-LRRK2 activity, reduces pS129-αSyn-positive inclusions, and prevents neurodegeneration, suggesting that restoring RIT2 levels may have therapeutic benefits. We are applying a long non-coding RNA (lncRNA)-based technology to increase endogenous RIT2 protein levels within the physiological range. The molecule comprises a sequence for target specificity by antisense pairing with the *RIT2* mRNA and a functional domain that fine-tunes endogenous translation. Targeting *RIT2* mRNA, where it is physiologically expressed, minimizes the risk of side effects from ectopic overexpression. We designed five Rit2-lncRNAs and delivered them into murine Neuro2A cells. We observed increased RIT2 levels without transcriptional changes for three molecules. Further, we show that these increased RIT2 levels trend to increase of p38-MAPK phosphorylation, a downstream target of RIT2, suggesting that lncRNA-dependent RIT2 augmentation has functional effects. We are now testing the most promising RIT2-lncRNA molecules in LRRK2-G2019S overexpressing SH-SY5Y human cells, which display pS129-aSyn inclusions and increased pS1292-LRRK2. Preliminary observations showed that RIT2-targeted lncRNAs lower both LRRK2 hyperphosphorylation and pS129-aSyn inclusions. Thus, our data support lncRNA-based modulation of RIT2 levels as a feasible neuroprotective strategy for PD.

ND 31

mGlu5 receptor negative allosteric modulation reduces the aberrant cellular reactivity and neurotoxicity of reactive human astrocytes differentiated from fibroblast of SOD1 and C9orf72 ALS patients

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BACKGROUND. Amyotrophic Lateral Sclerosis (ALS) is a multifactorial non-cell-autonomous neurodegenerative disease, affecting upper and lower motor neurons (MNs) with no effective cure. Glial activation and in particular the aberrant phenotype of astrocytes represent the main cause responsible of MNs loss and the metabotropic glutamate receptor type 5 (mGluR5) plays a key role in modulating glutamate excitotoxicity and astrocyte reactivity. Our recent *in-vitro* and *in-vivo* investigation demonstrated that genetic ablation or pharmacological modulation of mGluR5, by the selective negative allosteric modulator CTEP, significantly improves lifespan and disease progression in SOD1G93A ALS mice, and positively influences the reactive phenotype of spinal cord astrocytes and their neurotoxicity. Here we studied *in-vitro* the impact of mGluR5 modulation by CTEP on human astrocytes (i-Astrocytes) differentiated from inducible neural progenitor cells (iNPCs) of SOD1 and C9orf72 ALS patients and healthy donors.
RESULTS. *In-vitro* pharmacological modulation with CTEP did not alter the mGluR5 total expression in i-Astrocytes. RT-qPCR analyses, western blot and immunohistochemical experiments showed that 5 days *in-vitro* exposure to 100nM CTEP reduced the over-expression of astrogliosis (GFAP, S100β, C3) and neuroinflammation (NLRP3) markers ($p<0.05$; two-way ANOVA) in i-Astrocytes from ALS-patients vs. untreated or control cells. CTEP treatment was also able to restore oxidative stress conditions by promoting Nrf2 nuclear translocation, enhancement of the antioxidant enzymes activity (glutathione reductase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, catalase), and consequent reduction of ROS and malondialdehyde accumulation ($p<0.05$; two-way ANOVA) in ALS i-Astrocytes. Acute CTEP *in-vitro* treatment did not significantly changed the intracellular calcium mobilization in ALS and control i-Astrocytes. On the other hands, the reduced astrocyte reactivity translates into a beneficial effect towards iPSCs-derived MNs exposed to the conditioned medium of i-Astrocytes after CTEP exposure ($p<0.05$; one-way ANOVA).
CONCLUSIONS. Here we show that the *in-vitro* pharmacological negative modulation of the mGluR5 by CTEP positively affects the reactive phenotype of human i-Astrocytes derived from C9orf72 and SODA4V ALS patients, mainly by ameliorating the oxidative stress response of the cells. These results confirm our previous evidence studying SOD1G93A mice, further encouraging a translational application of mGluR5 modulators in clinical trials.

Keywords: iNPCs-derived astrocytes, mGluR5, iPSCs-derived MNs, oxidative stress, CTEP, astrocyte reactivity

Alteration of circadian clock genes in brain-infiltrating leukocytes in Alzheimer's disease

ND 32

SPEED TALK

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Alzheimer's disease (AD) is the most prevalent form of dementia in the world. The two classical histopathological hallmarks of AD are the accumulation of oligomeric aggregates of amyloid-β and hyperphosphorylated tau protein throughout the brain. Nonetheless, it has been established that neuroinflammation also plays a critical part in advancing AD. In line with this, we have previously uncovered the detrimental role of brain-infiltrating neutrophils during disease progression and their contribution to AD pathogenesis and cognitive decline. Additionally, it is now known that the circadian clock (CC), a cell-intrinsic diurnal process, is disrupted in approximately 80% of aged AD patients. Studies indicate that CC dysregulation occurs before cognitive symptom onset, implying circadian dysfunctions may contribute to AD pathogenesis. Our analysis of a single-cell RNA sequencing dataset revealed that neutrophils infiltrating the brain of triple transgenic AD-like mice (3xTg-AD) showed altered CC gene expressions compared to their wild-type controls. More specifically, the CC gene *Npas2*, a transcription factor responsible for inducing the expression of various CC and non-CC genes, was significantly up-regulated, whereas other CC genes showed significant down-regulation. Interestingly, immunofluorescent (IF) staining of bone marrow-derived 3xTg-AD neutrophils revealed the presence of NPAS2+ and NPAS2- neutrophils. Altogether, our data indicated deregulation of CC at the transcript level of AD brain-infiltrating neutrophils and suggested more closely examining how AD-related CC alterations influence the invasion of neutrophils in the AD brain. We aim to investigate whether these CC changes also persist at the protein level to deepen our insight into the potential role of the CC in the infiltration and neurotoxic activity exhibited by brain-infiltrating neutrophils. We believe that clarifying this complex interplay mechanism may aid in identifying new AD-targeting strategies.

Emerging roles of brain border macrophages in brain homeostasis and disease

NI 16

SPEED TALK

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Macrophages can be highly adapted to their tissue of residence where they perform specialized functions and maintain tissue homeostasis. Within the central nervous system, distinct subsets of tissue-resident macrophages have been identified, but many of their functions remain poorly understood. Microglia are the tissue-resident macrophages present within the brain parenchyma, whereas the other macrophage subsets, collectively known as border-associated macrophages or BAMs, are found in the dura mater, the leptomeninges, the perivascular spaces and the choroid plexus. It has been shown that specific signals from the macrophage niche can instruct macrophage precursors to adopt their tissue-specific profile based on signal-dependent transcription factors. However, the signals that are required for BAM subsets to adopt their respective profiles remain elusive. In this study, we demonstrate that the transcription factor c-Maf is a master regulator of brain macrophage identity and function. Using single-cell technologies, we show that c-Maf deletion changes all Lyve1-high BAMs into MHCII-high BAMs and pushes microglia into a more reactive state. Additionally, upon c-Maf knock-out, mice display changes in brain morphology, perivascular space size and cerebrospinal fluid (CSF) flow dynamics. Our findings offer new insights into the multifaceted role played by macrophages within the brain during homeostasis and disease.

Exploring astrocyte involvement in neurodevelopmental disorders: role of prenatal inflammation and PTX3

NI 17

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Maternal infections and foetal inflammation are recognized risk factors for neurodevelopmental diseases. Astrocytes are glial cells essential for synapse formation and refinement, but they also may react to inflammatory environment affecting neuronal network homeostasis. However, how an inflammatory episode occurring during foetal development influences astrocyte generation and phenotype remains largely unknown.

By using a mouse model of maternal immune activation (MIA) to mimic a viral or bacterial infection, with Poly(I:C) or LPS respectively, we show that astrocytes develop a long-lasting molecular signature related to the type of immune stimulation in MIA offspring suggesting that astrocyte generation is susceptible to an inflammatory environment. A single intraperitoneal injection of LPS or Poly(I:C) to wild type (WT) mice in the perinatal period, coincident with the period of astrocyte generation, induces a strong inflammatory response and results in behavioural indicators of altered neurodevelopmental trajectory. In this context, we aim at investigating in depth the impact of inflammation and immune mediators on astrocyte development at the single cell level and spatial resolution through Merscope spatial transcriptomic analysis in mice subjected to perinatal immune challenges. Among immune mediators, we are particularly interested in monitoring the expression of Pentraxin 3 (PTX3) in astrocytes. Indeed, astrocyte-derived PTX3 plays a key role in synapse maturation during brain development and hence, we are assessing its expression using RNAscope across various brain regions in MIA offspring. Furthermore, during my PhD, in collaboration with Ospedale Maggiore Policlinico di Milano and Institute of Neuroscience-CNR, we will investigate whether genetic or epigenetic variations of PTX3 in pregnant women and their infants associate with increased inflammatory profile and signs of neuronal damage in babies upon maternal SARS-CoV2 infection. This project aims at unveiling, for the first time, the contribution of astrocytes to the long-term alterations observed during brain development following prenatal inflammation and PTX3 specific role.

Organotypic brain and spinal cord slice cultures as an innovative model to study neurodegenerative and neuroinflammatory disorders

NI 18

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Neurodegenerative disorders, including Alzheimer's disease (AD) and multiple sclerosis (MS), affect millions of people worldwide. Despite the field's huge scientific effort, they remain one of the major clinical challenges due to their increasing prevalence and the limited therapeutical options available. Most of the knowledge about these conditions relies on data from animal models, whose results are often highly variable. In vitro systems represent a valuable alternative to animal studies, but they fail to fully recapitulate tissue architecture and pathogenetic mechanisms' complexity. Here, we used organotypic slice culture to study neurodegenerative and neuroinflammatory processes in brain and spinal cord (SC) slice cultures. To assess the vitality of the slices, we measured the release of lactate dehydrogenase, detecting a progressive decrease in its level during the culture days in both the brain and SC. We confirmed these observations using the AlamarBlue assay, which revealed a gradual time-dependent increase in slice metabolic efficiency. These data suggested that our organotypic slices recovered to a nearly homeostatic state after 12 and 5 days of culture for the brain and SC, respectively. Our preliminary data interestingly reported a significant increase of LDH release in organotypic SC cultures from mice with experimental autoimmune encephalomyelitis (EAE). We also showed by immunofluorescence staining that F4/80+ CD11b+ macrophages display a dynamic behavior, suggesting that an inflamed environment is maintained in these slices during the culture. Globally, our preliminary results show that organotypic slices are a highly tunable approach to studying the cellular and molecular mechanisms favoring the development of neurodegenerative disorders.

EBI2 drives lymphocyte migration through the blood-brain barrier in multiple sclerosis

NI 19

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Multiple sclerosis (MS) is a neurological disorder where peripheral immune cells invade the central nervous system via a damaged blood-brain barrier (BBB) and attack the myelin sheath surrounding axons, leading to neuronal death and neurodegeneration. The Epstein-Barr virus induced gene 2 (EBI2), a G-protein coupled receptor, is a chemoattractant driving immune cell migration. The oxysterol 7α,25OHC, the natural and most potent EBI2 ligand, allows EBI2+ cells to migrate towards a gradient of oxysterol. This ligand is synthesised from cholesterol by CH25H and CYP7B1 enzymes which further shows the importance of EBI2 in MS pathophysiology as the highest level of cholesterol in the body is found in the brain due to myelin sheaths, primary target for immune cells in MS. Here, we show that human brains and microvessels express EBI2 and the 7α,25OHC-synthesing enzymes, especially in MS. Interestingly, cerebrospinal fluid of MS patients during relapse shows decreased CH25H levels compared to non-MS patients and could then serve as an early biomarker for MS. Based on the role of EBI2 in immune cell migration and the presence of actors of the EBI2/oxysterol pathway in MS brain microvasculature, we used an in vitro model of the human BBB to investigate T cell migration towards the BBB, targeting this pathway. We first characterised this BBB in control and inflammatory conditions and the effect of inhibitors of the EBI2/oxysterol pathway on the integrity of the BBB. We then investigated migration, towards the BBB, of primary CD4+ T lymphocytes from patients during relapse or from non-MS patients. These results demonstrate that migration of lymphocytes from patients during relapse can be significantly reduced at the BBB targeting the EBI2 receptor. Taken together, these results show a potential early biomarker for MS but also increased evidence for the EBI2/oxysterol pathway as a potential therapeutic target to prevent immune cell migration towards the brain parenchyma.

Specialized pro-resolving lipid mediators modulate choroid plexus inflammatory activity

NI 20

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Neuroinflammation is a well-established feature of Multiple Sclerosis (MS) due to a loss of immune homeostasis and self-tolerance in which peripheral mononuclear cells enter into the brain passing through the well-known blood-brain barrier, but also through the less studied blood-cerebrospinal fluid barrier (BCSFB) formed by choroid plexus (ChP). Recent studies suggest that neuroinflammation could be due to a failure in resolving inflammation, the resolution of which is mediated by lipid mediators known as specialized pro-resolving lipid mediators (SPMs). Having seen the potential role of the choroid plexus in modulating the inflammatory and pro-resolving pathways and its permeability alteration under pro-inflammatory stimulus, we investigated the effect of specific SPMs (in particular resolvins and lipoxins) on the activation of the ChP and we discovered that RvD3 and LXB_4, either alone or in combination, are able to reduce ICAM-1 and CD62P expression on inflamed epithelial cells, an effect partially reversed by antagonizing their receptors. The same experiment was performed on the ChP endothelial cells and we observed an opposite effect. We then set up a 2D co-culture between inflamed epithelial cells of the ChP and peripheral blood cells of relapsing-remitting MS patients, and we found that RvD3+LXB_4 not only reduced the expression of the ligands of the adhesion molecules on leukocytes (i.e. PSGL1 and VLA-4) but also differentially modulated the trafficking of specific T-cell subsets such as Th1, Th17 and Tregs through the in vitro model of BCSFB. In conclusion, the ChP is a key neuroimmune interface where both inflammation and resolution of inflammation occur and where the modulation of these processes by means of key pro-resolving lipid mediators might be crucial for avoiding the excessive infiltration of pathogenic T cells.

Neural stem cells in meninges interact with immune cells and are modulated during the progression of experimental autoimmune encephalomyelitis (EAE)

NI 21

SPEED TALK

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Meninges are a crucial structure for the brain endowed with trophic, immune and neurogenic properties. They are able to sense, integrate, and respond to central nervous system (CNS) and external stimuli, and, importantly, the meningeal niche promptly responds to CNS injuries, including stroke, cerebellar ataxia, and spinal cord injury. Recently, meninges have been suggested as a novel neurogenic niche hosting neural progenitors (NPCs) that can migrate and integrate into the brain cortex. However, the contribution of the meningeal neural stem cell niche to brain diseases is still poorly explored. By exploiting the Experimental Autoimmune Encephalomyelitis (EAE), we studied the role of the NPCs meningeal population in meningeal inflammation and disease progression. We characterized the meningeal NPCs at different stages of the pathology (pre-onset, onset, peak and chronic) by combining immunofluorescence and single-cell transcriptomic analysis (scRNA). Meningeal NPCs respond to EAE progression by increasing their number, differentiation, and migratory properties. Interestingly, we found meningeal neural progenitors in close contact with immune cells. At the level of both brain and spinal cord meninges, we observed infiltrating NPCs. scRNA highlighted the different cellular populations involved in the EAE pathogenesis, including immune cells, endothelial cells, pericytes, and stromal cells. Through a ligand-receptor analysis, we identified potential crosstalk between stromal cells (i.e. neural cells) and immune cells, also confirmed by in vitro co-culture experiments. These results revealed that meningeal NPCs actively respond to the pathogenesis and progression of EAE, and unveiling the mechanism behind the dialogue with immune cells could be exploited as novel pharmacological targets.

A Novel Staining Technique for Thick Brain Slices to Assess Biocompatibility and Tissue Response of Neuroimplants

NI 22

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Immunofluorescence (IF) is able to detect and quantify multiple antigens within tissue, offering superior specificity and sensitivity compared to ex vivo methods like immunohistochemistry or Western blotting. Typically, IF staining is conducted on tissue slices ranging from 4 µm (paraffin-embedded sections) to 40 µm (cryosections). However, these standard thicknesses are insufficient for examining tissue responses on a macroscopic scale due to reduced staining and imaging efficiency beyond these depths. This limitation is particularly relevant for intracerebrally implanted neurostimulation devices, which are generally several hundred microns in size. For instance, the µBots developed in the European CROSSBRAIN project measure 100x100x100 μm^3. We optimized an IF staining method to characterize µBots in 150 µm thick brain slices, focusing on biocompatibility and neuroinflammation. This slice thickness ensures the µBots are contained within the tissue. Mouse brains were fixed with paraformaldehyde and embedded in OCT for cryosectioning. We tested antibodies against GFAP, RECA, and IBA1. To enhance cell membrane permeabilization and antigen penetration, we evaluated various protocols, exposing the tissue to different Triton concentrations (0.5%-1%) for 2 hours to 1 day. We also tested different incubation times for primary and secondary antibodies (1 to 4 days) and compared whole-mount tissue with IF performed in floating. Imaging efficiency was assessed using confocal and THUNDER microscopy. The optimal approach for precisely characterizing tissue responses to brain implants involved using confocal imaging on brain slices treated with extended exposure to higher Triton concentrations (1%) and prolonged incubation of secondary antibodies (4 days for all except DAPI, incubated for 1 day). This method provides enhanced clarity and detail in imaging, facilitating a more accurate assessment of biocompatibility and neuroinflammation associated with intracerebral devices.

Role of immune system and axonal damage: serological biomarkers in neurological sequelae post-SARS-CoV-2 infection

NI 23

SPEED TALK

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PURPOSE. SARS-CoV-2 infection produces significant post-acute sequelae and persistent symptoms for up to 9 months after infection. SARS-CoV-2 enters in the host cells by ACE2, which is expressed on cell surfaces in brain, heart, kidneys, and lungs and shed into the plasma. SARS-CoV-2 causes ACE/ACE2 balance disruption, resulting in new conformational complexes. Several factors and hypotheses regarding its pathogenesis have been described, including immune dysregulation and the development of autoimmunity. Recent studies highlight on Neuropilin-1 (NRP1), a transmembrane protein involved in neuronal development, which plays a role in the infectivity of SARS-CoV-2.

METHODS and RESULTS. 13 SARS-CoV-2 Long-COVID patients were enrolled during routine follow-up, along with 10 anti-SARS-CoV-2 vaccinated healthy donors (HD) and 13 Guillain-Barré Syndrome (GBS) patients. The presence of anti-ACE2 IgG and anti-NRP1 autoantibodies (Abs) in the serum was evaluated using ELISA and immunofluorescence tests. Flowcytometric analysis of NK and T cell compartment was performed. Anti-NRP1 Abs have been detected in 13 Long-COVID patients. Serum levels of anti-ACE2 IgG were statistically different among the Long-COVID neurological cohort compared to GBS patients and HD with no COVID-19 experience (23,04±8,26 vs 12±8,01 vs 13,54±5,08 U/mL; *p<0,05; **p<0,01).

Flowcytometric analyses revealed an increased proportion of Lin-CD34^{+}DNAM-1^{bright} inflammatory cell precursors (40,6±19vs13,3±10;) and CD4^{+}CD28^{+}NKG2D^{+}NKp30^{+} T cell subpopulation (0,25±0,19 vs 4,07±4,03) in 13 Long-COVID neurological compared to 10 HD samples.

CONCLUSION. The results suggest that SARS-CoV-2 infection can induce development of anti-ACE2 and anti-NRP1 Abs and the release of inflammatory precursors from bone marrow. Furthermore, anti-ACE2 are observed in vaccinated HD after controlled SARS-CoV-2 infection. Anti-NRP1 and anti-ACE2 Abs could be useful biomarkers to monitor Long-COVID development after primary infection.

Neuro-immune interactions in T-cell development: imaging mass cytometry uncovers B3AR+ stromal cells in EAE

NI 24

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The development of T-cells in the thymus is influenced by the interactions between thymocyte precursors, thymic stromal cells, and the sympathetic nervous system (SNS). Our previous research has shown that norepinephrine (NE) released by the SNS activates beta-3 adrenergic receptors (B3AR) on thymic stromal cells, increasing the production of chemokines and cytokines such as Cxcl12 and Il15, which drive T-cell maturation. In mice with experimental autoimmune encephalomyelitis (EAE), a T cell-mediated model of multiple sclerosis, elevated NE levels and altered gene expression in the thymus are observed. Still, the cellular players involved in these neuro-immune pathways are unknown.

To investigate the neuroimmune interaction happening between T lymphocytes and stromal cells, we analyzed the thymus of naive and EAE mice using imaging mass cytometry (Hyperion®) with a panel of 15 metal-labelled antibodies. Our analysis revealed distinct structures and cell populations in the thymus, highlighting the influence of the SNS. The cortical region contained small capillaries, proliferating Ki67+ immature T lymphocytes and B3AR+ cells, while the medullary region featured large arterioles, venules, and FoxP3+ Treg cells. CXCL12 and IL15 expression was associated with blood vessels, and Tyrosine Hydroxylase (TH) staining revealed noradrenergic nerve fibers and TH+ thymic cells. Single-cell analysis identified 23 distinct cell clusters, including 3 B3AR-expressing clusters. These B3AR-positive populations interact with each other and are significantly increased in EAE mice. Additionally, CD31+ vessels and a cluster of cortical $CD4^{high}$ T cells also increased in EAE. Overall, this analysis provided a comprehensive understanding of SNS-mediated neuro-immune interactions in the thymus, identifying B3AR+ cells and their interactions within the thymic cortex and medulla, and visualizing changes in the localization of maturing T lymphocytes in EAE.

Pharmacological inhibition of CDK9 in sepsis-associated encephalopathy: impact on microvascular endothelial function

NI 25

SPEED TALK

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Sepsis is an intricate condition displaying immune system overstimulation and hyperinflammatory state, causing organs damages including endothelial barrier impairment in the periphery and in the central nervous system. Endothelial disruption may contribute to sepsis-associated cardiovascular and neurological dysfunctions. Recent findings demonstrated cyclin-dependent kinase 9 (CDK9) promotes vascular dysfunction, however CDK9 pharmacological modulation on endothelial functionality has never been investigated. Here we tested CDK9 inhibitor LDC000067 effects to prevent sepsis-related and blood brain barrier damages and neuroinflammation. In vivo, sepsis was induced by cecal ligation and puncture (CLP) in C57BL/6OlaHsd mice. One hour after the CLP/Sham procedure, animals were randomly assigned to receive once either LDC000067 (50mg/kg) or vehicle intravenously. In vitro, sepsis-like stimulus was induced with LPS 500ng/ml+TNFα 5U/ml+IFNγ 10U/ml (LTI) single dose after FBS-deprivation on Human Brain MicroVascular Endothelial Cells (TY-10) in presence/absence of LDC000067. Experimental sepsis in vivo massively increased systemic concentrations of multiple organ dysfunction markers when compared to Sham animals but LDC000067 administration drastically reduced their blood levels. Sepsis-induced cytokines storm was also significantly counteracted by LDC000067 with CCL-2 and IL-6 displaying the biggest improvement. Similarly, LDC000067 was administered in vitro on TY-10 cell line: CDK9 activation was inhibited in a concentration-dependent manner and LDC000067's effects on endothelial barrier integrity were observed. Immunofluorescence showed that LTI induced massive claudin-5 degradation, but its expression was restored by LDC000067. Concurrently it halved LTI-evoked overexpression of ICAM-1. CDK9 may represent an innovative approach to counteract sepsis-induced microvascular dysfunction, by blunting the excessive inflammatory response and related endothelial dysfunction.

Microglia across neurodegenerative diseases: role of EVs-miRNA in neuroinflammation

NI 26

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Extracellular vesicles, EVs, a heterogeneous population of membrane vesicles, which contain, and transfer bioactive molecules play a role in many of the major pathological pathways altered in neurodegeneration, including Aβ aggregation, neuroinflammation, synaptic transmission, cell death, and senescence. Of note, most EV effects are mediated by encapsulated miRNAs.
On the one hand, with the progression of neuropathology, the inflammatory response of microglia can influence the expression of EV-miRNAs, whose release could promote neuroinflammatory processes.
On the other hand, EVs from neural stem cells, (NSC-EVs) have been explored for their ability to modulate neuroinflammation and neuronal-glial functions in neurodegenerative disorders.
In this regard, we proved that microglia have the capacity to self-sustain its active state, by releasing vesicular and non-vesicular pro-inflammatory factors contributing to the spreading of neuroinflammation.
We therefore demonstrated that the intracellular misregulation of selective inflamma-miRNAs in response to inflammatory stimuli is mirrored in the composition of EVs-miRNAs, proving their role in exacerbating the neuroinflammatory response *in vitro*.
Additionally, we aim to identify potential immunomodulatory NSC-EVs-miRNAs to prove that the contribution of EVs-miRNAs in neurodegenerative pathologies reflects the characteristic of the cells from which they are released.

Reduction in cholesterol supply by Mecp2 null astrocytes contributes to synaptic defects

NI 27

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Rett syndrome (RTT) is a progressive neurodevelopmental disorder mainly caused by mutations in the X-linked *MECP2* gene; it affects 1 in 10.000 live female births and represents the main genetic cause of intellectual disability in girls worldwide. Besides neurons, astrocytes have been identified as active contributors to RTT pathogenesis as Mecp2 knock-out (KO) astrocytes fail to correctly support neuronal maturation and synaptogenesis. Indeed, culturing wild-type (WT) neurons with KO astrocytes or treating them with KO astrocyte-conditioned medium (ACM) affects their synaptic phenotype. Of note, one of the key synaptogenic factors released by astrocytes is cholesterol which plays a crucial role in synapse formation and functioning; several data highlight a defective cholesterol metabolism in RTT supporting the hypothesis that abnormalities in astrocyte-produced cholesterol might contribute to synaptic dysfunctions. In this study, we report a downregulation of the genes involved in cholesterol synthesis and secretion in primary KO astrocytes and KO MACS-sorted astrocytes from P7 mice pups. Moreover, we demonstrate that cholesterol supplementation completely rescues synaptic defects not only in WT neurons treated with KO ACM but also in Mecp2 heterozygous (HET) neurons, that better recapitulate the pathological phenotype. From a therapeutic perspective, we also tested the effects of Trofinetide, the only FDA-approved drug for RTT, on cholesterol-related genes, observing an attenuation of their transcriptional defects in KO astrocytes. Interestingly, in line with several transcriptomic analyses in RTT samples, we report a strong downregulation of protein levels of Nsdhl, a key player in cholesterol biosynthesis, in different brain areas from KO, HET and Mecp-2^{Y120D}animals, shedding light on a possible correlation between *NSDHL* and Mecp2.

NI 28

Fluorinated oxysterol CF3-7alpha,25-OHC downregulates white blood cell count and enhances remyelination in the cuprizone model

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The EBI2 receptor (GPR183) is one of key regulators of the immune system. Its endogenous agonist, the oxysterol 7α,25-OHC, is synthesized enzymatically from cholesterol with CH25H and CYP7B1. EBI2 has chemoattractive properties and, apart from its immune functions, is also expressed and functional in CNS cells, including astrocytes, microglia, and oligodendrocytes. Together with its ligand, EBI2 has been implicated in chronic inflammatory and autoimmune diseases, including IBD, RA, T1D, and MS. Specifically in MS, EBI2 expression is increased in infiltrating lymphocytes and glial cells inside MS plaques and in memory lymphocytes in MS patients receiving natalizumab but not dimethyl fumarate. Type 1 interferons induce CH25H, the first enzyme in the ligand's synthesis, and reduced concentrations of oxysterol 25OHC, the precursor of 7α,25OHC, were found in the plasma of RRMS patients. Moreover, differential expression of EBI2 and CH25H in microglial cells, and CYP7B1 in astrocytes, was observed in MS brains. In animal models, upregulated levels of CH25H were reported in microglia in the cuprizone (CPZ) model and downregulated EBI2 expression in oligodendrocytes during remyelination in the same model. We previously observed a delay in MBP production in EBI2 KO pups during development and greater demyelination and less efficient remyelination in the KO mice in the CPZ model. Here, we tested a synthetic analogue of the agonist, CF3-7α,25OHC, in the CPZ model and found increased remyelination in the corpus callosum after two weeks of daily injections in WT mice. Notably, the lymphocyte and monocyte counts were reduced by 51% and 61%, respectively, in the CF3-7α,25OHC-treated group, imitating the mechanism of action of fingolimod. The *Ebi2* transcripts were significantly increased in the brains of CF3-7α,25OHC-treated mice in the CPZ model. In conclusion, EBI2 is a major immune system modulator which also regulates CNS glial cell function and inflammatory signalling in the CNS, making it a feasible drug target for the treatment of chronic inflammatory diseases such as MS.

Exploring the role of catecholamines in the control of neuronal disfunction in Multiple Sclerosis

NI 29

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Multiple sclerosis (MS) is a progressive neurodegenerative disease of the central nervous system (CNS) characterized by inflammation-driven synaptic abnormalities. Infiltrating T-cells and activated microglia cells are regarded as the main source of inflammatory mediators that contribute to synaptic dysfunctions and neurodegeneration in MS and in its animal model, experimental autoimmune encephalomyelitis (EAE). Notably, several findings suggest a significant role of catecholamines (CAs) in the activity modulation of CNS-infiltrating lymphocytes and of microglia cells. By means of a translational advanced experimental model based on a meld between mouse and human approaches (chimeric MS model), we aimed to explore the CAs immune-contribution to excitotoxic damage.

Firstly, using cytofluorimetry and electrophysiological recordings, we characterized CAs receptors (CAsRs) expression and function in T-lymphocytes derived from the spleen (peripheral) and CNS (infiltrating cells) of EAE mice. Pharmacological stimulation of DRD2, expressed on peripheral T-cells, counteracted the glutamatergic alterations mediated by EAE T cells in the striatum of healthy mice. Accordingly, we demonstrated that infiltrating EAE T cells promote similar glutamatergic alterations as those induced by peripheral EAE T cells.

Using transgenic mice with an inducible microglia-enriched deletion of the DRD2 ($CX3CR1^{CreERT2/wt}DRD2^{fl/fl}$) gene (HE), we investigated the role of CARs on microglial cells. The EAE clinical score was comparable between HE and wild-type (WT) mice, while the EAE anxious-like behaviors were mitigated in HE mice. In parallel, the increase in the duration of striatal glutamatergic events observed in WT mice was rescued in HE mice.

Overall, these data show that DRD2 plays a role in the modulation of EAE inflammatory synaptopathy. Ongoing experiments on different CAs receptors aim to clarify the involvement of other receptors in the EAE/MS pathology.

Tissue resident memory leukocytes alter neuronal functionality during neurodegenerative diseases

NI 30

SPEED TALK

Eleonora Terrabuio (1) - Enrica Caterina Pietronigro (1) - Vittorina Della Bianca (1) - Alessandro Bani (1) - Bruno Santos-Lima (1) - Elena Zenaro (1) - Barbara Rossi (1) - Gabriele Angelini (1) - Antonella Calore (1) - Pallab Majumder (1) - Nikolaos Vareltzakis (1) - Ermanna Turano (1) - Carlo Laudanna (1) - Gabriela Constantin (1)

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Alzheimer's disease (AD) is a neurodegenerative disorder affecting several millions of people worldwide. Classical neuropathological hallmarks of AD are the presence of amyloid beta (Ab) deposits and hyperphosphorylated tau protein. Neuronal dysfunctions and neuroinflammation are two other relevant factors in AD. Growing evidence demonstrate not only the chronic activation of microglial cells in AD brains, but also that brain-invading peripheral and adaptive immune cells strongly impact disease development. Despite this, the contribution of CD3+ T cells, and in particular of CD8+ T lymphocytes, in AD pathogenesis is still unclear. Recent studies demonstrated a detrimental role for CD8+ T cells in the AD course, reporting a clonal expansion of effector memory CD45RA+ (T_{EMRA}) CD8+ T cells in the cerebrospinal fluid of AD specimens and suggested a contribution of CD8+ T lymphocytes to neuronal dysfunction in mice with AD-like disease. However, no study investigated phenotypical and functional alterations of CD8+ T cells taking place in the AD brains during the early disease stages. Our results showed a dramatic increase of tissue-resident memory (Trm) CD103- CD8+ T cells in the brain of AD mice compared to WT control animals. Live imaging experiments revealed that neurons in contact with CD103- Trm CD8+ T cells isolated from AD mice showed significantly higher cytoplasmic Ca^{2+} levels compared to control neurons, clearly indicating that these cells can directly induce neuronal functional alterations. Notably, peripheral CD8+ T cell depletion strongly reduced brain detrimental Trm CD8+ T cells, improved cognition, and reduced neuropathological alterations in AD mice. We globally demonstrate that Trm CD8+ T cells are implicated in disease pathogenesis in 3xTg-AD mice and suggest that targeting the neurotoxic mechanisms exerted by brain leukocytes could interfere with the development of neurodegeneration in AD.

MACanalyzeR: a Computational Tool to Profile Immune Cell Dynamics in Spinal Cord Diseases at the Single-Cell Level

NI 31

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Spinal cord damage is mediated by infiltrating monocytes/macrophages. However, their role in response to pathophysiological conditions remains unclear. To address this, we developed MACanalyzeR, a novel bioinformatic tool dedicated to in-depth and comprehensive analysis of phagocytic cells in single-cell RNA-sequencing datasets. Here, we took advantage of our tool to profile the transcriptional dynamics of monocytes and macrophages in spinal cord injury (SCI) as well as in experimental autoimmune encephalomyelitis (EAE), a well-established model of multiple sclerosis. In order to gain a comprehensive understanding of each condition, we conducted a detailed analysis of a time-course spanning from the steady-state, through the development in the acute stages, and finally to the resolving phase. In both experiments we observed a significant infiltration of monocytes and macrophages, these populations were initially characterized by an inflammatory polarization state, followed by a progressive replacement by cells with healing behavior. The latter cells exhibit a transcriptional profile and foamy-like features typical of lipid-associated macrophages (LAMs). Nicely, the metabolic profiling performed by MACanalyzeR revealed a significant increase in pathways regulating lysosomal clearance and mitochondrial metabolism. To further investigate the mitochondrial metabolism of LAMs in spinal cord regeneration, we dug into the MitoCarta database. Of note, our tool revealed an increase in itaconate metabolism in LAMs of both SCI and EAE mice. In conclusion, MACanalyzeR turned out to be a solid package to study immune cell dynamics at the single-cell level, disclosing the involvement of LAMs in the healing stage of damaged spinal cord and also suggesting that mitochondrial itaconate metabolism could enhance inflammatory resolution during the acute phase of spinal cord injury.

Estimating Myelin Damage Based on Tractography

NIM 03

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We introduced a model called Myelin Streamline Decomposition accounting for lesions (MySD-Lesion), estimating myelin damage within a lesion. This model measures myelin-signal loss along axonal segments intersecting a lesion compared to those outside.
The objective of this study is to evaluate the correlation between myelin damage, as determined by MySD-Lesion, and disability in patients with multiple sclerosis (MS).
A total of 159 MS patients (mean age 46.3±14.3; 96 females; median Expanded Disability Status Scale (EDSS) 3.0) underwent brain MRI scans using a 3T MRI system. A deep learning method was used for white matter lesion segmentation, with manual corrections. Using MySD-Lesion, we generated a “myelin loss map” from magnetization transfer saturation (MTsat) (Myelin Volume Fraction). This map was used to quantify myelin damage within 1) all WML, 2) in remyelinated lesions (as identified using QSM) and 3) in WML affecting the corticospinal tract (CST). We computed the ratio of myelin loss in remyelinated lesions compared to other WML. To examine the correlation between EDSS (dependent variable) and myelin loss values, we applied a linear regression model adjusted for age, sex, and disease duration, using MySD-Lesion and MTsat values within lesions.
MySD-Lesion values in WML positively correlated with EDSS ($p=0.017$, $R^2=0.51$), while MTsat values did not ($p=0.25$, $R^2=0.50$). Similar results were observed for the subgroup of lesions in the CST (left: $p=0.019$, $R^2=0.53$) (right: $p=0.022$, $R^2=0.53$), while no significant correlations were found for MTsat values on either side (left: $p=0.293$, $R^2=0.50$; right: $p=0.071$, $R^2=0.51$). A higher remyelination ratio, reflecting less severe myelin damage, was associated with lower disability ($p=0.039$, $R^2=0.51$). The same analysis using MTsat values showed a similar trend ($p=0.058$, $R^2=0.51$).
In conclusion, the assessment of myelin based on MySD-Lesion proved to be more effective than using raw MTsat values in explaining clinical disability.

Role of 2-[18F]FDG-PET as a biomarker of upper motor neuron involvement in Amyotrophic Lateral Sclerosis

NIM 04

SPEED TALK

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Introduction. Amyotrophic Lateral Sclerosis (ALS) causes degeneration of upper (UMN) and lower (LMN) motor neurons. ALS diagnosis can be challenging, especially in predominant LMN phenotypes. Electromyography can replace LMN signs, while UMN involvement can be detected only by clinical examination, with possible support of magnetic resonance imaging (MRI), transcranial magnetic stimulation and neurofilaments. Our aim was to investigate the role of 2-[^{18}F] fluorodeoxyglucose (FDG)-positron emission tomography (PET) as an UMN biomarker in ALS. Methods. We built an UMN burden score (UMNBS). Performing a multiple regression analysis in SPM12 we evaluated the relationship between UMNBS and brain metabolism. We split ALS cohort based on the UMNBS median value (group A - under median, group B - above median). We ran a full factorial analysis including group A and B and healthy controls (HC), followed by group comparisons. Results. We included 118 ALS patients (group A and B, N=59), with a median UMNBS of 9.50 and a left lateralization of UMN signs. We found a negative correlation between motor cortex metabolism and UMNBS. Comparing each ALS group with HC, we found relative hypometabolism in the left frontal lobe and relative bilateral, right-prevalent hypermetabolism of cerebellum and corticospinal tracts (CSTs). The relative hypermetabolism in CSTs was more evident in group A. Conclusions. Motor cortex metabolism reflects UMN burden. CSTs metabolic changes could provide information about UMN involvement even in patients with predominant LMN phenotype. Our results, though at group level, pave the way for investigating the role of 2-[^{18}F]FDG-PET at a single-subject level, in combination with multiparametric MRI.

Neuroimaging Biomarker for Assessing Brain Circuit Function in Angelman Syndrome

NIM 05

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Angelman syndrome (AS) is a complex neurodevelopmental disorder caused by the loss-of-function of *UBE3A* in neurons. This condition manifests with profound cognitive impairment, behavioural alterations, seizures, and motor deficits. Currently, effective therapies for AS remain elusive, presenting considerable challenges for patients and caregivers. Despite ongoing investigations into various interventions, the accurate evaluation of their therapeutic efficacy is hindered by the absence of quantitative, reliable, and unbiased biomarkers for tracking disease progression and treatment response. We aim to bridge this gap by employing Intrinsic Optical Signal (IOS) imaging, a non-invasive neuroimaging technique of high translational value, to explore whether visually evoked responses can serve as a functional biomarker for monitoring the severity and progression of this disorder. Longitudinal monitoring of visual IOS (V-IOS) in Ube3a-deficient mice modelling AS (B6.129S7-Ube3atm1Alb/J) and control animals at postnatal day (P) 45 and P90 revealed a significant enhancement in the amplitude of V-IOS responses elicited by contralateral eye stimulation in heterozygous (HET) mice compared to wild-type (WT) control mice. This demonstrates the ability of V-IOS to differentiate mutant subjects and controls in the animal model of AS. Furthermore, we investigated the capability of V-IOS biomarker to predict disease severity. We found a correlation between V-IOS amplitude and behavioral performance in marble-burying and rotarod tests. Specifically, higher absolute amplitudes of V-IOS were associated with poorer performance in these behavioral tasks. Finally, we assessed the rescue effects of gene therapy on V-IOS measures of HET mice. Preliminary results indicate that the re-expression of the UBE3A protein in the brain of AS mice reverses the alteration in V-IOS amplitude. Taken together, these findings suggest that V-IOS could serve as a promising biomarker of brain function for AS.

MRI analysis of white matter in spastic ataxia: insights from the PROSPAX cohort

NIM 06

SPEED TALK

Ilaria Gabusi (1) - Alessandra Scaravilli (2) - Gaia Mari (1) - Matteo Battocchio (1) - Sara Bosticardo (1) - Simona Schiavi (1) - Benjamin Bender (3) - Christoph Kessler (4) - Bernard Brais (5) - Roberta La Piana (5) - Bart P. van de Warrenburg (6) - Mirco Cosottini (7) - Dagmar Timmann (8) - Alessandro Daducci (1) - Rebecca Schüle (4) - Matthis Synofzik (9) - Filippo Maria Santorelli (10) - Sirio Cocozza (2)

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Autosomal Recessive Spastic Ataxia of Charlevoix-Saguenay (ARSACS) and Spastic Paraplegia Type 7 (SPG7) are among the most common forms of spastic ataxias. Patients usually present with cerebellar ataxia, spasticity, and other pyramidal features. In this work, we aimed to analyze the white matter (WM) involvement using diffusion MRI (dMRI), evaluating both macro- and microstructural data.

We analyzed 37 ARSACS (M/F=21/16;33.4±12.4 years), 37 SPG7 (M/F=24/13;55.7±10.7 years), and 29 Healthy Controls (HC; M/F=13/16;42.1±17.2 years) enrolled in the PROSPAX multi-center prospective study. All participants underwent a standardized dMRI protocol and a neurologic examination, which included the Scale for the Assessment and Rating of Ataxia (SARA). After preprocessing, we harmonized various microstructural maps computed from the dMRI images, i.e., fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and weighted neurite density index (wNDI). We assessed differences in WM volume and microstructural metrics at a global level. Then, using the Tract-Based Spatial Statistics (TBSS) analysis, we examined voxel-wise differences in microstructure and their correlation with patients' clinical status.

Our analysis revealed a different behavior between ARSACS and SPG7 patients. Indeed, while SPG7 patients only show a moderate WM involvement, ARSACS patients showed a reduced global volume ($p<0.001$) and an alteration of all microstructural metrics (all with $p<0.001$), with no specific spatial pattern of damage but severe involvement of commissural fibers, which correlated with SARA scores ($p=0.004$).

Our findings indicate considerable WM involvement at a macro- and microstructural level in ARSACS, while SPG7 patients have relatively preserved WM macro- and microstructure. Subjects with ARSACS present a clinically significant widespread loss of neurite integrity, secondary demyelination, and overall reduction in cellularity and volume.

NIM 07

Exploring the relationship between volume and microstructural changes in multiple sclerosis lesions using advanced quantitative MRI

SPEED TALK

Martina Greselin (1) - Po-Jui Lu (1) - Lester Melie-Garcia (1) - Xinjie Chen (2) - Alessandro Cagol (1) - Riccardo Galbusera (1) - Esther Ruberte (1) - Mario Ocampo-Pineda (1) - Matthias Weigel (1) - Jens Kuhle (3) - Ludwig Kappos (3) - Habib Ganjgahi (4) - Cristina Granziera (1)

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Multiple sclerosis (MS) is characterized by the existence of focal lesions that continuously evolve. Currently, the link between alterations in lesion volume and lesion microstructure remains uncertain.The objective is to explore the correlation between the longitudinal volume alterations of White Matter Lesions (WML) and the corresponding microstructural tissue modifications using conventional and quantitative magnetic resonance imaging (qMRI). The dataset was composed by 3T MRI at baseline and 2 years follow-up in 67 people with MS (median Expanded Disability Status Scale =2,5 [0- 6,5]; median Age=49 [18-73]; 64% Female) encompassing 3D FLAIR (1x1x1 mm3), MP2RAGE for quantitative T1 maps (qT1,1x1x1 mm3), multi-shell diffusion MRI for neurite density index maps (NDI,1.8x1.8x1.8 mm3). WMLs were segmented on FLAIR/MP2RAGE using a deep-learning-based method, followed by manual correction. WMLs were categorized into shrinking lesions (SL) and enlarging lesions (EL) based on the voxel difference between WML masks at two-time points. To minimize potential registration/segmentation errors, we excluded lesions in the extreme parts of the distribution.The larger lesion mask (second-time point for EL and baseline for SL) was applied to calculate the mean qMRI measures in the two time points. A robust linear regression model was used to correlate the volume and qMRI changes. In EL (n=350), qT1 was positively correlated with volume increases (b=0.29 p-values=0.009) , while NDI shows a minor negative correlation (b=-0.31;p-value=0.025). In SL (n=321) a positive correlation was observed for qT1 (b=0.28; p-value=0.036).Our study shows a relationship between volumetric lesion changes and alterations of lesion microstructure in MS lesions. In EL, the volumetric growth correlated with a microstructural loss (increase in qT1 and decrease in NDI) in the lesion area at baseline. In SL, as expected, the reduction in lesion volume was related to microstructural repair (decrease in qT1).

NO 08

Discovery of a new selective inhibitor of Endoplasmic Reticulum Aminopeptidase 1 for targeting Hedgehog-dependent cancers

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The Hedgehog (Hh) pathway is essential for embryonic development and tissue homeostasis. Aberrant Hh signalling occurs in a wide range of human cancers, including medulloblastoma (MB), the most common pediatric brain malignancy, which shows a high drug-resistance to current therapies. Therefore, understanding the molecular mechanisms that regulate the HH pathway is crucial for the identification of new therapeutic targets and the development of more effective interventions. We identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a new positive regulator of the SHH pathway and an original therapeutic target for SHH-MB. However, the lack of availability for highly specific chemical inhibitors for ERAP1 has constrained the progress in this area. To identify novel selective and effective ERAP1 inhibitors, we performed a docking-based virtual screening of a library of natural compounds against crystallographic structure of the catalytic domain of ERAP1 and we identified an alkaloid compound, N1, as a potential inhibitor for ERAP1. We found that N1 directly binds ERAP1 and disrupts its function on HH pathway leading to a reduced signaling. Specifically, this coompound impairs the association of ERAP1 with the deubiquitylating enzyme USP47 promoting βTrCP protein stability and Gli1 degradation. Therefore, N1 blocks HH-MB growth both *in vitro* and *in vivo* in heterotopic and orthotopic allograft mouse models, demonstrating its ability to cross the blood-brain barrier.
These findings not only lay the foundations of a new era in ERAP1 inhibitors, but also provide a promising therapeutic approach for the treatment of HH-dependent tumors.

FLASH radiation effects on ocular tissues

NO 09

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Uveal melanoma is the most common primary intraocular malignancy in adults. Eye enucleation has long been the gold standard but adverse effects have shifted recommendations toward eye preservation and radiotherapy. This can also lead to complications like radiation retinopathy, retinal detachment and optic neuropathy. To overcome the limitations of conventional radiotherapy (CONVRT), flash radiotherapy (FLASHRT) shows promises. This novel technology involves the ultrafast delivery of radiation at dose rates much higher than those used in CONVRT, causing less damage to healthy tissues but achieving similar disease control (the so-called flash effect). In this study, we employed a dedicated Linear Accelerator (Linac) with a triode gun, enabling in vitro and in vivo studies and able to switch rapidly between ultra-high and conventional modalities under controlled conditions. We compared the effects of FLASH and conventional radiations in healthy ARPE-19 cells, modeling the human retinal pigment epithelium (RPE), and in the RPE of living mice, using various radiation protocols. Our aim is to develop a procedure for ocular melanoma treatment. We demonstrated the successful occurrence of a flash effect on ARPE-19 cells under specific conditions and discovered novel effects of the protocols used. We initiated an in vivo toxicity study on whole-brain irradiated mice using 20 Gy under conventional or flash methods, performing chronic and acute observations. Notably, we observed no significant differences in the morphology of RPE from FLASH versus CONV mice in both acute and chronic groups. This may be due to a dose exceeding the range necessary to generate a flash effect; the outcomes of lower doses are presently under investigation.

A novel strategy for glioblastoma treatment by natural bioactive molecules showed a highly effective anti-cancer potential

NO 10

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Glioblastoma (GBM) is a severe form of brain tumor that has a high fatality rate. It grows aggressively and most of the time results in resistance to traditional treatments like chemo- and radiotherapy and surgery [1]. However, recent studies suggest that combining different treatments against GBM can be more effective than using a single drug alone [2].
Biodiversity represents a big resource for human well-being, and, in addition, it provides several natural compounds that have shown great potential as anticancer drugs [3,4]. Many of them are being extensively researched and significantly slow GBM progression by reducing proliferation rate, migration and inflammation also modulating oxidative stress [5]
In our experiments, we explored some natural compounds (Succisa, Allium and Dianthus) and their properties observing a significant decrease in GBM cell number, partially given by a cell cycle quiescence. Furthermore, we reported a reduced cell migration ability accomplished by morphological cytoskeleton changes, which highlighted even a mesenchymal-epithelial transition, and metabolic studies showed an induced cell oxidative stress modulation and a massive metabolic rearrangement.
Therefore, we suggest/propose a new therapeutic option to overcome the limitations of conventional treatments and thus improve patient outcomes.

Keywords: Glioblastoma multiforme, Biodiversity, natural compounds, anticancer drugs

Modulating the Gut-Brain Axis: The Impact of Fecal Material Transplantation on Glioblastoma

NO 11

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Glioblastoma multiforme (GBM) has been identified as the most lethal type of brain tumor, characterized by a low lifespan of 12.1 to 14.6 months after diagnosis, and a 5-year survival rate as low as 5%. It has been observed that glioblastoma bearing patients often host gut microbiota of different richness and diversity compared with healthy individuals. Furthermore, the important role of gut-brain axis in shaping the glioblastoma development has made it a popular target to modulate. Among several approaches, fecal material transplantation (FMT) is one of the most direct ways to modulate the gut microbiota composition. FMT is a procedure of transplanting fecal material from a healthy donor to a diseased individual, after being pre-treated with broad spectrum antibiotics (ABX) to ensure that new microbiota get established. To assess if FMT from healthy mice to glioblastoma bearing mice can eventually influence the tumor development, we treated mice orthotopically injected with murine glioma cells GL261 with terminal stool of healthy age-matched mice or vehicle through oral gavage. Treatments were performed three times a week for two weeks in total with or without ABX pre-treatment. FMT from healthy mice in the presence of antibiotic pre-treatment reduced tumor growth, increasing circulating CD8-positive T cells and CD107-positive NK cells measured by FACS and increasing the ability to directly kill glioma cells by NK cells isolated from the spleens of FMT treated mice compared with control mice. These preliminary data show that mice receiving a novel microbiota experienced an immune response that increased the amount of circulating killer immune cells, resulting in an anti-tumor effect on glioma growth. Preliminary data showed that FMT treatment reduced tumor growth with respect to the mice receiving only PBS, particularly in the presence of ABX pre-treatment. In these same mice the $CD8^+$ T cells and CD107+ NK cells were significantly higher in the circulation than the other groups. Whereas circulating NK cells showed higher cytotoxic activity compared to controls. These preliminary results suggested that a higher immune activation was possibly induced in mice treated with healthy FMT previously depleted with ABX.

Development of new antibodies against glioblastoma

NO 12

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Glioblastoma (GBM) is the most diffuse and aggressive neoplasm of the nervous system. It is characterized by aggressive growth and high rates of recurrence. Despite the advancements in conventional therapies, the prognosis for GBM patients remains poor. ErbB receptor tyrosine kinases are involved in several cellular processes, such as proliferation, differentiation, cell survival, migration, and invasion. Among them, ErbB3 is overexpressed in GBM tissue and, after binding with its specific ligand, Neuregulin-1 (NRG1), phosphatidylinositol 3-kinase (PI3K)/AKT pathway is activated. That's why a promising new GBM therapeutic approach has considered ErbB3 as a target. In fact, recent studies show how the use of anti-ErbB3 monoclonal antibodies can have positive effects in fighting tumors. There is a consideration to be made regarding the complicated physiological characteristics of intracranial tumors. These include the presence of the blood-brain barrier (BBB), which leads to insufficient penetration of therapies. For these reasons the aim of this project is to evaluate the effective BBB crossing of new anti-ErbB3 monoclonal antibodies, using different approaches. First, we use an *in vitro* model of the BBB composed of murine bEnd.3 endothelial cells and primary murine astrocytes. This co-culture system creates a barrier that reaches a trans-endothelial electrical resistance (TEER) measured in the absence or presence of murine GBM cells GL261. For *in vivo* experiments we will test the best administration route of new anti-ErbB3 monoclonal antibodies to reach the brain in GBM-bearing, by labeling and visualizing anti-ErbB3 and by evaluating its efficacy on tumor volume and mice survival.

NO 13

Beetles-derived cantharidin as potential therapeutic agents for solid tumor treatment: design and development of antibody-drug conjugates

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Cantharidin (CTD) is a beetle's derived terpene with several healing properties whose exploitation is limited due to its toxicity. Here, we have investigated the anti-tumoral effect of CTD on glioblastoma (GB), the most common type of primary malignant brain tumor characterized by a very severe prognosis. The aim of this study is, on the one hand, to analyze the cellular mechanisms underlying the cytotoxic effects of CTD in GB cells and, on the other one, to find a way to reduce its important side effects. However, CTD resulted to be cytotoxic also for normal human glial cells (HA), thus indicating its side effects. To reduce the toxicity of CTD and increase its specificity, we proposed the use of advanced biotechnological drug-delivery systems, with a focus on Antibody-Drug Conjugate (ADC), and sought to identify possible tumor markers (e.g., System XC-, CD44, and EGFR) as potential targets to selectively direct the effects of CTD. We are focusing on CD44, which is significantly more expressed in glioma cells, and we are evaluating the expression of CD44 splicing isoforms to understand their possible role in tumor progression. Results indicate how CTD reduces glioma cell viability in dose-dependent manner. This cell loss of viability could be explained by a significant increase in lipid peroxidation, an important marker of ferroptosis, after CTD stimulation. To find a tumor marker we demonstrated the expression of the target genes in the U373 cell line with immunofluorescence assay and with Western Blot analysis that show that all selected markers are more expressed in GB cells compared to HA. Finally, we evaluated some CD44 isoforms that we divided in CD44s, that is the smallest isoform, and CD44v (all the other isoforms) expressed respectively for 85% and 15%, our next focus will be on the expression of all the CD44v.

NO 14

Molecular study of Ferroptosis in glioma-resistant models and its implications in HIF-1α and miRNA675-5p modulation

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Glioblastoma (GBM) is the most frequently encountered astrocyte-derived brain tumor in adults. It is characterized by a poor prognosis due to its highly aggressive behavior, and for this reason, the median survival is 14.6 months. GBM is treated now in clinics with a combination of Temozolomide (TMZ)-based chemotherapy and radiotherapy. Resistance is still a challenge since TMZ sets off a resistance behavior in some GBM cells. In these conditions it is possible to see that cells do not respond to TMZ but rather increase in cell viability.
Recently Ferroptosis, an iron-dependent cell death mechanism, has been described as efficacious in GBM, increasing oxidative stress and enhancing cell death.
MiRNA675-5p is a hypoxic-miRNA involved in the modulation of the master gene of hypoxia, HIF-1α resulting as a pro-oncogenic miRNA in tumors. It was found that the miRNA675-5p inhibition is sufficient to impair, in vitro and in vivo, HIF-1α activity and cell proliferation and tumor growth.
Three different Ferroptosis-inducers (Erastin, RSL3 and FIN56) have been tested in TMZ-resistant human cell lines in vitro, through cellular and molecular assays. Resistant cells showed specific features characterizing a cell in ferroptotic state after all treatments. In particular, decrease in the cell viability, HIF-1α and cell motility, was observed with a significant increase in oxidative stress. Notably, a consequent miRNA675 downregulation has been observed in all cell lines following the reduction of HIF-1α.
The induction of Ferroptosis in GBM-affected patients could be a process to improve tumor cell death, and to overcome the resistance to the standard treatment with TMZ. Meeting clinical needs, the treatment with Ferroptosis inducers would be employed in combination with the standard treatment, bypassing the TMZ-resistance; might be essential in designing innovative therapeutic approaches.

Surgery-induced ischemia in residual glioblastoma induces tumour plasticity and aggressiveness

NO 15

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Glioblastoma (GBM) is a highly aggressive and invasive tumor of the central nervous system (CNS). With a five-year survival rate of only 6.9% and a median survival period of eight months, it has the lowest survival rate of any CNS tumor. Treatment includes surgical resection, fractionated radiation, and concomitant adjuvant chemotherapy with temozolomide. Although the ability of GBM cells to withstand chemoradiation is well documented, few studies have been conducted on the molecular alterations that follow surgery, their function in the plasticity of tumor cells and the microenvironment (TME), or their connection to treatment-resistant tumors. Thus, the goal of this study was to investigate the histopathological and transcriptome alterations that resulted after microsurgical resection of TME and leftover tumor cells. To this end, we developed a new mouse model for GBM surgical resection guided by stereotaxic fluorescence that replicated the measures taken in patients. Syngeneic GBM cell lines CT2A and GL261 were orthotopically implanted in C57Bl6j mice's brains, while P3 xenografts were injected into immunosuppressed nude mice. Bulk and scRNA-seq, digital pathology, and intravital microscopy revealed that surgery induces ischemic damage, leading to remodelling of the TME under hypoxia, with subsequent immunosuppression and tumor cell plasticity. Furthermore, our findings revealed that microsurgical resection induced strong proneural-to-mesenchymal transition (PMT). Finally, we found that exposure to ischemia caused substantial alterations in TME related to the immune system and vascular function, which may compromise the response to treatment. Overall, our findings demonstrate that although surgery is an essential treatment to increase the chances of survival of patients with GBM, it also causes cellular alterations that facilitate glioblastoma resistance to treatment.

NP 15

Unveiling the Role of a Wnt Signaling Factor in Acetylcholine Receptors Expression and 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 we use it as a model to study the ability of the peripheral nervous system (PNS) to remodel and regenerate after damage. This study examines the effects of Botulinum Neurotoxin type A (BoNT/A), a potent bacterial exotoxin that inhibits acetylcholine (ACh) release at the motor nerve terminal, inducing prolonged NMJ paralysis and stimulating PNS remodeling through motor axon terminal (MAT) sprouting and novel neuromuscular synapse formation. 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. Advanced bioinformatic analyses revealed expression changes of several genes and molecular axis, including that of a Wnt pathway transcript whose expression increases uniquely in the soleus during critical sprouting phases. This protein, known to be a secreted factor, interacts with receptors implicated in NMJ formation and maintenance by clustering ACh receptors (AChRs). Consistently, the addition of this factor to cultured primary myotubes significantly enhanced the signal intensity of AChRs and their clustering, as shown by confocal microscopy experiments, while Real-Time PCR analysis confirmed that the protein upregulates the expression of both adult and fetal AChR isoforms. Importantly, the neutralization of this factor with a specific antibody *in vivo* strongly reduced the synaptogenesis of novel NMJ induced by BoNT/A-through sprouting. Conversely, direct injection of the protein alone increased the density of AChRs, both in the soleus and EDL muscles. Overall, our findings suggest that this Wnt-signaling factor plays a crucial role in AChR expression and clustering in NMJ maintenance and remodeling.

Cellular and molecular characterization of retinal degeneration in a novel mouse model of cone-rod dystrophies

NP 16

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Purpose: Cone dystrophies and Cone-Rod dystrophies (CORDs) are severe forms of inherited retinal diseases characterised by progressive degeneration of photoreceptor cells that can lead to complete blindness. To date, no treatment is available to stop the progression of the disease. Photoreceptor viability is strictly dependent on the levels of the second messengers cGMP and Ca^{2+}, the intracellular concentrations of which are finely regulated by guanylate cyclase-activating proteins (GCAPs) and their GC targets. Several mutations in the genes coding for GCAPs have been *associated with autosomal dominant* CORDs. Among these, p(E111V) GCAP1 variant has shown to lead to increased intracellular Ca^{2+} and cGMP levels [1,2] and recently, we confirmed that the delivery of E111V-GCAP1 protein in mice induces a disease-like electrophysiological phenotype [3], consistent with constitutive cGMP synthesis and increased Ca^{2+} level. In this work, we investigated the role of the E111V-GCAP1 and its involvement in the CORD-related phenotypes in a newly developed knock-in mouse model. • **Methods**: Both heterozygous and homozygous CORD mouse models and wildtype C57Bl/6 J mice of both sexes were analysed at different time points in order to investigate: i) morphological changes in the thickness ratio between the outer nuclear layer (ONL) and the inner nuclear layer (INL), ii) the expression of specific retinal proteins iii) and behavioral differences. For each point, mice were anaesthetised and euthanised via cervical dislocation and their retinas were extracted through a corneal incision. Then retina sections were processed for Immunofluorescence technique and from the same eyes retinas were also homogenised for RNA extraction to measure the expression levels of GCAP1 and GCAP2. In detail, RNA of each animal was firstly used for cDNA synthesis using a reverse transcription kit and then Real-time PCR amplification was performed. In addition, we subjected each mouse genotype to a comprehensive behavioral test battery and electrophysiological tests [4]. To assess the potential of liposome-mediated delivery of GCAP1 in a mouse model, the human recombinant protein was encapsulated into a liposome with a lipid composition similar to that of the disks present in the Rod Outer Segment (ROS-like). • **Results**: We present the preliminary results on the morphological and behavioral characterisation of disease progression in both hetero and homozygosis mice with respect to the wildtype and the possible involvement of GCAP2 isoform in compensating for the dysregulation induced by the disease-associated E111V-GCAP1. In addition, the possibility of using direct or liposome-mediated administration of recombinant human GCAP1 to modulate the phototransduction cascade in mouse rods is presented. • **Conclusion**: In this work, we characterised for the first time a novel both heterozygous and homozygous CORD mouse model and the time course of retinal degeneration. In addition, we show how the efficient delivery of functional recombinant WT GCAP1, either with or without the use of liposomes, could be promising for the treatment of retinal diseases. • **References:** [1] Marino V et al. (2018) Hum Mol Genet. 27(24), 4204-4217; [2] Dell'Orco D et al. (2019) Sci Rep. 9(1), 20105; [3] Asteriti S et al. (2023) Cell Mol Life Sci. 80(12), 371; [4] Cangiano L, Asteriti S. (2023) Int J Mol Sci, 24(14),11346

NP 17

Design of an innovative in vitro 3D bioprinted blood vessel model in fluid-dynamic condition to study blood-brain barrier

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The blood-brain barrier (BBB) is a multifunctional structure that protects brain from external injury constituted by neurovascular units (NVU). Its function is obstacle the entry of many molecules, including therapeutic compounds, making difficult the cure of brain diseases. In this regard, several in vitro BBB models have been proposed to mimic in vivo conditions such transwell, and organ-on-chip. In our previous study, we presented a dynamic in vitro 3D BBB model made by a double chamber bioreactor (LB2, IVTech) with brain endothelial cells and neural compartment separated by a porous membrane, useful for our drug delivery studies. In the present study we used a single chamber bioreactor Livebox1-6 (LB1-6, IVTech) designing, bio-fabricating and placing in dynamic flow a three-dimensional blood vessel model which resembles more closely the in vivo condition. We used an extrusion-based bioprinter, BIOX (CellInk), to produce the blood vessel able to withstand continuously in flow for 24h without undergo flux changes that bring to medium leakage. Our model satisfies Schwabb operational parameters of thickness, filaments distance and pore diameter at different printing pressure and speed. This model can be useful to study both the pathophysiology of BBB and also in different research fields to mimic the fluid dynamic complexity associated with blood vessels of organs and tissues.

Does Emotion Matter in Attentional Capture?

NP 18

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Attentional mechanisms prioritize relevant information from our environment while regulating the processing of irrelevant information. However, certain stimuli with salient features (e.g., feature singletons or emotionally salient stimuli) can involuntarily grab our attention, regardless of their relevance to current goals, in turn impairing performance. While the interference caused by these task-irrelevant salient stimuli has been clearly demonstrated with simple shapes, it is unclear how this applies to picture stimuli with emotional valence. We studied whether emotional distractors can be attentionally suppressed by using a visual search task, where participants were asked to locate the target with a distinct outline shape among other stimuli and indicate a dot's location (above or below) relative to the target. The task included two display types: simple geometrical shapes (squares) and pictures. 60% of the search displays included a salient distractor, either a neutral or a negative picture in the Picture display type, and a red or a green singleton item in the Square display type. Consistent with previous findings, our results indicate that the presence of a salient distractor significantly impacts performance. Participants exhibited lower accuracy and slower responses when a distractor was present versus absent in the display, irrespective of the display type. Furthermore, the emotional valence of the distractor exerted an additional influence on performance, with decreased accuracy in the presence of a negative distractor compared to neutral and no-distractor trials. Significantly, the difference between negative and neutral distractors persisted throughout the experiment. In summary, our research demonstrates a robust capture of attention by emotionally salient stimuli (negative), resulting in persistent difficulty in visual search.

The Role of CXCR4 on Perinatal Ischemic Stroke

NP 19

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Perinatal ischemic stroke involves the occlusion of cerebral blood vessels and is the most common cause of unilateral cerebral palsy in infants. Perinatal stroke occurs during the brain maturation period, and knowledge about both its impact on brain functions and treatment alternatives is still limited. C-X-C chemokine receptor type 4 (CXCR4) is a receptor for the C-X-C motif ligand 12 (CXCL12) and is found on the membrane of neuronal cells. Previous studies on adult ischemic stroke have shown that CXCR4 acts as a neuroprotective factor and triggers angiogenic responses, mostly in the perilesional (penumbra) region. However, nothing is known about the possible role of CXCR4 in the outcome of perinatal stroke. In this study, we aimed to characterize the role of CXCR4 expression in a mouse model of perinatal ischemic stroke and to correlate it with the motor and cognitive outcomes observed. For this purpose, we induced ischemic stroke at post-natal day 14 (P14) in CD1 mice using the distal Middle Cerebral Artery Occlusion (dMCAO) model, and we performed a battery of behavioral tests on different days after the stroke. Moreover, we checked for the expression of CXCR4 79 days after the stroke. We found that CXCR4 expression at day 79 post-stroke was significantly lower in the stroke group, and these individuals presented significantly worse motor balance and coordination than the sham group. However, it is still necessary to characterize the changes in CXCR4 expression during the stroke onset to be able to correlate these changes with the observed outcomes.

Astrocytes diversity across mammals: from gene expression to morphology

NP 20

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Are you smart? Then, it is probably also because of your astrocytes! Less famous than their neighboring neurons, astrocytes have been lately shed in light for their role in brain physiology and pathology, and for their primate-specific features. In this context, a comprehensive comparison of the different subtypes of astrocytes across evolution is pivotal for revealing their involvement in cognitive abilities. We have previously: (1) investigated the distribution of astrocyte subtypes across mammals through immunostaining of the prefrontal cortex of Primates (chimpanzee, rhesus macaque, human), Carnivora (tiger, lion, leopard), Artiodactila (cow, tursiops), Rodentia (mouse) and Chiroptera (Seba's short-tailed bat); (2) reconstructed the single-cell morphology with an algorithm-driven segmentation and Image-J plugin Neurotracer analyzer; (3) described the primate-specific features of two particular subfamilies of astrocytes: (i) Interlaminar astrocytes (ILAs), which are characterized by an increase in complexity and density in primates, and (ii) Varicose-Projection astrocytes (VP-As) which until now where described primarily in humans. We now moved to the next step, digging deeper into their single-cell features and functions: (1) Through spatial transcriptomic technique, we will provide an analysis of differential gene expression of astrocytes, with a special focus on ILAs, across different species (i.e.,human, mouse and cattle) and across different cortical layers; (2) uncover the role of astrocytes in the neuroinflammation by treating human IPSC-derived astrocytes with interleukin-1 beta (IL1beta) and tumor necrosis factor alpha (TNFα), able to recreate the inflammation morphology typical of astrocytes in neurodegenerative disorders. These results will be fundamental to better understand the roles of these peculiar astrocyte subtypes: ILAs and VP-As.

Environmental enrichment reduces anxiety-like behaviour and changes the microbial community composition of mice

NP 21

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Environmental enrichment (EE) is a husbandry method known to provide enhanced somatosensory, locomotor and cognitive stimulation. It has been shown to exert significant effects on the behavior and cognitive function improving rodent behavior, altering neuronal plasticity, and promoting stress resilience of rodents. Concurrently, emerging research highlights the intricate bidirectional communication between the gut microbiome and central nervous system function, known as the microbiota-gut-brain axis. The aim of this work is to investigate the influence of EE on mouse behavior in conjunction with alterations in the gut microbiome composition. Four-week-old C57BL6/J mice were co-housed for four weeks in order to homogenize their microbiome. Afterwards the mice were split into EE and standard environment (SE) housing. Through a series of behavioral assays and microbiome profiling techniques, we demonstrate that exposure to enriched environments significantly modulates mouse behavior reducing anxiety-like behaviors in the open field test (OFT). Furthermore, analysis of the gut microbiome via 16S Illumina Sequencing reveals distinct alterations in microbial diversity and composition in enriched mice compared to standard-housed controls. Our findings suggest a reciprocal relationship between environmental enrichment, behavior, and the gut microbiome. In future experiments, we want to assess causality by using antibiotics models and stool transplantation experiments to provide insights into potential mechanisms underlying the therapeutic effects of EE on neurological and psychiatric disorders. Understanding these interactions may pave the way for novel interventions targeting the gut-brain axis to promote gut and brain health.

NP 22

Orexin receptor 2-dependent modulation of dopaminergic cells in ventral tegmental area. Implication for narcolepsy with cataplexy

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The neuropeptides orexin A (OxA) and B (OxB) are synthesized by hypothalamic orexinergic neurons that broadly innervate the ascending arousal system as well as its forebrain targets. Orexins regulate active wakefulness and specific sleep phases, by acting through the G protein-coupled receptors Ox1R and 2R. OxR1 has a higher affinity for OxA, whereas OxR2 has similar affinity for the two peptides. Ox neuron degeneration causes narcolepsy with cataplexy (NC), which is characterized by daytime sleepiness episodes of sudden loss of muscle tone (cataplexy), associated with strong emotions. How Ox1R and 2R cooperate in regulating motivated behavior and how disrupting their action leads to NC is poorly understood. The dopaminergic neurons of the ventral tegmental area (DA^{VTA}) are likely central players in these mechanisms, because of their implication in arousal and their functional interplay with orexins. DA^{VTA} neurons are densely innervated by Ox fibers, but the specific effects of Ox1R and 2R at pre- and postsynaptic sites are uncertain. To determine the cell-autonomous effects, we study by patch-clamp methods DA^{VTA} cells dissociated from murine brain slices (2^{nd} to 3^{rd} postnatal week). In WT, while 100 nM OxA produced a ~60% increase in the spontaneous firing frequency, 100 nM OxB decreased it from 2.9 ± 0.7 to 1.4 ± 0.46 Hz ($p = 0.03$; $n = 8$). A similar result was obtained by using the Ox2R-specific peptide OxB-AL (200 nM). No effect was observed by applying 100 nM OxB in DA^{VTA} cells from Ox2R-deficient mice. We are now testing whether OxB modulates the DA^{VTA} cell's complement of K^+ channels, such as the A-type, K_{ir} and K_{Ca}. Regardless of the specific mechanism, our results suggest that Ox2R activation tends to inhibit DA^{VTA} neuron firing, which could explain why mice lacking Ox2R in DA^{VTA} cells show potentiated θ waves in wakefulness, pointing to disinhibition of the VTA-septo-hippocampal pathway.

Funded by UNIMIB (2021-ATE-0042 to AB) and SNSF (31003A_182613 to AV).

Network-Wide Control of Circuit Architecture of Cultured Neurons

NP 23

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Culturing neurons is a indispensable tool for examining the nervous system, and has advanced to a tremendous degree in the last decade. When combined with micro-electrode array (MEA) technology, which allows for non-invasive electrophysiological recording of cultured neurons, it facilitates straightforward readouts of neuronal dynamics and enables high-throughput experiments. However, one substantial impediment to the field is the lack of control of how neurons form circuits, which has been shown to be almost entirely random.
We have demonstrated a new approach to tackling this problem, by controlling neurite growth using microstructured polydimethylsiloxane (PDMS) membranes. In order to constrain neurite outgrowth across an entire population of neurons, we developed a new way of making perforated membranes of PDMS through which the neurons may be seeded and distributed across the microstructure. We discovered that different patterns can produce different radial distributions of neurite growth, which are in many cases asymmetric, meaning neurons can be directed to grow more "forward" than "backwards". We also present some preliminary results of electrical activity of these neurons grown on a micro electrode array.

The gut-brain axis: the role of the microbiota as a mediator of the enriched environment

NP 24

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The role of gut commensal microbes in maintaining host health and contributing to disease is increasingly recognized. The gut-brain axis model explores the interactions between the gut and brain and their mutual influence. Our recent study investigated the impact of an enriched environment (EE) on these interactions. We discovered that an EE alters both the microbial profile and Short-chain fatty acids (SCFAs) concentrations in animals compared to standard-housed mice (SE). Notably, administering SCFAs (formate and acetate) produced behavioral and molecular changes in the brain akin to those observed in EE. To delve deeper into the microbial contributions, we utilized a specific bacterial cocktail (Bacteroides gallinarum, Parasutterella excrementihominis, Catabacter hongkongensis, Alistipes senegalensis, Clostridium kluyveri) identified in EE mice to replicate the effects of the environment in the brain of SE mice. Furthermore, we conducted fecal material transplantation (FMT) from EE donors to examine whether the observed modifications were due to interactions between microbes and metabolites. Our findings revealed that both interventions reshaped the gut microbial community, reduced anxiety-like behaviors, and affected hippocampal neurogenesis and neurotrophin gene expression.

Focused Ultrasound Aided Magnetic Nanoparticles Delivery to the Brain for Targeted Neurostimulation

NP 25

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Magnetic nanoparticles, when exposed to an external magnetic field gradient, can activate mechanosensitive and thermosensitive neural receptors. In this study, we developed novel magnetic nanodiscs (MNDs) for targeted brain delivery via the blood-brain barrier (BBB). We employed focused ultrasound (FUS) in conjunction with systemically administered microbubbles (MBs) to facilitate the safe opening of the BBB and enable precise delivery of MNDs to the brain. The MNDs were synthesized through a solvothermal method, producing hematite nanodiscs with a hexagonal lattice. Specifically, 0.27g of $FeCl_3$, 0.80g of sodium acetate, 10ml of ethanol, and 600µl of water were heated in an autoclave reactor. Post-synthesis, the nanodiscs were washed with deionized water, resuspended in trioctylamine (0.2ml per mg of hematite) and oleic acid (100mg per mg of hematite), and heated in a hydrogen atmosphere to convert them to magnetite. The resultant MNDs had an average diameter of 127nm, with their transformation from nonmagnetic hematite to magnetic magnetite confirmed via X-ray powder diffraction and transmission electron microscopy (TEM). In vivo delivery of MNDs was conducted on fully anesthetized mice using FUS (1.5 MHz, 0.8 MPa acoustic pressure). Thirty seconds prior to FUS application, 150µl of SonoVue® microbubbles were administered systemically through the caudal vein. The disruption of the BBB was assessed by evaluating Evans Blue uptake in the brain. Mice were sacrificed 20 minutes post-procedure, following perfusion with PBS and 4% paraformaldehyde. Extracted brains were post-fixed in paraformaldehyde overnight, and potential tissue damage induced by FUS was evaluated using Haematoxylin-Eosin staining. Our findings suggest that FUS can safely open the BBB. Future TEM imaging will be employed to evaluate the interaction of MNDs with brain tissue and to compare the efficiency of FUS-mediated delivery of MNDs against traditional methods, such as intracranial injection.

From Bone Fragility to Neural Vulnerability: Understanding the Link between Osteogenesis Imperfecta and Neural Impairment

NP 26

Enrico Pelloni (1) - Claudio Casali (1) - Roberta Besio (2) - Gloria Milanesi (1) - Wendy Perez Franco (2) - Federica Gola (1) - Ludovica Gaiaschi (1) - Margherita Cavallo (1) - Antonella Forlino (2) - Maria Grazia Bottone (1) - Fabrizio De Luca (1)

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"Osteogenesis imperfecta (OI), often called brittle bone disease, is group of genetic disorders that causes changes in connective tissue composition and metabolism, leading to bone fragility. Most of the times, the mutations involve the type I collagen, and the pathophysiology of this disorder is highly diverse, implicating a range of genetic variables and a variety of phenotypic symptoms, including neurological impairment.

Our research focused on the possible alteration that occurred in the Central Nervous System (CNS) of an in vivo model of OI, using male and female adult Brtl$^{-/+}$ mice. Histochemical techniques were employed to investigate the morphological changes of CNS, through Hematoxylin & Eosin and Picrosirius red stainings. A wide range of microscopy techniques, such as bright-field, polarized light, and transmission electron microscopy (TEM), were used to investigate the modification of brain tissue organization, cellular changes, and ultrastructural alterations. Parallelly, we focused our attention on the potential changes in the cellular autophagy pathway, using immunohistochemical reactions to analyze the levels of expression of Beclin-1, p62, and LC3B markers.

By doing so, we detected a strong alteration of CNS cytoarchitecture, with a profound change in the expression of collagen fibres, clearly detectable in mutant mice. The investigated markers cellular autophagy appeared strongly correlated to the severity of the disease. Through this work, it is possible to enhance our understanding of the link between genetic anomalies, bone fragility, and neurological involvement in OI, paving the way for future research."

POSTER SESSION

3

Exploring Distinct Metabolic Signatures Associated with Acquired Epilepsy

EBN 10

Greta Volpedo (1) - Antonella Riva (1) - Eray Sahin (2) - Rossella Di Sapia (3) - Federico Moro (3) - Ilaria Lisi (3) - Elisa Zanier (3) - Pasquale Striano (4) - Annamaria Vezzani (3) - Teresa Ravizza (3)

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Background: Emerging research implicates microbiota-gut-brain (MGB) axis dysfunction in neurologic disorders, including epilepsy. Metabolites produced by the gut microbiota affect brain activity, possibly modulating excitotoxicity and seizures, yet the underlying mechanisms remain unclear, leading to a lack of prognostic/diagnostic biomarkers and therapeutic targets for epilepsy. Using a status epilepticus (SE) rat model induced by intra-amygdala kainite injection, we found lipid metabolism dysregulation. To further investigate whether these unique metabolic changes are also associated with other forms of acquired epilepsy, we analyzed the metabolic profiles in a post-traumatic epilepsy (PTE) mouse model.

Methods: 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-traumatic brain injury (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 through untargeted metabolomic analysis.

Results: By performing metabolite and pathway enrichment analysis we show substantial metabolic variation between epileptic and non-epileptic animals in both murine models, including significant differences in lipid metabolism. These unique metabolic signatures could contribute to seizure onset and disease progression, highlighting the influence of MGB axis dysfunction on epileptogenesis.

Conclusion: This translational study highlights the existence of unique metabolic signatures associated with PTE development in mice. Understanding the role of the MGB axis in shaping these metabolic profiles offers promising avenues for identifying novel biomarkers and therapeutic targets for epilepsy, addressing the unmet clinical need in the management of this debilitating disease.

EBN 19

Unraveling the roles of oligodendrocyte progenitor cells in the development of the cortical inhibitory system

SPEED TALK

Martino Bonato (1) - Maryam Khastkhodaei Ardakani (1) - Anna Incerti Tinterri (1) - Niccolò Di Cintio (1) - Arianna Sturlese Verduri (2) - Ferdinando Di Cunto (1) - Eleonora Vannini (2) - Francesco Ferrini (3) - Annalisa Buffo (1) - Enrica Boda (1)

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Recent studies have revealed an unexpected contribution of oligodendroglial cells to inhibitory circuit establishment and function. Yet, the entire spectrum of oligodendroglia-interneuron interactions as well as the impact of oligodendroglia loss or dysfunction on the development of the inhibitory system are still open issues. Here, we exploited two mouse models where the germinal or the oligodendroglia-specific deletion of citron kinase (i.e. Cit-k KO or Sox10Cre::Cit-k fl/fl mice) leads to the selective ablation of cortical oligodendrocyte progenitor cells (OPCs) during the first two weeks of life. Cit-k KO mice display an impaired activity-dependent inhibition onto cortical pyramidal neurons and a reduced lifespan due to lethal seizures. Although not showing a spontaneous epileptic phenotype, Sox10Cre::Cit-k fl/fl mice are more vulnerable to epileptogenic drugs compared to their wild-type littermates, indicating that OPC loss was associated with an altered excitation/inhibition balance in both models. In Cit-k KO mice, the pharmacological rescue of cortical OPCs – although not restoring myelination - resulted in a reduced epileptic phenotype and in a significant rescue of the cortical inhibitory neurotransmission. This positive outcome was particularly enhanced when OPC repopulation was achieved by wild-type OPC transplantation, leading to the abrogation of susceptibility to epileptogenic drugs and to a remarkable lengthening of mouse lifespan. Mechanistically, OPC rescue/graft were associated with a strong upregulation of markers indicative of interneuron maturation. Overall, our data indicate that, at neonatal stages, the loss of OPCs is associated with alterations of the cortical inhibitory system which can be rescued by oligodendroglia restoration.

EBN 20

Upregulation of Negr1 converges into core impaired processes in autism spectrum disorders

SPEED TALK

Mariam Marie Chellali (1) - Amos Fumagalli (2) - Olga Shevtsova (2) - Giulia Colombo (2) - Giovanni Morelli (2) - Ilias Ziogas (2) - Pier Francesca Proceddu (2) - Andrea Barberis (2) - Angelo Reggiani (2) - Alessandro Gozzi (3) - Yuri Bozzi (4) - Giovanni Piccoli (4) - Laura Cancedda (5)

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Diverse neurodevelopmental syndromes (e.g. Fragile X) are caused by alterations in specific genes leading to pervasive impairments comorbid with autism (i.e. social deficits and repetitive behaviors). Although all ASDs (autism spectrum disorders) converge into common core behaviors, genetic variants in autism are very heterogeneous. Here, we describe a cleavable cell adhesion molecule (Negr1) as upregulated in the brain of 5 diverse mouse models of ASDs (including Fragile X) and postmortem brains from people with autism or Fragile X, and causative of core ASD behaviors and brain alterations when up-regulated in wild-type (WT) animals *in vivo*. In particular, we found that Negr1 upregulation in the prefrontal cortex of WT mice was sufficient to induce disruptions in sociability, repetitive behaviors and neuronal morphology. Moreover, we found that Negr1 is excessively cleaved in the Fragile X mouse model. Supporting the relevance of the latter finding, we found that overexpression of the soluble form of Negr1 in WT animals is sufficient to cause social deficits, but do not affect repetitive behaviors. Altogether, our results on the causal link between Negr1 upregulation and ASD core features in WT mice, together with our findings on Negr1 dysregulation in people with autism and in diverse ASD mouse models, may explain how a wide variety of ASD genetic variants converge into a unique core group of impaired processes during brain development. Our results also indicate regulation of Negr1 pathway as a possible target for future therapies and assessment of Negr1 levels in biological fluids as a potential biomarker for patient stratification in future clinical trials.

EBN 21

Brain Organoid Platform for Discovering New Therapeutic Strategies to Promote Neural Maturation in Allan-Herndon-Dudley Syndrome (AHDS)

SPEED TALK

Francesca Ciarpella (1) - Benedetta Lucidi (1) - Giulia Pedrotti (1) - Chiara Santanatoglia (1) - Eros Rossi (1) - Elisa De Tomi (2) - Giovanni Malerba (2) - Giorgio Malpeli (3) - Emanuela Bottani (1) - Ilaria Decimo (1)

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Thyroid hormone T3 plays a crucial role in early brain and overall central nervous system (CNS) development, influencing the proliferation, differentiation, and migration of neural progenitors. Beyond its essential function in nervous system development, T3 is a key regulator of CNS metabolism and mitochondrial activity. Hypothyroidism during the fetal stage can lead to neurodevelopmental defects, as seen in Allan-Herndon-Dudley Syndrome (AHDS). AHDS is a rare X-linked disorder caused by mutations in the SLC16A2 gene, which encodes the monocarboxylate transporter 8 (MCT8), a specific transporter for the thyroid hormone T3. We developed a brain organoid model that mimics the pathological scenario of AHDS to study neurodevelopmental defects and identify new metabolic targets to promote neuronal maturation. Immunofluorescence and whole transcriptomic analysis revealed that AHDS organoids displayed a sustained proliferative and stemness profile, with a high number of neural progenitor cells, indicating a delay in neuronal maturation. Additionally, astrocytes were the most prevalent cell type, highlighting the reduced neuronal commitment. The impaired neuronal maturation in AHDS organoids was further confirmed by functional assessments, which showed low spontaneous calcium activity. Importantly, we observed disruptions in fatty acid metabolism and mitochondrial dynamics in AHDS organoids. By pharmacologically modulating mitochondrial metabolism with Nicotinamide Riboside (NR) in AHDS organoids, we were able to reduce stemness and glial cell content, partially restoring normal neural development. These findings suggest that enhancing mitochondrial metabolism can mitigate the neurodevelopmental impacts of AHDS and ameliorate neural development.

The role of Protocadherin 9 in depression and anxiety: evidence from a knockout mouse model

EBN 22

Ilaria di Iasio (1) - Mariaelvina Sala (1) - Maria Passafaro (1) - Edoardo Moretto (1)

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Major Depressive Disorder (MDD) is one of the most disabling psychiatric disorders in the world. Common treatments act on the monoaminergic systems, but recent researches have shifted towards the glutamatergic one, due to its crucial involvement in the pathophysiology of depression and anxiety. Recent studies have linked genetic variations altering Protocadherin 9 (PCDH9) expression with these pathologies. In addition, PCDH9 knockout (KO) mouse models show reduced number of glutamatergic neurons and impaired social behaviours. We performed a preliminary panel of behavioural tests to explore whether the deletion of PCDH9 can induce a depressive phenotype. Interesting results concerns the anhedonia and depressive-like behaviour. Both male and female KO mice showed a trend towards shorter duration of grooming in the spray test and increased immobility time in the tail suspension test. In addition, in the marble burying test, a reduced number of marbles buried could be measured, which could be interpreted as a reduction in interest and motivation. Another remarkable observation concerns the anxiety phenotype in KO females which exhibited a trend towards reduced number of entries and time spent in the open arms in the elevated plus maze test. Exploratory behaviour also seemed affected more in KO females as seen in the hole board test in which the number and duration of head-dips were lower, although not significantly. In conclusion, these preliminary results suggest a possible PCDH9 gene deletion effect on depressive-like behaviour and more curiously on the KO female anxiety state. In addition to investigating further these behavioural alterations, we will analyse candidate proteins within different brain areas, based on a prior transcriptomic analysis. This study will elucidate potential molecular pathways at the basis of depressive states linked to PCDH9 reduced expression.

The Mouse Lipid Brain Atlas

Luca Fusar Bassini (1*) - Halima Hannah Schede (2*) - Giovanni D'Angelo (3**) - Gioele La Manno (1**)

* These authors contributed equally to this work; ** These authors jointly supervised this work.

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The architecture of the mammalian brain has been described in terms of its anatomy, physiology, connectivity, and cell types. As lipids play key roles in cellular processes, including synaptogenesis, signaling, and energy storage, a spatial description of the brain in terms of lipids and metabolism is imperative. Here, we utilized Matrix-Assisted Laser Desorption Ionization Mass Spectrometry Imaging (MALDI-MSI) to measure hundreds of lipids across hundreds of brain sections from multiple adult mice. The result is the first 3D lipidomic atlas of the mouse brain at quasi-cellular resolution. The atlas reveals an unprecedented patterning of lipids at the micrometric scale. First, we integrated with the Allen Brain Atlas to describe the metabolic organization in the context of core anatomical knowledge of the brain. Then, we explored the potential of lipids to reveal a new axis of heterogeneity. A lipid-based unbiased analysis exposed a fine-grained spatial zonation of the lipidome, with 1000+ meaningful clusters that we termed lipizones. Lipizones recapitulate known anatomical structures, but they can also extend across anatomical boundaries. Lipizones reveal an unprecedented heterogeneity in the white matter. In addition, we compared metabolomics with transcriptomics, and modeled the activity of biochemical pathways and reactions in space. Finally, leveraging this atlas, we investigated brain-wide compositional changes in a CERT1 metabolic disorder model, demonstrating the atlas's potential for translational research in understanding metabolic brain disorders.

EBN 24

SPEED TALK

Unveiling the molecular mechanism of intestinal metabolite para-cresol in modulating neuroinflammation and synaptic dysfunction: implications for autism spectrum disorder

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Autism spectrum disorder (ASD) is a heterogeneous group of neurodevelopmental disorders sharing similar behavioural patterns. Many individuals with ASD also display gastrointestinal disturbance, probably linked to changes in the composition and activity of intestinal bacteria, leading to the overproduction and release of toxic metabolites into the systemic circulation. They may reach the central nervous system (CNS) and trigger microglia activation, which releases inflammatory cytokines, ultimately impairing neuronal function. The microbiota-gut-brain axis has been proposed to play a crucial role in the pathogenesis of ASD. Para-cresol (pCres) is one of these intestinal metabolites and might be a potential contributor to ASD, as its urinary level is elevated in autistic children under the age 8 and correlates with symptom severity. Here we aimed to investigate the effect of pCres in different brain-cell types to speculate on their specific contribution to ASD synaptic dysfunctions. Immunocytochemistry assays showed a significant decrease in excitatory (Vglut1, PSD95) and inhibitory (VGAT, Gephyrin) synaptic markers in pCres-treated primary neurons during synaptogenesis. Such effects are exacerbated in SHANK3 knockdown neurons whose synapses are more susceptible. On the other hand, pCres also induced a dose-dependent inflammatory response in both astrocytes and microglia, characterized by the overexpression and release of inflammatory cytokines and chemokines, such as IL6, IL1β, CCL3. Interestingly, we observed that glial-derived secretome induced synaptic alterations similar to direct pCres treatment. Our results suggest that pCres exerts cell-specific effects on astrocytes and microglia, and the release of inflammatory cytokines and chemokines upon pCres treatment might represent a trigger event for synaptic dysfunction.

EBN 25

Generation and characterization of hippocampal cerebral organoids as tool for regenerative medicine

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Cerebral organoids are emerging as state-of-the-art tools for the study of neurodevelopment, disease modelling, and recently, they have drawn the attention on their potential use in the regeneration of damaged brain, overcoming all the drawbacks of neural stem cells (NSCs) transplantation e.g., low survival rate and poor differentiation. Indeed, cerebral organoids are 3D *in vitro* structures, able to recapitulate both functionally and structurally the *in vivo* brain complexity. Based on the protocol already published in our lab, we succeeded in generating rat cerebral organoids starting from NSCs isolated from the subgranular zone (SGZ) which progressively organize and differentiate reaching maturation in 32 days in vitro (DIV).

Immunofluorescence characterization of cerebral organoids revealed a progressive neuronal maturation and differentiation (DCX^+, $TUBb3^+$ cells), and different cellular composition ($GFAP^+$ cells), mirroring the *in vivo* neurodevelopment. Moreover, at later stage of culture, we observed mature neurons ($MAP2^+$ cells) forming synapses and functional neuronal network, evaluated by high-density multi-electrodes array recordings.

As proof of concept of their potential use as regenerative tools, we set up organoids' transplantation grafting immature organoids (14 DIV), obtained from hippocampal NSCs transduced with LV-GFP, into the brain of healthy adult rodents. Organoid-derived cells were successfully detected 30 days after transplantation in different brain regions (i.e cortex, hippocampus) expressing markers of neuronal differentiation, thus suggesting their integration and maturation within the host brain.

Overall, these data highlight the possible application of organoids for regenerative medicine purposes opening, as future perspectives, the possibility of producing rat CA3 region-specific organoids, for restoring neural circuitry following transplantation into epileptic animals.

EBN 26

SLC6A1 KO ZEBRAFISH MODEL: an innovative tool to identify new therapeutical approaches for myoclonic-astatic epilepsy

SPEED TALK

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Myoclonic-astatic epilepsy (MAE) is a paediatric epilepsy syndrome characterized by intellectual disability, behavioural disorders as well as the daily frequency of seizures and resistance to pharmacological treatments. Since 2015, autosomal dominant mutations of the gene SLC6A1 – which encodes for the GABA transporter (GAT)-1 - have been identified in MAE patients. Using an out-of-frame approach, two knock-out (KO) mutant zebrafish lines - named slc6a1a^{av1} and slc6a1b^{av2} – were generated by CRISPR-Cas9 technology. Although the KO zebrafish animals resulted to be vital, fertile, and able to produce viable offspring, the depletion of 50% of GAT-1 leaded to a developmental delay as well as to alterations of the locomotor activity after light/dark stimuli exposition. Moreover, neurophysiological analyses of local field potentials (LFPs) were performed on Slc6a1 KO mutants, focusing on the spontaneous frequency spectra activity and the presence of specific electrographic signatures. Finally, a target-driven drug screening was performed by testing compounds belonging to the family of inhibitor of the histone deacetylase (HDAC), known to be active on transcriptional regulation of SLC6A1. In particular, a 24-hour exposure to 0.01 mM Sodium Phenylbutyrate, resulted in a significant augmentation of Slc6a1a/b expression in zebrafish embryos. On this basis, this model may provide a promising tool to identify new potential therapeutical approaches and unveil the pathological mechanisms associated to SLC6A1 alterations.

EBN 27

Targeting Mitochondrial Calcium Uptake: Investigating MCU Enhancers in FLVCR1-Related Neurological Disorders

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FLVCR1-related disorders are rare genetic conditions caused by mutations in the FLVCR1 gene, ranging from severe neurodevelopmental defects like congenital hydrocephalus to milder sensory neuropathies like Posterior Column Ataxia and Retinitis Pigmentosa (PCARP) and Hereditary Sensory and Autonomic Neuropathies (HSAN). FLVCR1 has been identified as a choline importer and in addition to this function, as a scaffold protein of the IP3R3-VDAC complex regulating mitochondrial Ca2+ influx from the ER at Mitochondria Associated Membranes (MAMs). However, the mechanisms behind the disease remain unclear, and a therapy is lacking. In this study we aim to investigate potential therapeutic interventions for FLVCR1 related diseases. Our findings reveal that both FLVCR1 loss and mutations are associated to reduced and altered ER-mitochondria tethering, impaired mitochondrial calcium uptake and mitochondrial dysfunction. Moreover, evidence showed that mitochondrial calcium uniporter (MCU) overexpression fully rescued the defective phenotype, thus suggesting that FLVCR1-patients might benefit from therapeutic approaches promoting calcium uptake to restore mitochondrial energy metabolism. To further investigate this, we are currently testing MCU enhancers, like Kaempferol and Amorolfine. Preliminary data indicate that Kaempferol treatment in Flvcr1-null neural progenitors, significantly improved TCA cycle enzymes activity, ETC activity and ATP levels and rescued both mitochondrial and cytosolic lipid peroxidation levels. Furthermore, it significantly promoted cell proliferation. MCU emerged as a potential therapeutic target and its modulators seem promising. However, further investigation is needed to fully elucidate the therapeutic benefits of targeting MCU and to advance novel treatments for these neurological conditions. To address this, we are developing a new model of Induced Pluripotent Stem Cells derived Sensory Neurons established from patient-derived fibroblasts.

ND 33

Neurofilaments heavy chains (NfH) as potential biomarker of neurodegeneration in progressive multiple sclerosis

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Neurofilaments light chains (NfL) are largely used as a biomarker in neurodegenerative diseases, including Multiple Sclerosis (MS). Their levels increase in cerebrospinal fluid (CSF) and blood proportionally to the degree of axonal damage in acute MS and may predict disease evolution. It remains still unclear whether other MS-specific biomarkers could better reflect neurodegeneration in progressive MS (p-MS).

Re-analysis of previously performed Illumina microarray gene expression and single-cell sequencing of grey matter (GM) obtained from post-mortem MS and control cases was carried out. The protein levels of neurofilaments heavy chains (NfH) and NfL were measured in paired CSF of 27 post-mortem MS cases and correlated with clinical/neuropathological data, and with levels of other 80 CSF inflammatory mediators.

Greater down-regulation of NfH, as compared to NfL, gene expression was measured in GM lesions of post-mortem p-MS compared to controls, linked to meningeal inflammation and extensive cortical damage.

Significant correlation between NfH and NfL CSF levels (R=0.722, p=0.00002) was observed in the same MS cases. NfH levels correlate significantly with degree of lesion activity (R=0.632, p=4.045 x10^{-4}), number of RIM+ lesions (R=0.805, p=4.071x10^{-7}), percentage of GM demyelination (R=0.799, p=5.978x10^{-7}), number of meningeal follicles (R=0.801, p=5.059x10^{-7}), degree of meningeal inflammation (R=0.822, p=1.492x10^{-7}), degree of perivascular inflammation (R=0.761, p=4.136x10^{-6}). The correlations between NfL and the same neuropathological parameters were less strong.

Significant correlation was found between CSF NfH and: GFAP (R=0.87, p<0.001), NfL (R= 0.721,p=6.292 x10^{-5}), CXCL13 (R=0.589,p= 0.001), INF-gamma (R=0.581, p=0.001), IL9 (R=-0.688,p<0.0001), INF-beta (R=-0.568, p=0.002), sTNF-R2 (R=-0.541, p=0.003).

Summarizing, our preliminary analysis suggests that NfH, compared to NfL, may reflect more specifically the neuro-axonal damage occurring in progressive MS.

Decoding Neuronal Vulnerability: Integrative Analysis of D1R- and D2R-MSNs Responses in Huntington's Disease

ND 34

Guendalina Bergonzoni (1) - Miguel Pellegrini (1) - Aurora Savino (2) - Martina Lazioli (1) - Lorenzo Graziani (3) - Denise Ferrarini (1) - Tammy Gillis (4) - Nicola Andrea Casiraghi (1) - Jarne Geurt (5) - Sarah Geurs (5) - Michele Tebaldi (1) - Isabella Pesce (1) - Valeria Poli (2) - Remo Sanges (3) - Alessandro Romanel (1) - Thierry Voet (5) - Vanessa C. Wheeler (4) - Erik Dassi (1) - Marta Biagioli (1)

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Understanding the molecular mechanisms driving selective neuronal vulnerability to different neurodegenerative disorders remains a crucial, but unsolved question. Here we explored the case of Huntington's disease (HD), where the striatum and, specifically, dopamine receptor 2 (D2R) medium-sized spiny neurons (MSNs), exhibit an increased susceptibility to the CAG-repeat expansion mutation. To unravel differences between D2R- and D1R-MSNs, we employed a multidimensional approach, integrating transcriptional, morphological, genomic, and somatic instability analyses. Specifically, we resourced to *Htt* CAG knock-in mouse models harbouring 18 (*HttQ20*: "control") or ~190 (*HttQ175*: "HD") consecutive CAG repeats, expressing tdTomato and EGFP under the control of *Drd1* and *Drd2* promoters, respectively. First, comprehensive transcriptomic analyses following FACS-sorting of dissociated MSNs, revealed distinct gene expression profiles, indicating a significant upregulation of oxidative phosphorylation and translation pathways in D1R-MSNs at pre-symptomatic stage. Secondly, morphological analyses revealed an outnumbering of D1R-MSNs in the HD condition, particularly in the ventral-medial striatum. Interestingly, D2R-MSNs instead, present an increased nuclear accumulation of mutant huntingtin aggregates. Finally, while no large copy number variations were detected at genomic level in either population, a significant greater somatic instability in D2R-MSNs was noted as early as 8 weeks of age. In summary, our integrative study suggests that the distinct vulnerability of MSNs in HD might result from a combination of an early transcriptional compensatory-response of D1R neurons together with an early susceptibility of D2R neurons, correlated to increased somatic instability and mutant huntingtin aggregation.

ND 36

Air pollution and neurodegeneration: an in vitro study of the role of astrocytes in magnetite nanoparticle-induced neurotoxicity

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Air pollution has been identified as a potential contributing factor in the increased prevalence of neurodegenerative diseases (NDs) such as Alzheimer's disease (AD). Recent studies have indicated that in urban areas with high levels of traffic and industrialisation (*e.g.*, Mexico City), the incidence of dementia in younger individuals has increased. Among the traffic-related air pollutants, magnetite nanoparticles (MNPs) can be inhaled and directly reach the brain, where they can promote the formation of reactive oxygen species (ROS) and induce oxidative stress (OS), a condition often associated with AD. In the brain, astrocytes can efficiently counteract OS through the activation of an antioxidant response, thereby exerting protective or detrimental effects on neurons. To investigate the biological impact of MNPs in the context of NDs, we employed an astrocyte-neuron cocultures grown in the presence or absence of amyloid-β (Aβ). Our findings indicate that MNPs induce a significant and dose-dependent reduction in astrocyte viability, on the contrary we observed no direct effect on the viability of neurons. Moreover, oligomeric and fibrillar forms of Aβ1-42 peptide exert distinct effects on cell viability. The oligomeric form of Aβ reduces astrocyte viability in a dose-dependent manner, whereas the fibrillar form of Aβ reduces it by 50% at all concentrations tested. Instead, the two forms of Aβ resulted in the same significant and dose-dependent reduction on neuron viability. The co-treatment of MNPs with the oligomeric form of Aβ (MNPs+Aβ) induces a further significant reduction in astrocyte viability. Furthermore, we observed a significant reduction in neuron viability cultured with the conditioned medium of astrocytes previously exposed to MNPs and Aβ. MNPs+Aβ induced a further significant reduction in neuronal viability when compared to Aβ alone. The present findings collectively suggest that MNPs pollution can intensify the toxic effects of Aβ.

ND 37

Ultramicronized-palmitoylethanolamide restores cerebral metabolism and enhances anti-aging klotho expression in 3xTg-AD mice

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Growing evidence suggests that declining brain metabolism contributes to aging and neurodegenerative diseases such as Alzheimer's disease (AD) by promoting synapse loss, neuronal death, excitotoxicity and cognitive deficits. Recent findings have shown that the longevity protein klotho, which is released by neurons upon glutamatergic stimulation, can stimulate astrocytic lactate formation and release. Subsequently, lactate can be used by neurons as a preferential energy source during activity. In this way, astrocytes regulate cerebral blood flow and ensure a rapid source of oxygen and glucose to neurons. Cerebral klotho levels also appear to be dependent on the availability of taurine, another important metabolite that regulates neuron excitability and thus protects against excitotoxicity. Here, we examined the expression of klotho and measured lactate, taurine, and glutamate/glutamine levels in 3xTg-AD mice in the areas significantly affected in AD, namely the frontal cortex and hippocampus. We also investigated the effects of a 3-month treatment with ultramicronized palmitoylethanolamide (um-PEA) on these parameters. Indeed, we demonstrated that um-PEA improved learning and memory in 3×Tg-AD mice, decreased Aβ formation and phosphorylation of tau proteins, and promoted neuronal survival by restoring glutamatergic transmission. In vivo MRI/MRS experiments showed altered lactate, taurine and glutamine/glutamate levels in 3xTg-AD mice. In addition, ex vivo results showed a significant reduction in klotho protein levels and its cleaving metalloproteases as well as changes in the expression of proteins related to the synthesis, degradation and uptake of lactate and taurine in 3xTg-AD mice compared to controls. Overall, our results have revealed new and previously unexplored effects of um-PEA which, in combination with its already proven high safety in humans, make um-PEA a promising co-adjuvant treatment for AD.

ND 38

Spermidine treatment affects gene expression in mouse model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive loss of upper and lower motor neurons. Significant contributors to the disease's onset and progression include alterations in lipid metabolism and oxidative stress, both linked to mitochondrial dysfunction. Despite extensive research efforts to understand ALS etiopathology, no therapies currently exist to halt its progression. Recent studies have highlighted crucial metabolic and mitochondrial changes related to ALS development and progression both in neuronal and muscular tissues. These changes indicate a shift from glycolysis (glucose as the primary energy source) to β-oxidation (fat as the primary energy source) in ALS mouse models. Spermidine (Spd) supplementation has shown neuroprotective effects in various *in vivo* neurodegenerative models. In a mouse model of multiple sclerosis, oral Spd supplementation attenuates disease progression and enhances visual functions by reducing demyelination in the optic nerve and spinal cord and decreasing retinal ganglion cell loss. Additionally, Spd has demonstrated neuroprotective properties in mitigating α-synuclein neurotoxicity, a hallmark of Parkinson's disease, in model organisms such as fruit flies and nematodes and in rescuing motor dysfunction in mice with frontotemporal lobar dementia. Given the neuroprotective effects of Spd, we treated the SOD1-G93A mouse model of ALS at the onset of the symptoms. Spermidine was administered for 60 days, followed by RNAseq analysis of the gastrocnemius muscle . Moreover, mice force has been evaluated during the treatment by grip strength test. Our findings revealed that Spd administration counteracts the physical and molecular changes occurring in the muscles of the ALS mouse model. In conclusion, our data suggest that Spd positively affects the ALS mouse phenotype, paving the way for future studies aimed at improving patient outcomes.

ND 39

A comprehensive functional and omics approach in patient-derived dopaminergic neurons to identify specific molecular signature associated to Parkinson's disease

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Genetic understanding of Parkinson's disease (PD) is quickly increasing with the emerge of the next-generation sequencing (NGS). Using whole-exome sequencing analyses we recently discovered a polygenic model of inheritance associated with familial and sporadic PD. The aim of this study was to explore the functional role of the most promising genetic variants in a large set of human iPSC-derived dopaminergic neurons.

We focused our study on the role of more than 10 likely pathogenic variants (singularly or in combination) in 10 novel PD candidate genes (*AIMP2, HMOX2, SLC6A3, KIF21B, LRRK2, RHOT2, TMEM175, TOMM22, TVP23A, ZSCAN21*).

We planned to study functional and molecular alterations in mesencephalic dopaminergic (mdDA) neurons derived from PD patients and controls by patch–clamp recording and integrated omics (transcriptomic, proteomic and lipidomic) approach.

We found that all the genes analysed were expressed at undifferentiated state and during neurons differentiation. Interestingly, we observed that the mutated genes showed a significant different level of expression in patients derived cells with respect to control cells. Preliminary functional data revealed a defect in current recording in patient-derived neurons when compared to healthy subjects. Specifically, we discovered that cells derived from patients heterozygous for mutations in *SLC6A3* and in *HMOX2* genes displayed an electrophysiological immature profile compared to those of other patients and controls.

The integrated multi-omics analysis to identify specific molecular signatures associated with PD mutated alleles is still under investigation.

In conclusion, our approach could extend the knowledge about the interplay between genetics and metabolism in Parkinson's disease and might pave the way for developing novel therapeutic strategies.

ND 40

Correlation between the β amyloid and the cognitive and memory impairments in 3xTg-AD model of Alzheimer's disease

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Alzheimer's diseases (AD) is the most prevalent neurodegenerative cause of dementia worldwide associated with progressive neurodegeneration in specific brain area, that cause neural dysfunction from synapses to networks. Recent studies identified biomarkers for a preclinical stage of AD. Different transgenic mice models are studied to dissect the mechanisms involved in AD pathology. Here, we focused our project on the triple-transgenic mouse 3xTg-AD, a model that exhibit both Aβ and tau pathology, as well as synaptic dysfunction, that reflect the human form. 3xTg-AD model described extracellular plaques first appearing at 6-months of age until 12 months of age. Here, the triple transgenic mouse model 3xTg-AD will be subjected to different therapeutic approaches such as monoclonal antibodies. At different stages of the AD progression, we correlate the level of β amyloid in the liquor of 3xTg-AD treated with monoclonal antibodies and we will evaluate cognitive and memory deficits, including spatial navigation capacity, electrophysiological activities and imaging *in vivo*. All the data will be compared with those of controls.

ND 41

Trazodone, dibenzoylmethane and tauroursodeoxycholic acid do not prevent motor dysfunction and neurodegeneration in Marinesco-Sjögren syndrome mice

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There is no cure for Marinesco-Sjögren syndrome (MSS), a genetic multisystem disease linked to loss-of-function mutations in the *SIL1* gene, encoding a BiP co-chaperone. We previously found that the PERK kinase inhibitor GSK2606414 delayed cerebellar Purkinje cell (PC) degeneration and the onset of ataxia in the woozy mouse model of MSS. However, GSK2606414 is toxic to the pancreas and does not completely rescue the woozy phenotype. The present study tested trazodone and dibenzoylmethane (DBM), which partially inhibit PERK signaling with neuroprotective effects and no pancreatic toxicity. We also tested the chemical chaperone tauroursodeoxycholic acid (TUDCA), which can protect MSS patients' cells from stress-induced apoptosis. Mice were chronically treated for five weeks, starting from a presymptomatic stage. Trazodone was given 40 mg/kg daily by intraperitoneal (ip) injection. DBM was given 0.5% in the diet ad libitum. TUDCA was given either 0.4% in the diet, or 500 mg/kg ip every three days. None of the treatments prevented motor dysfunction in woozy mice, assessed by the beam walking and rotarod tests. Only trazodone slightly boosted beam walking performance. However, immunohistochemistry found no reduction in the number of CHOP-positive PCs, or increased PC survival, indicating no neuroprotective inhibition of PERK signaling. Pharmacokinetic studies excluded that the lack of effect was due to altered drug metabolism in woozy mice. These results indicate that trazodone, DBM and TUDCA, at dosing regimens active in other neurodegenerative disease mouse models, have no disease-modifying effect in a preclinical model of MSS.

Impact of physical exercise on immune-madiated synaptic toxicity in Multiple Sclerosis

ND 42

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Physical exercise is known to improve clinical symptoms and overall wellness in Multiple Sclerosis (MS), and is linked to vagus nerve stimulation through the cholinergic anti-inflammatory pathway (CAP). Recently, T cell-mediated synaptopathy has emerged as a key mechanism of neurodegeneration in MS.

In this study, we evaluated the effects of exercise on MS immune-mediated synaptic damage by analysing: i) T cell-mediated excitotoxic damage using electrophysiological recordings; ii) the T cell immunometabolic profile using cytofluorimetric analysis and intracellular staining; iii) clinical and synaptic (striatal and hippocampal) defects induced by experimental autoimmune encephalomyelitis (EAE) model; iv) the role of CAP stimulation during exercise, performing splenectomy and unilateral cervical vagotomy in the EAE animal model.

Results showed that in MS patients, exercise partially reversed T cell-mediated glutamatergic alterations by modulating the lymphocyte immunometabolic axis. Similarly, in the EAE model, exercise improved the frequency of striatal spontaneous excitatory postsynaptic currents (sEPSCs) and restored normal synaptic potentiation after LTP induction. EAE mice that engaged in voluntary exercise showed improvements in motor disability, cognitive functions, and anxious behavior. Splenectomy and cervical vagotomy negated the beneficial effects of exercise on T cell-mediated synaptotoxicity in the striatum and hippocampus, confirming that exercise-induced vagal stimulation helps improve synaptic parameters in EAE mice by modulating T lymphocyte activity.

In conclusion, the study demonstrates that exercise rebalances the immunometabolic axis, which is disrupted in MS and underlies altered T cell responses. The CAP pathway appears to mediate these benefits, although further research is needed to clarify the exact mechanisms.

Raman Spectroscopy analysis of salivary alpha synuclein for early diagnosis of Parkinson disease

ND 43

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Parkinson's disease (PD) is a complex neurodegenerative disorder that is still lacking a robust biomarker for early diagnosis and monitoring. Recent studies on blood and other liquid biopsies showed that α-synuclein (α-syn) is mainly present as oligomers and fibrils in people with PD (pwPD) and as monomeric conformation in healthy controls (HC). For this reason, α-syn is extensively studied as promising biomarker for early PD diagnosis, although the variations in its concentration in liquid biopsies have not lead to its validation as diagnostic biomarker.
The aim of this study is the development of an analytical method based on Raman Spectroscopy (RS) to detect salivary pathological α-syn in people with PD (pwPD) compared to healthy subjects (HC).
Saliva was collected from 14 pwPD and 12 HC. RS acquisition was performed both on fresh and post-freezing saliva, in wet and dry conditions, and on two types of substrates, aluminium and Calcium Fluoride. Saliva was then ultracentrifugated to concentrate α-syn and pooled samples were preparated according to Hoehn and Yahr scores, age and gender. After acquisition, the spectra were processed and statistical analysis was performed.
The acquisition of saliva spectrum on aluminium substrate in dry condition was proved to be the most reproducible condition to obtain a good signal-to-noise ratio and it was preferred for further analyses as it is also more easily translatable to the clinical setting. Raman analysis of saliva showed differences in multiple spectral ranges between pwPD and HC and a shift in the peak potentially attribuible to α-syn in the spectral range between 1600 and 1700 cm^{-1}, despite remarkable biological variabilty among pwPD.
In conclusion, the RS-based label free analysis on saliva samples showed to be an effective method for the detection of α-syn, having the potentiality to be transferred to the clinical setting to improve the diagnosis and monitoring of PD.

ND 44

Cortical neural synchronization disrution in patients with dementia associated with Parkinson's disease and symptomatic Huntington's disease

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Background. Parkinson's disease (PD) and Huntington's disease (HD) are neurodegenerative disorders that impact the basal ganglia, resulting in movement-related symptoms and possible cognitive decline or dementia. Resting-state electroencephalographic (rsEEG) rhythms serve as indicators of neurophysiological mechanisms linked to brain arousal fluctuations. The hypothesis suggests that rsEEG sources may exhibit more significant abnormalities in patients with symptomatic Huntington's disease (S-HD) compared to those with dementia caused by Parkinson's disease (PDD). This differentiation could provide insights into the distinct pathophysiological profiles of these conditions and their impact on brain function.
Methods. Clinical and rsEEG data were collected from 18 S-HD, 16 PDD, and 25 Healthy participants, matched for demographics, education, and gender. The cortical rsEEG sources across different frequency bands were estimated using eLORETA software.
Results. The results revealed a reduction in the amplitude of posterior alpha rhythms (approximately 8-12 Hz) and an increase in widespread low-frequency bands (specifically delta, <4 Hz, and theta, about 4-7 Hz) in both the PDD and S-HD groups compared to the healthy participants. Additionally, the S-HD group showed significantly greater reductions in rsEEG alpha 2 rhythms in the frontal and temporal regions when compared to the PDD group.
Conclusion. These findings suggest that the cortical sources of rsEEG rhythms may reveal distinct abnormalities in the neurophysiological mechanisms related to brain arousal in PDD and S-HD patients. Such rsEEG markers could prove clinically valuable for disease staging, monitoring progression, and aiding in drug discovery. Furthermore, understanding these differences could enhance our ability to tailor interventions and therapies to the specific needs of patients, potentially improving outcomes. By integrating rsEEG findings with other clinical assessments, healthcare providers might gain a more comprehensive understanding of the underlying pathophysiology, leading to more effective management strategies.

ND 45

Development of AAV-mediated gene therapy for Marinesco-Sjögren syndrome and preliminary efficacy test in woozy mice

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Marinesco-Sjögren-syndrome (MSS) is a rare multisystem disease of infancy causing cerebellar ataxia and myopathy. Loss-of-function mutations in the gene encoding SIL1, a BiP co-chaperone, are found in most MSS patients. SIL1 impairment results in accumulation of unfolded proteins in the endoplasmic-reticulum (ER), triggering maladaptive activation of the unfolded protein response (UPR). Woozy (*wz/wz*) mice carry a spontaneous *Sil1* mutation and develop cerebellar atrophy with Purkinje cells (PCs) degeneration and skeletal muscle myopathy reminiscent of MSS. This study aims at developing adeno-associated viral vectors (AAVs) expressing wild-type SIL1 and test whether they can prevent UPR activation and PC loss in woozy mice. The mouse SIL1 WT or eGFP cDNAs was cloned in the pAAV-CAG-GFP vector under the control of the CMV-early enhancer/chicken-β-actin (CAG) or the PC-specific L7-6 promoter, packaged in AAV9 or PHP.eB capsids. The AAVs were tested *in vitro* to assess transgene functionality and expression pattern. To test whether AAV- mediated SIL1 expression prevented or ameliorated development of ataxia, we injected newborn woozy mice with 5*10^11 vg/mouse of AAV-CAG-SIL1 or AAV-L7-6-SIL1 in the jugular vein. Mice were trained on the accelerated-rotarod and beam-walking tests during the fourth week of life and their motor performance was monitored weekly for five weeks. No difference was observed in development of early motor deficits between AAV- and vehicle-injected woozy mice. Western blot analysis of brain tissue indicated transgenic SIL1 expression similar to that in heterozygous (*wt/wz*) mice. Further investigations are ongoing to determine whether different AAV administration routes, such as intracerebroventricular injection, result in better SIL1 expression levels and rescue the woozy phenotype.

ND 46

Novel theranostic nanobubbles: combining magnetic guidance for precise delivery and imaging with local iron chelation in neurodegenerative disease models

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Neurodegenerative diseases, such as Alzheimer's and Parkinson's, pose complex challenges, with their triggers still largely unidentified. Recent research points to brain iron dysregulation as a potential key factor. Specifically, iron accumulation has been associated with oxidative stress and the abnormal protein aggregation characteristic of these diseases. Current therapies, unfortunately, offer limited benefits, underscoring the pressing need for novel approaches. In this context, we present an innovative theranostic approach based on nanobubbles (NBs) for local iron chelation, while utilising superparamagnetic iron oxide nanoparticles (SPIONs) for magnetic guidance and imaging. By linking deferoxamine (DFO) to glycol chitosan (GC), we were able to develop GC-DFO NB (166.6 ± 23.2 nm) capable of chelating Fe^{3+} up to 640 µM in the presence of 50 µM SPIONs (18.6 ± 2.3 nm). In vitro studies further demonstrated the ability of GC-DFO NB to prevent amyloid β misfolding and reactive oxygen species (ROS) production in the presence of Fe^{3+} ($p<0.001$). Additionally, after 24 hours of incubation of NBs with Fe^{3+}, NBs were shown to rescue the spontaneous firing potential of a primary hippocampal network compared to Fe^{3+} alone ($p<0.05$). Our current focus is on developing a new NB formulation that can optimise iron chelation and enable reversible SPIONs conjugation.

IL-9/IL-9 receptor signaling is active in murine motor neurons

ND 47

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Scientific research in recent years has revealed a complex, and still incompletely understood, interaction between the immune system and the nervous system. In this context, interleukin (IL)-9 is a cytokine produced by the immune system. Recently, it is emerging a neuroprotective role of IL-9 in neuronal cells. In fact, IL-9 regulates differentiation of murine hippocampal progenitor cells and promotes neuroprotection in cortical neurons by reducing the pro-apoptotic factor Bax. The neuroprotective role of IL-9 is consistent with clinical data: levels of IL-9 in the cerebrospinal fluid of multiple sclerosis patients inversely correlate with levels of Neurofilaments Light chain, index of neurodegeneration. We hypothesised that IL-9 could have a beneficial role in other neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), which is a disease characterized by degeneration of motor neurons, especially in the spinal cord. The response to IL-9 is mediated by its specific receptor. First, we found that IL-9 receptor (IL-9R) is expressed in spinal cord tissues and embryonal spinal cord cultures from wild-type (WT) and G93A (ALS mouse model) mice. Moreover, confocal microscopy analysis revealed that IL-9R is mainly expressed in motor neurons. In addition, we validated this data on NSC-34 motor neuron-like cell line. It is known that IL-9/IL-9R binding activates STAT and AKT pathway. Thus, we stimulated with IL-9 embryonal spinal cord cultures and NSC-34 motor neuron-like cell line, and we investigated IL-9/IL9R pathways. Results indicate that IL-9 significantly activate STAT3 and AKT phosphorylation in both cell types, demonstrating the responsivity of murine motor neurons to IL-9. Overall, our data indicate that IL-9 has a potential neuroprotective role on motor neurons, with important implication in ALS disease. Further studies are directed to elucidate IL-9 role in murine and human motor neurons.

ND 48

Characterization of macrophage activation and effects of Ambroxol treatment in GBA-associated Parkinson's disease

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Glucocerebrosidase (GCase) is a lysosomal enzyme encoded by the GBA1 gene, and heterozygous mutations in this gene, which cause a defective GCase activity, are the most important risk factor for Parkinson's disease (PD). Since defects in GCase activity seems to contribute to disease-associated neuroinflammation, in this study we investigated the impact of GBA mutations on macrophage activation by comparing three experimental groups: healthy controls, PD patients with GBA1 mutations (GBA-PD), and patients with sporadic PD. Moreover we explored the effects of Ambroxol, a promising candidate in therapeutic strategies for GBA-PD patients, on macrophage alterations.

Macrophages were differentiated from patient's blood monocytes. The culture medium was enriched with growth factors and cytokines to stimulate the macrophages towards the M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotype. To assess the influence of GBA1 mutation on macrophage activation, we evaluated the expression of surface markers associated with M1 and M2 phenotypes by flow cytometry, and the release of pro- and anti-inflammatory cytokines in the cell medium using ELLA™ technology. GCase activity was measured by a fluorometric assay.

The preliminary data obtained from 26 samples suggest that the expression of cytokines and chemokines differs between the experimental groups in baseline conditions. We observed a trend towards an increase in CCL2 and CXCL10 in GBA-PD, while TNF-α appear to be increased in both PD groups; IL-6 levels seems to be lower in PD groups. Ambroxol treatment effectively reduces the levels of pro-inflammatory cytokines in the PD groups.

In conclusion, this data provides insights into possible alterations in macrophages in presence of GBA1 mutations. Future analyses in a greater court of patients and controls will be crucial to confirm these results and to determine whether treatment with Ambroxol can restore physiological conditions in macrophages of mutation carriers.

CD4+ T cells in Alzheimer's disease: modulating role of sleep

NI 33

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Accounting for 54% of all dementias in Europe, Alzheimer's Disease (AD) is the most common neurodegenerative disorder affecting brain cognition. Still, the pathophysiology of the disease remains largely unclear, particularly in the context of adaptive immunity. While inflammation is a well-recognized aggravating factor, $CD4^+$ T cells can either exacerbate or mitigate AD symptoms depending on the brain-infiltrating subpopulations. Here, we propose the construction of an age- and region-specific atlas of brain infiltrating $CD4^+$ T cells subtypes in wild-type and AD mouse models (i.e. APP/PS1) exploiting RNAscope and IF technologies. Once completed, we will validate the atlas by analyzing the impact of sleep deprivation on variations in $CD4^+$ T cell subtypes. Sleep fragmentation and insomnia are among the earliest signs of AD and contribute to memory impairments, Aβ production, and tau aggregation processes. The construction of a validated $CD4^+$ T cell atlas for AD will enable us to explore the specific roles of T cells at different disease stages and provide the possibility of targeted interventions.

NI 34

Choroid plexus is a neuro-immune interface critically involved in the resolution of neuroinflammation: implications for multiple sclerosis immunopathogenesis

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Uncontrolled or unresolved inflammation is associated with many widely occurring diseases both in the periphery and in central nervous system (CNS). During neuroinflammation, both resident and infiltrated immune cells are activated and contribute to disease onset and progression. Immune cell infiltration within the CNS occurs through the well-known blood-brain barrier, but also through the blood-cerebrospinal fluid barrier formed by neighboring choroid plexus (ChP), which is formed by epithelial and endothelial cells. Recent studies show that ChP is altered in several neurodegenerative diseases in terms of permeability and release of inflammatory mediators. By means of flow cytometry, we characterized both epithelial (HIBCPP) and endothelial (iHCPEnC) ChP cells in terms of their ability to biosynthesize via different enzymes pro-inflammatory and specialized pro-resolving lipid mediators (SPMs) and to respond to them via their receptors in a pro-inflammatory environment. We found a differential capability of HIBCPP and iHCPEnC cells to produce SPMs but also to potentially respond to resolvins and lipoxins. This was also confirmed by a lipidomic analysis of 60 different lipid mediators performed on their supernatants obtained under different inflammatory conditions. Considering the role of ChP in the recruitment of immune cells from the blood into the CNS and the repercussions this may have in neurodegenerative diseases such as multiple sclerosis, we then performed a 2D co-culture and an inverted culture between inflamed HIBCPP and peripheral blood cells from healthy donors and multiple sclerosis (MS) patients, and we found an enhanced recruitment of specific subsets of immune cells from the blood into the CNS according to the disease phase. In conclusion, our data identify a role for the ChP as a key neuro-immune interface in modulating the inflammation-resolution processes, suggesting that it could be involved in MS pathogenesis.

New wireless implantable neuromodulating devices: evaluation of minimally invasive implantation strategies

NI 35

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The CROSSBRAIN EU project has introduced innovative wireless implantable devices known as microbots (µBots, 100x100x100 μm^3) to address the heterogeneity of brain disease by applying various neurostimulation modalities. This approach aims to provide personalized treatments, a capability that currently technologies lack, through the µBots' small size, wireless control technology, and design that enables highly precise modulation of specific brain regions and targeted resolution of specific spatiotemporal events. This study investigated an implantation method to minimize the invasiveness of µBots within the brain during their applications. Experiments were conducted *in vitro* using 0.6% agarose gel, which mimics the properties of brain tissue, with silicon (Si) dummy µBots that replicate the shape and size of actual µBot. Various wires of 100µm (gold, tungsten and optical fiber) were used as a shuttle to guide the positioning of the dummy µBots, coated with different biocompatible polymers (PEOX and PEG) at various concentrations to protect brain tissue integrity. These polymers also facilitate the release of the dummies due to their water-soluble properties. Implantation tests were conducted to determine the best wire/polymer combination, focusing on low entry force and optimal polymer dissolution time to ensure stable positioning of the dummies in the brain. Our investigation revealed that the dummies were successfully implanted in the agarose gel under all conditions, with high variability in dissolution time (ranging from 3 to 10 minutes at 22°C) depending on polymer concentration and thickness. The insertion force varied based on the wire materials. Following wire extraction from the implantation area, the dummies remained in the positions where they were attached to the shuttle and implanted. These results indicate that the strategy involving a shuttle coated with a dissolvable polymer could be used for the controlled delivery of µBots inside the brain.

Human Umbilical Cord-Mesenchymal Stem Cells Promote Extracellular Matrix Remodeling In Microglia

NI 36

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Human mesenchymal stem cells (h-MSCs) modulate immune response and are promising candidates for cell therapy in neuro-inflammatory brain disorders affecting both the adult and the premature infant. Recent evidence indicates that, through their secretome, h-MSCs direct microglia, the brain resident immune cells, toward pro-regenerative function, but the mechanisms underlying microglial phenotypic transition are not yet understood. The aim of this study was to identify by qPCR and RNA-seq the molecular pathways mostly influenced by h-MSCs and explore their impact on microglial functions by ICC and live imaging. To this end, murine isolated microglia were inflamed with a cytokine cocktail (IFNγ, IL1β and TNFα), co-cultured or not with h-MSCs in transwell and stained for immunofluorescence analysis, treated for RNA extraction or prepared for live imaging. qPCR and ICC analysis revealed that h-MSCs impact on microglia is complex, inducing protective markers (*Arg1* and *Socs3*) and counteracting some inflammatory traits but also increasing some inflammatory pathways. Transcriptomics data showed that the most relevant pathway altered by h-MSCs secretome is related to extracellular matrix (ECM) remodeling, leading to higher ECM deposition and microglial motility, as indicated by increased expression of ECM components (fibronectin and transglutaminase 2) and higher path length and speed of microglial cells. These results suggest that transcriptional and functional changes regulating ECM remodeling may be the key towards the pro-regenerative microglia transition, which contributes to the protective effects of h-MSCs in experimental model of neuroinflammatory diseases. Further research is required to validate this hypothesis *in vivo* and to understand how microglia-mediated ECM modulation contributes to better outcomes in models of neuroinflammatory diseases. (This work is funded by H2020 PREMSTEM project).

Combined neuropathology and in situ gene sequencing characterization of meningeal inflammation in progressive multiple sclerosis

NI 37

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Chronic inflammation compartmentalized in central nervous system niches, such as meningeal tertiary lymphoid-like structures (TLS), is suggested to play a key role in progressive multiple sclerosis (pMS) and to be associated with a “surface-in” gradient of neuronal loss, microglia activation in grey matter lesions (GML) and normal appearing with matter (NAGM) and rapid and severe disease progression. We aimed to better characterize the inflammation on formalin-fixed paraffin-embedded (FFPE) sections from 60 post-mortem MS progressive cases and 10 controls, stained to obtain semi-quantitative immune cells count. Using spatial transcriptomics-in situ mRNA sequencing (ISS) technology (CARTANA) we analysed 157 immune-related gene expression in meningeal TLSs of 4 TLS+ MS, 4 TLS- MS and 4 controls. Differential expression analysis was performed using the limma package in R. Meningeal cell count demonstrated significantly increased numbers of B cells in 26/60 examined cases with the highest degree of meningeal inflammation (>50 cells/field). B cell number was significantly higher than macrophages and T cells. ISS analysis revealed: a significant increase (adjp < 0 .1, fold change > 1.5) of 5/157 genes (NFKBIA, SELL, ICAM, RASGRP2, PSMB8) in all MS cases with respect to CTR; a significant increase of 4/157 genes (IGKC, IGHM, RASGRP2, CD81) in TLS+ MS with respect to CTR; no genes were found differentially expressed in TLS- MS respect to CTR. Immunohistchemistry validation analysis demonstrated that meningeal TLS B cells were characterized by expression of the germinal center B-cell markers CD10 and CD81, the proliferation marker Ki67, light immunoglobulin chains (with overexpression of K chains), immunoglobulin M, in combination with elevated expression of antigen-presentation marker CD11c. Our data suggest sustained intrathecal accumulation and expansion of B cells with active immunoglobulin production in MS meningeal TLS even in progressive MS phase.

Subarachnoid hemorrhage triggers T-cell infiltration associated to microglial activation and neuronal death in mice

NI 38

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Subarachnoid hemorrhage (SAH) triggers neuroinflammation including the recruitment and activation of immune cells. T-cells in particular, have been shown to promote the evolution of secondary injury following ischemic stroke, however their role in SAH is still unclear. In this study we aimed to evaluate T-cells' recruitment in the parenchyma of SAH injured mice to next investigate their specific role in injury progression. Adult C57BL/6J mice were subjected to SAH by blood injection in the prechiasmatic cistern or sham procedure and sacrificed at 3 or 7 days-post-injury (dpi). Sensorimotor deficits were longitudinally evaluated by Garcia modified test up to 7 dpi. Diffusion weighted imaging (DWI) was performed at 3 dpi. Brain histopathology was performed at 3 and 7-dpi. Gene expression was assessed by RT-PCR in the hippocampus and lesion area at 7-dpi. SAH mice displayed sensorimotor deficits up to 7-dpi. The DWI analysis at 3 dpi showed increase hyperintense areas close to the lesion, indicating the presence of vasogenic edema. Infiltration of CD3+ T-cells was observed in the lesion of SAH mice only, and was associated with increased neuronal death and IBA1 stained area in the lesion and in the hippocampus. The gene expression analysis showed increased levels of specific lymphocytic and microglial genes in the same areas at 7-dpi. SAH induced functional impairment and neuroinflammation marked by vasogenic edema and microglial activation associated to T-cells' infiltration in brain parenchyma. Further studies will aim to better elucidate T-cell's contribution to injury evolution following SAH and to attribute it to specific phenotypes.

184 -Optimization and comparison of laboratory methods and correlation with clinical phenotype: anti-NF155, CNTN1, CASPR1 antibodies

NI 39

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Background: Autoantibodies (auto-Abs) against paranodal proteins, such as neurofascin-155 (NF155), contactin-1 (CNTN1), and contactin-associated protein-1 (CASPR1), define a subgroup of patients with immune-mediated neuropathies (IMN) and distinct phenotypes: subacute onset, ataxia, tremor and failure to respond to IVIG. Guidelines recommend testing paranodal Abs in patients with Chronic Inflammatory Demyelinating Polyradiculoneuropathy (CIDP) resistant to standard therapies and in those with acute-onset Guillain-Barré Syndrome (GBS). Current study aims to validate an indirect immunofluorescence (IIF) Research Use Only fixed Cell-Based Assay (CBA) and to determine appropriate cut-off for in-house ELISA.
Methods: 83 serum samples from patients with IMN (30F, 53M; mean age 61yrs) were tested for anti-NF155, -CNTN1 and -CASPR1 (23 samples) IgG by in-house ELISAs and IIF CBA (Euroimmun). ELISA was reactive if OD values were >mean+3DS of the healthy controls. Tissue-Based Assay on rat sciatic nerve fibers (TBA) is being optimised.
Results: ELISA showed reactivity in 12/83 samples. Anti-NF155 Abs were detected in 3/81, CBA negative; 2/3 exhibited compatible phenotype. Anti-CNTN1 were detected in 4/82: 2 samples with higher OD values were positive in CBA; 1 sample was positive in TBA but negative in CBA; 1 sample was negative in CBA; all these patients had chronic neuropathies with ataxia. Anti-CASPR1 were detected in 5/23: only 1 with higher OD was positive in CBA. All patients with anti-CASPR1 had GBS, confirmed in long follow-up. By CBA we detected anti-CNTN1 Abs in 2 samples (non-reactive ELISA). Overall between-test agreement was 84,3%.
Conclusion: To diagnose Ab-associated paranodal neuropathies, current guidelines require at least two positive tests between ELISA, CBA and TBA. Our preliminary data highlight a certain level of analytical discordance between in-house ELISA and CBA; however some cases align with clinical phenotype. Further studies are needed.

High-fat diet drives glutamatergic synaptic damage by shaping the gut microbiota and T cell dynamics in Multiple Sclerosis

NI 40

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High-fat diet (HFD) induces a systemic inflammatory condition that can aggravate multiple sclerosis (MS), an unpredictable, demyelinating autoimmune disease of the central nervous system (CNS). HFD effect on inflammatory synaptic dysfunctions is still unexplored despite their role in the silent MS progression.
Here, we studied this aspect in both a large cohort of patients with MS (N=226) and its mouse model, the experimental autoimmune encephalomyelitis (EAE). We observed that overweight or obese patients with MS exhibited higher disability and glutamate levels in the CNS, contributing to silent disease progression. We also showed in the EAE that HFD worsened clinical manifestations, neuroinflammation, and glutamatergic synaptic transmission. Unexpectedly, HFD triggered glutamatergic synaptic alterations in control mice resembling those observed in EAE. Mechanistically, interleukin-1beta (IL-1β) and tumor necrosis factor (TNF) were identified as pivotal mediators in the process. A multi-omics approach revealed that HFD altered blood-brain-barrier permeability and gut microbiota composition, redistributing adaptive immune cells from the periphery into the CNS and leading to glutamatergic synaptic dysfunctions. Importantly, a biphasic dietary supplementation of prebiotics (resistant starch, fibers and oligosaccharides) and probiotics (multistrains of Lactobacillus and Bifidobacteria) was able to reverse both immune and synaptic effects of HFD.
Altogether our findings suggest that reducing dietary fat intake can offer protection against immune-inflammatory synaptic damage and MS progression.

Abbreviations: BMI: Body-mass index; CFA: Complete Freund's Adjuvant; CSF: cerebrospinal fluid; EAE: experimental autoimmune encephalomyelitis; IL-1beta: interleukin 1-beta; L-Glu: L-glutamate; MS: multiple sclerosis; TNF: Tumor Necrosis Factor

Blood-Brain Barrier Dysfunction in Cerebral Arteriovenous Malformations. A Murine Model of Hypoperfusion-Reperfusion Injury Assessed with Contrast-Enhanced Dynamic MRI

NI 41

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Introduction: Cerebral arteriovenous malformations (AVMs) behave as low-resistance circuits, with vascular steal and chronic hypoperfusion of the perimalformative tissue, and dynamic vascular overload with increased tangential tension and endothelial damage. The inflammatory theory and blood-brain barrier (BBB) dysfunction in the evolution of the disease are supported by ex vivo demonstrations. Having a model that allows evaluating dynamic vascular changes in vivo would be of great clinical and translational interest.
Objectives: Generate a murine model that mimics the hypoperfusion-reperfusion damage typical of perimalformative tissue before and after surgical intervention. To evaluate BBB alteration in vivo using dynamic contrast-enhanced MRI (DCE-MRI).
Material and methods: First stage: ligation of both external carotids (ECA) and end-to-side anastomosis between internal jugular–common carotid (Y-C). Evaluation of BBB in the hypoperfusion phase with DCE-MRI (7-Teslas) on days +1, +7 and +21. Second stage: closure of anastomosis by ligation ("treatment"). Evaluation in the reperfusion phase with DCE-MRI at 24 hours.
Results: Of 17 animals operated on, 12 survived with a patent Y-C fistula until day +21. The BBB permeability constant (K_{trans}) increased from day+1 (0.091±0.075) to day+21 (0.248±0.168), and decreased after 24h after fistula closure, without reaching basal levels (0.145±0.170). .
Conclusions: The described model seems to reproduce the dynamic BBB damage induced by the low resistance arteriovenous circuit, loco-regional hypoperfusion and venous overload. The BBB alteration is more evident on day +21 of evolution. After 24 hours of "treatment", a decrease in BBB permeability is observed, but not its full normalization. DCE-MRI seems valid to evaluate BBB permeability in this model, and could be used in the study of disease-modulating drugs.

Deep Learning-Powered Microglia Activation Analysis in a Spinal Cord Injury Model

NI 42

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Spinal cord injury is a devastating condition that can result in significant motor and sensory impairment. Microglia, the resident immune cells of the central nervous system, play a critical role in the response to damage. However, the accurate quantification of microglia activation is challenging due to the heterogeneity of morphology and the lack of standardized methods. In this study, we developed a deep learning-based approach for the automated quantification of microglia activation.

We evaluated the performance of our approach on a dataset of histological images of microglia in injured and uninjured tissue.

We first preprocessed them manually delineating the perimeter of microglial cells. The total number of cells was segmented by U-Net deep neural network, which is a state-of-the-art architecture specifically designed for biomedical image segmentation, after a training phase that improved the algorithm's predictions and achieved accurate results. After the prediction we used a post processing pipeline to improve segmentation in high cell density regions such as the injury and to classify the morphology using different morphometric parameters. Deeply investigating the morphological differences among the microglial cells recorded, we could identify some characteristic clusters. Our study also investigated whether Rolipram, an anti-inflammatory drug, loaded with an innovative nanogel could modulate microglial morphology. The results suggest that encapsulation within the nanogel effectively delivers Rolipram to microglial cells, thereby altering their morphology and function to promote recovery.

The present study successfully proposes a robust discriminative machine learning model to predict distribution and morphology of activated microglia in an acute damaged spinal cord tissue, as well as allowing the assessment of the fine ramifications. This pipeline effectively eliminates artifacts while preserving the integrity of cellular structures.

Sex-based differences in a mouse model of experimental colitis housed in environmental enrichment

NI 43

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Ulcerative colitis, a chronic inflammatory bowel disease, is often associated with mental health disorders exacerbated by chronic stress. Environmental enrichment has been shown to alleviate stress and inflammation in mice, therefore we hypothesized that it may mitigate the adverse behavioral and neurobiological outcomes associated with experimental colitis. To study this, male and female mice were kept in 2 different housing conditions, conventional small cages (SE) or an enriched environment (EE), for 8 weeks. Subgroups were treated with dextran sulphate sodium (DSS) to induce experimental colitis and readouts comprised a battery of behavioral tests and inflammation assessments. Analysis revealed that male DSS-treated EE-housed mice experienced less weight loss, had less colonic inflammation and had a lower disease activity index than SE-housed animals. In contrast, DSS-treated females in EE exhibited more severe symptoms than those in SE, suggesting sex-dependent differences. Both male and female DSS-treated mice, irrespective of housing, displayed reduced locomotion in the open field test. DSS-treated males in SE showed less anxiety-like behavior in the elevated plus maze compared to controls, an effect not observed in EE. DSS-treated females displayed increased anxiety-like behavior regardless of housing, and a decrease in social behavior. DSS-treated animals also showed increased numbers of innate immune cells in colon, brain and blood, indicating inflammation all along the gut-brain axis. In conclusion, EE appears to provide partial protection against experimental colitis in males but exacerbates inflammation in females. Further experiments will clarify which biological pathways contribute to the observed sex-dependent effects.

Moyamoya Angiopathy: novel insights into plasma proteome profiling

NI 44

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Moyamoya Angiopathy (MA) is a rare cerebrovascular disorder, defined by progressive steno-occlusive lesion in internal carotid arteries and the development of a vulnerable network of collateral vessels at the base of the brain. MA affects young adults and children, exhibiting recurrent ischemic/hemorrhagic strokes, cognitive impairment and neurological sequelae. MA is considered a multifactorial disorder, being its development and progression affected by genetic, environmental, and immunological features. The only available treatment refers to surgical revascularization. Since MA pathogenesis is still unclear, a better understanding is expected from the molecular profiling of patients' plasma.

An explorative analysis was performed on plasma samples from MA patients recruited at Fondazione IRCCS Istituto Neurologico C. Besta (n= 24). Age/sex matched healthy donors (HD; n= 25) were used as controls. Plasma-proteome relative quantification was performed at Centro Cardiologico Monzino by using the Proximity Extension Assay technology on Olink® Neurology/ Neuroexploratory Target 96 panels. Additionally, ELISA assays were carried out on a validation set of samples from MA patients (n=20) and HD (n=20).

By means of dual-recognition multiplexed panels, we have been able to evaluate the plasma expression of 184 proteins. Four proteins (i.e., Growth/differentiation factor 8, GDF-8; Brevican core protein, BCAN; Carboxypeptidase A2, CPA2; and Proepiregulin, EREG) were found as significantly modulated in MA plasma samples *vs* HD. Specifically, GDF-8, BCAN and CPA2 relative expression decreased in MA *vs* HD; on the contrary, an increased relative expression of EREG was found in MA *vs* HD. These findings have been preliminarily validated by ELISA assays.

Comprehensively, we preliminarily performed a plasma proteome profiling of MA patients. These initial findings strongly prompt us to shed light on the role of these proteins as putative MA key players and novel circulating biomarkers.

How to study T cells trafficking in neuroinflammation: a methodological approach

NI 45

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A complex interplay between the immune system and the brain exists, and it is implicated both in the maintenance of brain physiology and in the development of neuroinflammatory and autoimmune diseases, such as Multiple Sclerosis (MS). The interplay between the adaptive immune system and the neurovascular interface is laborious to investigate mainly because of the limited number of infiltrating cells.
We developed a microfluidic Neurovascular-Unit (NVU) model to investigate neuro-immune interactions ex vivo combining primary cells either human or isolated from wild type and genetic animal models. This represents an accessible tool to investigate immune cells transmigration, both under physiological and pathological conditions. The NVU prototypes challenged with TNFα on the brain side, displayed reduced TEER levels, increased barrier permeability and a higher immune cells infiltration rate, indicating a proper responsivity of the barrier to a neuroinflammatory context.
To dig deeper into pathophysiological mechanism, we set up a personalized human BBB in vitro model to study the interaction of primary endothelial cells with autologous immune cells in patients with MS.
In vivo, we exploited the adoptive transfer of EGFP+ T cells to identify immune cells infiltrating brain parenchyma from the periphery. The number of transmigrating leukocytes was quantified with TaqMan real-time PCR assay that has been set up for the detection of $EGFP^+$ cells in tissue samples. To specifically immunophenotype infiltrating immune cells, we set up an isolation protocol followed by multi-color flow cytometry and absolute number counting, thus allowing the characterization of immune cells subsets crossing brain borders in comparison with peripheral circulating cells.
Altogether, the exploitation of these techniques would allow the investigation of the immune-brain axis with the final ambition to improve the understanding of brain immunity and its involvement in brain pathophysiology.

Funding: Italian Multiple Sclerosis Foundation (FISM grant 2019/R-Single/032).

Role of interleukin 6 in the pathogenesis of Rett syndrome: focus on astrocyte-neuron crosstalk and its therapeutic implication

NI 46

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This abstract is not available online on request of the presenting author

SonIC: An Artificial intelligence architecture to safely deliver drugs to the brain through focused ultrasound

NI 47

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Microbubble-assisted focused ultrasound (MB-FUS) is a promising technique for temporarily opening the blood-brain barrier (BBB) to facilitate drug delivery to the brain. This method leverages the interaction of injected microbubbles with ultrasound waves, inducing cavitation that safely loosens tight junctions. However, at high acoustic pressures (APs), microbubbles can degrade and undergo inertial cavitation (IC), potentially emitting harmful high-energy microjets. Despite these risks, high APs are believed to enlarge gaps in endothelial walls, enabling the delivery of large drugs (>100 nm). The behavior of microbubbles can be monitored through secondary acoustic emissions.

We introduce SonIC, a closed-loop system designed to adjust FUS parameters in real time during sonication to mitigate IC phenomena. SonIC employs an artificial intelligence (AI) architecture to distinguish between safe and potentially harmful IC activities. A custom software communicates with the FUS generator to adjust parameters based on SonIC's feedback. The AI system was trained using data from a realistic phantom model, simulating microbubble flow and hindrance within vessels, with a transducer operating at 1.5 MHz and APs ranging from 0.20 to 2 MPa. Microbubble activity during sonication was monitored using a Passive Cavitation Detector. For analysis, the IC Dose (ICD) was quantified across 350,000 time windows of 3 ms each, normalized against a baseline signal acquired at 0.2 MPa without microbubbles, and classified as safe or harmful based on a predetermined threshold. SonIC's performance was evaluated with training, validation, and test splits of 70%, 10%, and 20%, respectively. The model demonstrated exceptional performance in IC detection, with accuracy, sensitivity, specificity, and F1 score all exceeding 99%.

Future experiments will test SonIC with in vivo data and evaluate the closed-loop system's efficacy in reducing ICD and adverse effects during BBB opening experiments on animals.

NIM 08

Evaluation of Sleep Quality in Patients With Pituitary Adenomas: comparison between Acromegaly and Non-Functioning Pituitary Adenomas

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Acromegaly is associated with sleep disorders, particularly obstructive sleep apnea syndrome (OSAS). In non-functioning pituitary adenomas (NFPA), low sleep quality has been reported, likely due to optic chiasm compression. In this study we aimed to investigate sleep quality in patients with pituitary tumors, focusing on acromegaly (GH group) and NFPA. One hundred-eleven patients were included (GH, n=55; NFPA, n=56). Sleep quality was assessed using the Pittsburgh Sleep Quality Index (PSQI), Insomnia Severity Index (ISI) and Epworth Sleepiness Scale (ESS). Tumor size at diagnosis and disease activity, evaluated within 3 months of questionnaire administration, were recorded.

The two groups did not differ as of age (GH: 62±12 years, NFPA: 59±15 years), sex (GH: 55% females; NFPA 46% females) and prevalence of macroadenomas (GH: 71%; NFPA: 75%). No significant difference was observed in the prevalence of OSAS (GH=5, 9%; NFPA=2, 4%).

No significant difference in total PSQI score (p=0.163), ISI score (p=0.095) and ESS score (p=0.693) was observed between GH and NFPA groups. However, the GH group had significantly higher score in the component 5 (sleep efficiency) of the PSQI (p=0.008), although no correlation between age-adjusted IGF-1 values and sleep efficiency was found. In the GH group, females showed a trend towards higher PSQI scores (p=0.057), but no difference in the ISI (p=0.226) and ESS (p=0.777) scores compared to males. Of note, female sex was a major determinant of sleep quality in the NFPA group, showing higher scores for global PSQI (p=0.002) and ISI (p=0.009), but similar ESS (p=0.732), compared to males.

In conclusion, we did not observed differences in the overall sleep quality between patients with acromegaly and NFPA. However, patients with acromegaly showed worst sleep efficiency than NFPA. Patients' sex seems to have a more relevant role in sleep quality in NFPA than acromegaly.

Improved neuromelanin models as new tools for study MRI contrast

NIM 09

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The current diagnosis of Parkinson Disease (PD) is possible after pathogenesis is at an advanced stage with evident symptoms, when a significant number of dopaminergic and noradrenergic neurons in the substantia nigra pars compacta (SNc) and locus coeruleus (LC), respectively have died. It is imperative that the disease be diagnosed at earlier stages, that the response to therapies and progression of disease is monitored. Recent advancements show that detection of neuromelanin (NM), a dark pigment accumulating in aging in healthy SNc and LC neurons, by magnetic resonance imaging (MRI) is feasible and convenient. However, mechanisms of NM-associated contrast remain unclear. Typically assumed is a shortened water longitudinal relaxation time (T_1) that is caused by paramagnetic relaxation enhancement due to metals bound to NM. However the metal ions present in NM are heterogeneous (mostly Fe,Cu) and distributed in different sites, and this results in changes in magnetic behavior. For these reasons, suitable synthetic melanins could represent a valuable tool for MRI sequence calibration. We have previously shown that it is possible to synthesize models of the NM structural core leading to soluble compounds where the melanic, protein and metals contents can be controlled and quantified. Little is known about how parameters observable with nuclear magnetic resonance (NMR) can be modulated by metal ions distribution at different binding sites. The aim of our study was to clarify how iron accumulation and/or NM loss occurring in Parkinson could affect MRI contrast. In order to elucidate these mechanisms, metal analysis, electron paramagnetic resonance (EPR) and NMR-based relaxometry were conducted in this work. Compared to previous studies, the main objective of this work was to examine a more realistic model of human NM containing also β-lactoglobulin (βLG) as protein component; and that, in addition to varying iron concentrations, also included varying concentrations of copper.

NIM 10

Retinal Synaptic Volume Alterations in Early Alzheimer's Disease Revealed by Voxel-Based Morphometry with Optical Coherence Tomography

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Background: Synaptic dysfunction drives cognitive decline in Alzheimer's Disease (AD). The lack of non-invasive biomarkers for quantifying synaptic loss remains a challenge. Recent work suggests the inner plexiform layer (IPL) of the retina may serve as such a biomarker in inflammatory demyelination. Voxel-based morphometry with Optical Coherence Tomography (VBM-OCT), an innovative non-invasive neuroimaging method, has shown high sensitivity in detecting focal retinal alternations. This study compares the sensitivity of VBM-OCT with the traditional OCT in measuring IPL volumetric changes in early AD.

Methods: The patient groups consisted of individuals with subjective cognitive decline (SUB, N=5, two males, age=61.6±5.8), and individuals with CSF biomarkers-based diagnosis of cerebral amyloid angiopathy (ANP, N=10, six males, age=70.9±10.3). Bilateral macula OCT scans were acquired from each participant, and the IPL thickness was analyzed using both the traditional Early Treatment of Diabetic Retinopathy Study (ETDRS) grid and VBM-OCT.

Results: When comparing healthy subjects with individuals affected by cognitive decline, no significant differences were detected with the ETDRS grid, while VBM-OCT revealed distinct patterns of IPL change in the patients' group. The SUB group exhibited focal thickening of the IPL (mean=2,0 µm) compared to HCs, while the ANP group showed focal IPL atrophy (mean=-3,3µm). Notably, significant parafoveal IPL atrophy was observed in the ANP group compared to the SUB group (mean=-6,6µm).

Discussion and Conclusion: The IPL contains the dendrites of the retinal ganglion cells and their synapses with bipolar cells and other neurons; thus, the IPL volume change may reflect synaptic quantity and dendritic architecture changes. Our preliminary analysis indicates that IPL thickness changes occur in opposite directions in the two patient groups, suggesting biphasic changes in IPL thickness in our two groups of patients, potentially due to different pathogenic mechanisms.

The Force Awakens: PIEZO1 as a novel oncogene in glioma

NO 16

SPEED TALK

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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 and a high recurrence rate (75-90%). Moreover, there have been only moderate improvements in survival rates for patients with this disease in recent decades, and current therapeutic options have limited ability to alter the course of disease. 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. First, we have analysed a cohort of GBM patients, and we have observed that PIEZO1 is a marker of worse prognosis exclusively in male patients. Furthermore, PIEZO1 overexpression in glioblastoma U251 modified cell line increases cell migration and clonogenicity, which are processes closely related with GBM aggressiveness. PIEZO1-overexpressing U251 cells also show a reactive-like phenotype, according to our RNA-Seq and Western Blot results. We have also created a novel and unique transgenic mouse model of Piezo1 overexpression specifically in astrocytes (GFAP-Cre/Tg.Piezo1 and GFAP-Cre/Tg.Piezo1/p53$^{lox/lox}$) and we have observed that these mice present a reduced overall survival and a high incidence of glioblastoma. The tumours developed in our mouse model are characterized by their aggressive and reactive phenotype. All these results postulate PIEZO1 as a new GBM biomarker and as the first discovered *bona fide* glioblastoma driver oncogene, and with it, in a potential therapeutic target for this disease.

NO 17

SPEED TALK

Involvement of DNA repair in high-grade glioma recurrence: mechanistic insights into the nucleotide excision repair pathway in glioma stem cells

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High-grade gliomas (HGGs) are the most common primary brain malignancies, accounting for about 50% of brain cancers. Grade 4 IDH wild-type glioblastoma (GBM) is the deadliest and most difficult-to-treat, since tumor recurrence and therapy resistance remains a significant challenge. GBM Stem Cells (GSCs) are a critical subpopulation responsible for tumor initiation, progression, and recurrence. Although GSCs exhibit enhanced double-strand break repair capabilities, their proficiency in repairing DNA double-helix distorting lesions from platinum-based chemotherapeutics or oxidative stress is poorly understood. The Nucleotide Excision Repair (NER) pathway repairs various DNA lesions, including those mentioned above. NER operates through two sub-pathways: Global Genome Repair (GGR) for non-transcribed DNA and Transcription-Coupled Repair (TCR) for transcribed strands. These sub-pathways differ in lesion recognition but converge to complete the repair process. The resumption of transcription post-repair is crucial in TCR. Our aim is thus to mechanistically characterize the NER pathway in GSCs compared to bulk Glioblastoma cells (GBM). To evaluate NER efficacy in GSCs and GBM cells, we conduct golden standard NER proficiency assays following UV lesion induction, analyzing key NER factors' repair process, timing, and damage accumulation rates. Patient-derived glioma stem cell lines (GSCs), and the differentiated counterpart, are used in these experiments. Our initial findings reveal distinct responses to UV-induced DNA damage in GSCs and highlight significant differences in the transcription resumption step post-repair between GSCs and GBM cells. Furthermore, we evaluated the timing and efficiency of gap refilling by the DNA replicative machinery in both cell types. These promising results elucidate the contribution of NER efficacy to GSC drug resistance, providing insights that could inform novel therapeutic strategies to overcome chemoresistance in glioblastoma.

Plasma derived EVs of glioma-bearing mice contain promising biomarker for an early Glioblastoma diagnosis

NO 18

SPEED TALK

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Glioblastoma (GBM) is the most deadly human brain tumors. Currently, clinicians used to recognize the tumor mass invasive techniques of imaging and histological analysis. An early detection is the challenge to increase the survival of patients. In the last decades, liquid biopsies (plasma, saliva, urine, cerebrospinal fluid) represented a way for the diagnosis and the cure of different diseases, including tumors. In fact, it is not invasive to collect liquid biopsies, it is less stressful for the patient and it helps monitor changes in patients during the pharmacological treatment.
During tumor growth, neural cells communicate each other and with periphery releasing growth factors and extracellular vesicles (EVs). EVs are composed of bilayer membranes, distinguished on biogenesis, size and contain specific lipids, proteins and nucleic acids that can change the functions of recipient cells. Among these factors, there are miRNAs involved in the tumori genesis, like miR21.
In this study, we analysed the expression of miR21 in the EVs isolated from brain, plasma and urine precociously collected after glioma inoculation.
The miRnomic analysis of EVs isolated from brain and plasma of glioma-bearing mice, revealed miR21 as the most expressed seven days after inoculation.
We also observed a correlation between the increase of miR21 into the brain EVs with the progression of the tumor growth. Otherwise, in plasma EVs, we observed only a peak of miR21 expression, one week after inoculation. By using a xenogeneic glioma, we distinguished miR21 expression in tumor-derived vs host-derived EVs and observed a similar pattern of miR21 expression with markedly higher expression in EVs derived from the host.
Ongoing experiments will identify the biological meaning of the early increase of mir21 in the plasma EVs of glioma-bearing mice as well as getting to the conclusion that in plasma EVs miR21 could represent that relevant and promising biomarker to anticipate the GBM diagnosis.

NO 19

Antibiotic-induced gut dysbiosis promotes tumor progression in glioma-bearing mice and SCID mouse model of human glioma

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In the last few years a growing concern about gut-brain axis has disclosed the relevance of gut-derived metabolites in the intercellular cross-talk occurring in brain parenchyma, both in physiological and pathological context. Gut dysbiosis induced by the administration of non-absorbable antibiotics (ABX) has been proven responsible for shaping brain microenvironment in a syngenic mouse model of glioma, contributing to tumor growth and progression. Upon ABX treatment, we described increased glioma stemness and enhanced vasculogenesis. Using a SCID mouse model of human glioma, we are now validating the correlation between gut microbiota alteration and glioma progression in terms of tumor volume and vasculogenesis. We report that ABX treatment induces (i) an increase in cancer size; (ii) trans-differentiation of glioma cells into endothelial precursor cells, boosting vasculogenesis.

Ketogenic diet induces an inflammatory reactive astrocytes phenotype reducing glioma growth

NO 20

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Glioblastoma multiforme (GBM) is the most common and aggressive primary brain tumor in adults, characterized by rapid growth and low survival rates. The average life expectancy of patients with newly diagnosed glioblastoma is approximately ~18 months. Therefore, developing new therapeutic strategies that enhance existing treatments or limit tumor growth is necessary. Preclinical studies have indicated that a ketogenic diet (KD) may exhibit beneficial effects in patients with GBM; however, KD is currently used as a co-adjuvant to standard therapies for GBM. Although KD provides an alternative energy source not available for GBM cells, the direct effects of its metabolites, such as beta-hydroxybutyrate (BHB), on the biology of tumor cells are not yet fully understood. GBM can modify the phenotype of stromal cells, creating a microenvironment that helps carcinogenesis and tumor progression. In the tumor brain microenvironment astrocytes and microglia play an important role, for this reason, there is an unmet need to deepen our studies on their involvement in glioblastoma progression, thanks to epigenetic reprogramming, certain substances introduced with diet could affect gene expression, especially of those genes involved in cells' proliferation and growth.
Using a syngeneic mouse model of glioma, we have investigated the role of a restricted ketogenic diet on tumor volume and tumor microenvironment. Also by using different in vitro approaches, we were able to mimic the ketogenic diet treating cells with the main ketone body produced during ketosis: β Hydroxybutyrate (β-HB). In the present work, we aimed to analyze: i) the β-HB consumption ascribable to cancer cells and glial cells, ii) the glioma proliferation rate with or without β-HB stimulation, iii) the phenotype acquired in vitro by glial cells mimicking ketosis condition, iv) the potential role of astrocytes in reducing glioma cells' proliferation through in vitro assays and co-culture experiments.

Keywords: Glioblastoma, Ketogenic Diet, β Hydroxybutyrate, Astrocytes

A pan-sigma receptors modulator as a novel therapeutic strategy to fight glioblastoma

NO 21

SPEED TALK

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Glioblastoma multiforme (GBM) is the most common and malignant primary tumor that affects the central nervous system (CNS). Despite the improvement in neurosurgery and neuro-oncology research there are currently no truly effective cures for GBM. The survival rate is still about 15 months after diagnosis, underlying the urgent need to develop new therapeutic strategies. In the early 2000s, the sigma receptors family was identified by the international scientific community as potential new target for antitumor therapy. This hypothesis was based on their role in tumor growth, progression, and aggressiveness, given the high levels of expression observed for both σ1 and σ2 receptors in various tumor tissues and metastatic lesions. Furthermore, the σ1 receptor is currently being evaluated as a target for the treatment of chronic pain, particularly neuropathic pain, a condition frequently encountered in cancer patients. For these reasons, we tested a new pan-σ receptor modulator, which has demonstrated efficacy on different patient-derived glioblastoma 3D cell lines, established in our lab. We compared the gene expression levels of σ1 and 2 receptors in our cell lines with cytotoxic effect of the molecule. We strongly correlate the higher efficacy of the compound with an augmented expression of sigma receptors by comparing digital PCR and MTS cell viability assay. We also demonstrated that, with up to 10μM of the pan sigma modulator, there is no or low toxicity in the zebrafish model. We than established a PDOX glioblastoma mouse model by orthotopic injection of the same patient-derived cell lines tested in vitro to validate the efficacy of the compound in vivo. These findings, taken together, corroborate the hypothesis that sigma receptor modulators could be a supportive strategy to fight GBM.

The role of hydrogen sulfide on glioblastoma growth: a gut-brain approach

NO 22

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SPEED TALK

Glioblastoma multiforme (GBM) is the most common and deadly malignant brain tumor, with a low life expectancy and poor efficacy of standard therapies, leading often to relapse. However, it is already known that lifestyle changes, such as restricted diets and fasting, have an impact on GBM growth. Less clear is the effect of red meat intake on the tumor. One observational study found that people who consume a diet high in animal protein and fat show changes in the microbiota composition, particularly an increase in common hydrogen sulfide (H_2S) producing bacteria. This metabolite appears to counteract GBM growth. Recent studies highlight the role of the gut-brain axis in altering the GBM microenvironment and growth. Our aims are to investigate: 1) the effect of a standard animal-protein diet on tumor growth; 2) diet-induced gut microbiota modification; 3) the possible involvement of H_2S in tumor progress. To assess such hypothesis, we fed mice isocaloric diets with standard content of proteins derived from red meat (protein diets) or animal-derived proteins (control) and we orally administered amino-oxyacetic acid (AOAA), an inhibitor of H_2S biosynthesis. After two weeks on the diets, we orthotopically injected murine glioma cells (GL261). Three weeks after the injection, we collected stool for each group to assess H_2S concentration and brain for tumor volume. Our results show an increased concentration of H_2S in the faeces and a decreased tumor volume in mice fed the animal protein diet compared to controls, this effect disappeared in mice treated with AOAA. These results are consistent with our in vitro data showing a reduction in GL261 viability following treatment with sodium hydrosulfide (NaHS), an H_2S donor. These results suggest that a standard red meat intake may have an antitumor effect on GBM compared to a standard animal-derived protein diet and that this effect is H_2S-dependent.

Manipulating GABAergic mechanisms to delay glioma invasion of peritumoral microenvironment

NO 23

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The interaction between tumor cells and the main components of the tumor microenvironment (TME) seems to guide the progression of glioma. Several recent studies have highlighted that the synaptic activity of pyramidal neurons potently drives Glioblastoma (GB) proliferation. However, the role played by other neuronal cell types is still poorly understood. Here we tested whether the chemogenetic manipulation of peritumoral PV-interneurons could control GB growth. To address this issue, AAV-hSyn-DIO-hM4D(Gi)-mCherry or AAV-hSyn-DIO-hM4D(Gq)-mCherry were injected in the motor cortex bilaterally with respect to the GL261 injection site in PV-Cre mice. In order to manipulate PV-interneurons activity, animals will be randomized to receive CNO or vehicle intraperitoneally twice a day from 12 to 22 day post tumor induction. As readouts, we: i) evaluated the tumor volume using Magnetic Resonance Imaging (MRI), ii) performed a longitudinal monitoring of motor capabilities using specific motor tests, iii) assessed the survival, iv) determined the GB proliferation rate of glioma-bearing mice after different treatments. We found that PV-interneuron inactivation exacerbates glioma symptoms. Indeed, PV-inactivated GB mice showed worse motor performances and about the 43% of them had tonic-clonic seizures with respect to the vehicle GB mice. Despite that, no difference was observed in animal median survival or tumor volume among the two groups. On the other hand, MRI data showed a very significative reduction in the tumor volume at 28 days after glioma induction in PV-activated GB mice. These findings were further confirmed by the decrease in the proliferation rate of Ki67-positive cells present in the tumor area. Taken together, these data suggest that the activation of parvalbumin inhibitory circuitry in the tumor-adjacent zone could delay GB growth, protecting peritumoral tissue from GB proliferation and invasion.

NO 24

Polysialic Acid sustains the temozolomide-induced undifferentiated state of glioblastoma cells

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Glioblastoma (GBM) is a high-incidence malignant brain tumor characterized by pleomorphic cells with high capacity to migrate and invade the surrounding parenchyma. Neural Cell Adhesion Molecule (NCAM) can promote GBM cells motility by decreasing overall cell adhesion when post-translationally modified with polysialic acid (PSA), a carbohydrate consisting of long sialic acid chains. PSA is considered an oncodevelopmental antigen; in fact, high-grade tumors with extraordinary spreading ability exhibit high levels of PSA. GBM aggressiveness is also due to the presence of cancer stem cells, which show low vulnerability to chemotherapeutic agents. Therefore, it is necessary to study the involvement of PSA in GBM cell resistance to chemotherapy.
The aim of this study is to better understand the role of PSA in the de-differentiation induced by temozolomide (TMZ) in U87-MG cells.
Our results show that, after chemotherapy, U87-MG cells had higher PSA levels and enhanced polysialyltransferases expression. Moreover, Real-Time PCR analysis showed that TMZ induced the expression of the stemness genes Oct4, Sox-2, and Nanog. By lowering PSA levels with the sialic acid analog, F-NANA, we interfered with PSA expression in U87-MG cells, as confirmed by Western Blot analysis. In addition, Real-Time PCR analysis confirmed that inhibition of PSA levels by F-NANA administration significantly interfered with GBM cell de-differentiation process induced by chemotherapy.
In conclusion, we have gained important evidence on the biological function that PSA exerts in GBM cells exposed to the gold-standard of chemotherapy, TMZ. This drug may also induce pharmacological resistance promoting the acquisition of a more undifferentiated phenotype sustained by PSA overexpression. Our observations potentially provide relevant directions for novel therapeutic strategies based on the pharmacological inhibition of this post-translational modification in a destructive tumor as GBM.

Tumor-associated macrophages-educated promote neuronal regeneration in vivo and in vitro

NP 27

SPEED TALK

Ilaria Barone (1) - Giulia Prounto (1) - Ludovica Sagripanti (1) - Zulkifal Malik (1) - Alessia Amenta (1) - Sissi Dolci (2) - Loris Mannino (2) - Alessandra Campanelli (2) - Eros Rossi (2) - Emanuela Bottani (2) - Francesca Ciarpella (2) - Isabel Karkossa (3) - Elisa Setten (4) - Benedetta Savino (4) - Nicola Piazza (2) - Stefano Gianoli (4) - Giuseppe Busetto (5) - Alex Pezzotta (6) - Marzia Di Chio (2) - Alessandra Castagna (4) - Nicolò Martinelli (2) - Federico Boschi (7) - Adam Doherty (8) - Maria Teresa Scupoli (9) - Chiara Cavallini (2) - Giorgio Malpeli (10) - Vincenzo Silani (10) - Marco Sandri (2) - Patrizia Bossolasco (10) - Anna Pistocchi (6) - Marco Endrizzi (8) - Kristin Schubert (3) - Guido Francesco Fumagalli (2) - Massimo Locati (4) - Francesco Bifari (1) - Ilaria Decimo (2)

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We identified and characterized tumor-associated macrophages (TAMs) as relevant mediator for cancer nerve growth. We show that both human and mouse TAM are endowed of a specific neurogenic signature, exhibit direct role in promoting neurite outgrowth, neural development, and axonal regeneration. We highlighted TAM neuro-reparative mechanistic functions by: i) setting-up a new protocol for human co-culture of SHSY-5Y differentiated neurons and human TAM (hTAM);ii) validating hTAM potency in promoting neuronal arborization both in SHSY-5Y neurons and human Motoneurons derived from iPSCs; iii) defining osteopontin (SPP1) as a relevant mediator of hTAMs neuro-regenerative property supported by corroborating data from RNA interference and pharmacological approaches. To better understand molecular mechanisms of TAMs regenerative properties, we are now identifying and functionally validating SPP1 signalling axis both *in vivo* and *in vitro*.

Neural differentiation of the SH-SY5Y human neuroblastoma cell line on P3HT thin polymer film

NP 28

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In the promising field of bioelectronics, the combination of electronics and biology has paved the way for new applications in many medical fields, including implantable devices. Developing techniques, capable to monitor and control the biological systems efficiently and instantaneously, is crucial for many biomedical applications, including drug delivery, electrophysiological recording, and regulation of intracellular activities. In this context, conductive polymer-based systems (CPs), provide a useful scaffold to develop multifunctional nano systems able to mimic the properties of biological tissues offering a platform for electrical stimulation, particularly important for targeting differentiation of cells into neurons and glial cells, opening up new options for neural regenerative therapies. Based on the above reported evidence, in this study we investigated the properties of a semiconductive polymer P3HT based-substrate on the neuroblastoma SH-SY5Y cells, in terms of cell adhesion, proliferation, biocompatibility and neural differentiation. For this aim, we performed cytotoxicity tests, immunohistochemical analyses with specific neuronal markers, such as β-III Tubulin, MAP2, NF-H, and DAPI staining. Our preliminary results highlighted that the new P3HT-based substrate show a good biocompatibility, and high capability to induce neuroblastoma cells adhesion, proliferation and differentiation, from 1 to 15 days even without addition of retinoic acid. These data taken together suggest that the P3HT-based substrate represents a promising first step toward the development of biocompatible and functional interfaces for use in future biomedical applications, especially for designing new medical devices for neural tissue engineering.

Advancing Stroke Rehabilitation: Personalized Neurostimulation Using Spiking Neural Networks

NP 29

SPEED TALK

Marta Carè (1) - Federico Barban (2) - Mattia Di Florio (2) - Dania Vecchia (2) - Sam Zahedi (2) - Romain Beaubois (3) - Timothée Levi (3) - Michela Chiappalone (2)

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Stroke is a major global cause of death and disability, but current therapies are limited. Standard post-stroke rehabilitation with physical therapy often falls short. Impaired communication between unaffected brain areas contributes to motor deficits, making the restoration of this communication crucial for motor recovery.

Emerging evidence suggests that tailored stimulation patterns, which align with the dynamics of brain networks, can enhance plasticity and improve motor recovery. However, clinical therapies that use standardized stimulation protocols frequently yield inconsistent results. This inconsistency may stem from the lack of personalized stimulation protocols that consider the unique dynamics of each patient's neural system.

This study seeks to enhance neurostimulation techniques by tailoring electrical stimulation based on individual electrophysiological characteristics. By applying principles of neuromorphic engineering, we aim to develop devices that seamlessly interface with the neural system for treating neurological disorders like stroke. Specifically, we developed and tested a hardware-based Spiking Neural Network (SNN) designed to deliver neural-like stimulation patterns in an open-loop fashion. The neurons in the SNN were modeled using the Hodgkin-Huxley formalism, with parameters sourced from neuroscientific literature. Our experimental setup involved deeply anesthetized healthy rats to evaluate the effects of SNN-driven stimulation. We monitored neuronal firing activity in both the primary somatosensory and rostral forelimb areas before and after stimulation. Our findings indicate that SNN-based neurostimulation can significantly increase spontaneous neuronal firing at both monitored sites, a result previously observed only with closed-loop stimulation.

This study marks an important step toward translating neuromorphic-based devices into clinical applications, offering a promising avenue for personalized neurostimulation therapies.

Cortical hypomyelination is associated with cognitive impairment in a mouse model of oligodendroglia-specific deletion of Citron Kinase

NP 30

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Although extensive evidence correlates myelination and cognition, the mechanisms linking these two processes are not yet fully understood. Here we addressed this issue in a mouse model of delayed myelination and regionalized hypomyelination, i.e. a conditional oligodendrocyte-specific Citron-kinase (Cit-k) mouse mutant (Sox10Cre::Cit-k fl/fl mice).
Cit-k is a serine-threonine kinase involved in DNA repair and cytoskeletal dynamics, whose loss-of-function results in microcephaly and severe CNS hypomyelination in both humans and mice. Cit-k loss cell-autonomously induces forebrain oligodendrocyte progenitor cells to undergo cell death or enter a senescent state, leading to a diffuse impairment of myelin deposition at juvenile stages. Myelin defect is only party compensated in adult mice, where hypomyelination persists in the cerebral cortex – but not in other brain areas.
To assess whether such region-specific hypomyelination was associated with functional alterations, we characterized the behavioral phenotype of the conditional mutants at adult stages. While motor, sensory and emotional aspects appeared unaltered, we observed significant defects in the memory domain. Specifically, Sox10Cre::Cit-k fl/fl mice displayed impaired working and short term memory, as assessed in the Y maze and Novel Object Recognition test, respectively. Conversely, in the Morris Water Maze test, mutant mice did not exhibit significant spatial memory defects. Ongoing experiments are now aimed at unveiling possible alterations in long-term memory retention and retrieval and expand also to the associative fear memories domain.
Overall, our data indicate that the regionalized hypomyelination resulting from the oligodendroglia-specific deletion of Cit-k is associated with specific cognitive defects, in line with the idea that myelin alterations can compromise network activity leading to functional impairment. Further experiments will investigate the mechanistic substrates of our observations.

Homeostatic plasticity in response to short-term monocular deprivation in the mouse primary visual cortex

NP 32

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In adult humans, short-term monocular deprivation enhances responses from the deprived eye, leading to increased ocular dominance in binocular rivalry, a process attributed to homeostatic plasticity mechanisms. This phenomenon has not been previously studied in animal models. In this study, we characterized 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 120-minute occlusion of the eye ipsilateral to the recorded brain side. We found that short-term deprivation led to significant increments in the responses to the deprived eye stimulation, as computed by the contralateral to ipsilateral eye (C/I) VEP ratio, a paradigmatic measure of ocular dominance properties of visual cortical neurons. The decrease of the C/I VEP ratio resulted from an increase in ipsilateral VEP amplitudes accompanied by a simultaneous decrease in contralateral VEP amplitudes, in agreement with with the observations in human subjects. Strikingly, the same effect was also evident in in young mice during the critical period for binocular visual development, i.e. within a temporal window classically described as dominated by competition between the two eyes. These results underscore a great potential for homeostatic plasticity in the visual cortex of both adult and young mice, which may have translational implications for treating visual disorders such as amblyopia.

NP 33

SPEED TALK

Deciphering the interactions across different attentional control mechanisms during target selection: Insights from Behavioural and EEG Experiments

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The natural environment exhibits consistent patterns, making it repetitive and somewhat predictable. Statistical learning (SL) enables us to recognize these regularities from past experiences and use them to focus our attention on elements relevant to our goals. However, it is unclear whether SL operates jointly with, or independently from, other experience-independent attentional control mechanisms, namely top-down and bottom-up control. To critically test their interaction during the target selection process, in three related experiments, behaviour and EEG activity were recorded during a visual search task. In Experiment 1 the combined influence of top-down control, modulated via endogenous cueing (neutral/valid), bottom-up control, through the introduction of a salient item, and SL, induced by an imbalance in target probability (high/low) across locations, was behaviourally assessed. Experiments 2 and 3 focused on how the N2pc, an EEG marker related to target selection, was affected by the interplay of SL with top-down and bottom-up control, respectively. Results showed that both SL and bottom-up control enhanced behavioural performance for targets at high- (vs. low-) frequency locations and for salient (vs. non-salient) targets. Critically, an interaction revealed that the benefit of top-down control for validly cued targets could override the SL effect (Experiments 1 and 2). Additionally, EEG results indicated a greater N2pc for validly cued and salient targets, but only when they were at low-frequency locations, likely compensating for the lower attentional resources allocated to those locations due to SL (Experiment 3). In conclusion, while top-down control and SL closely interact, SL appears to be mostly independent of bottom-up signals.

NP 34

Antidepressant effects of PFC transcranial direct current stimulation in mice are coupled with the modulation of the dorsal raphe nucleus serotonergic activity

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Transcranial direct current stimulation (tDCS) is gaining increasing consideration as an effective non-pharmacological alternative for a variety of psychiatric and neurological disorders. In major depressive disorder (MDD), it was observed that anodal tDCS (A-tDCS) applied over the left dorsolateral prefrontal cortex improves depressive symptoms in drug-resistant patients; however, its underlying mechanisms are still unclear. One of the main neurotransmitters involved in the pathophysiology of MDD is serotonin (5-HT), released by neurons originating in the Dorsal Raphe nucleus (DRN). In rodents, the prefrontal cortex (PFC) directly innervates the DRN, and the activation of this pathway has already been shown to have an antidepressant effect. Our previous study revealed that both A-tDCS and cathodal tDCS (C-tDCS) over the left PFC induce an acute inhibitory response of DRN 5-HT neurons, resembling the acute effects exerted by SSRIs. Building on that, the present investigation aims to investigate the chronic effects of repetitive tDCS over the PFC on the DRN 5-HT neurons in young female mice. We found that 1 month after stimulation onset, A-tDCS but not C-tDCS increases the firing rate of DRN 5-HT neurons with respect to controls. These electrophysiological effects were associated with a higher 5-hydroxyindoleacetic acid, the primary metabolite of 5-HT, in the midbrain of the A-tDCS mice. In addition, in the forced swim test (FST) used to assess depressive-like behaviors in rodents, lower immobility time in the A-tDCS group was showed, suggesting a correlation between antidepressant effects of A-tDCS and modulation of 5-HT neurotransmission. As a final remark, we observed a different neural activation within the DRN in response to the FST. Overall, the modulation of the top-down projections from the PFC to the DRN could be a key target for the antidepressant effects of tDCS.

Development of the water grasping task to detect behavioral motor deficits in a mouse model of stroke

NP 35

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Ischemic stroke represents one of the main causes of death and disability worldwide. Harming the Central Nervous System (CNS) of the patients, it causes a clot that occludes a cerebral blood vessel with following local ischemia and cellular damage at the specific brain site. Specifically, after stroke, the nervous tissue around the wound, the perilesional tissue, undergoes rearrangements and modifications which underlie neuronal plasticity mechanisms. The recovery outcomes of the patients depend on the reorganization output of this brain area. In this scenario, our research aims at the characterization of the stroke model in adult mice, validating a new behavioral protocol to define the sensory-motor impairments induced by an ischemic stroke event. To address this aim, we induced the stroke lesion in mice, by performing the distal middle cerebral artery occlusion (dMCAO), which produced a tissue damage in the somatosensory and motor areas, and assessed the behavioral outcome by using the water grasping test. This is a behavioral task developed from Gallinanes and colleagues that consists of a 3D-printed chamber with a slit on one of the walls, through which water-restricted mice are compelled to extend their lesioned contralateral forelimb to grasp a drop of water. The water grasping test has been carried out before (baseline) and after stroke induction weekly, at different time points. In order to develop a scoring system able to assess motor impairment, we trained a neural network to extract grasp frames from the raw video, creating short clips for each grasp. We then used these clips to track the trajectory of the forelimb segments with the DeepLabCut package, in order to extract features about position, movement, trajectory and success rate. The validation of the water grasping task in a mouse model of stroke will enable the stratification of the behavioral recovery outcomes, making it a more suitable test to describe sensory-motor impairments after stroke.

Advanced Carbon-engineered Organs-on-a-Chip: Innovative Nanotools-based platforms for Brain Injury Repair

NP 36

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Advanced Carbon-engineered Organs-on-a-Chip: Innovative Nanotools-based platforms for Disorders of the Central Nervous System (CNS) are one of the grand health challenges of this century. Therapeutic development, however, remains limited by poor understanding of the brain. The Neurovascular Unit (NVU) is a complex and specialized structure, composed of multiple cell types: vascular endothelial cells, pericytes, astrocytes, and neurons. These cells are involved in maintaining brain metabolic homeostasis and in its protection against toxins. Because of its role as the gatekeeper of brain functionality, impairments to the NVU have recently been associated with several CNS disorders, including traumatic brain injury. However, the precise role of each NV component is not yet fully established. In modern neuroscience there is an increasing interest in the development of nanotechnologies, as promising therapeutic tools. In particular Carbon-based materials, such as Carbon Nanotubes (CNTs) and Graphene, have been shown to modulate synapse formation and cell excitability, which suggests them as interesting candidates for the amelioration of the NVU altered functionality. Here we describe how CNTs impact primary neuronal networks through the use of electrophysiological and immunofluorescent techniques. Furthermore, we developed an Organs-on-a-Chip (OoC) platform which will allow us to study cell-cell interactions in a more human-relevant model. The adaptability and excellent conductive properties of these nanotools, together with their ability to modulate neuronal activity, might open the way to modern therapeutic strategies for several neurological conditions.

The intracellular trap: dissecting the mechanism of action and neuroplastic potential of molecules targeting 5-HT receptors

NP 37

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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} have emerged as promising rapid-onset antidepressant agents. Fast-acting antidepressants, *i.e.*, psilocybin and its active metabolite psilocin, produce a sustained therapeutic effect after the administration of a single dose. This clinical effect is thought to be mediated by the promotion of long-term 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. The mechanism by which this prolonged effect is produced is still under investigation. Recently, it has been proposed that psilocin diffuses inside the cell, where it could promote neuroplasticity through the activation of intracellular 5-HT_{2A} receptors, rather than by conventional signal transduction originating at the plasma membrane. An intriguing hypothesis is that, once inside the cell, psilocin could be protonated and retained inside intracellular acidic compartments, leading to its accumulation and explaining its sustained activity. To test this, we developed a biologically active fluorescent-labeled psilocin derivative that we aim to use as a probe for exploring the intracellular distribution of psilocin. By combining pharmacological and imaging interventions both *in vitro*, on primary cortical neurons and neuronal cell lines, and *ex-vivo*, on mouse brain slices, we shed new light on the intracellular localization of psilocin. Our approach may be useful to characterize the neuroplastic activity of new 5-HT_{2A} agonists and to comprehend the contribution of intracellular acidic compartments in the pharmacology of other fast-acting antidepressants.

CXCR4 in neurophysiology and neurodegeneration

NP 38

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The ability of the peripheral nervous system to regenerate after injury relies on intrinsic peripheral neuron qualities and a permissive environment. A key role is played by the CXCL12α-CXCR4 molecular axis. CXCR4, a G protein-coupled receptor, aids motor axon elongation during development from the ventral horn in the spinal cord to its final target. Its expression declines after reaching the target but increases in adult motor axons upon nerve damage. Indeed, the receptor is re-expressed by the nerve upon compression, and reappears at the motor axon stump following a localized injury to presynaptic nerve terminals. In such conditions, Schwann cells (SCs) release its natural ligand, CXCL12α. Activation of CXCR4 by this chemokine or the agonist NUCC-390 accelerates re-innervation and neuromuscular recovery. For this reason, understanding CXCR4 dynamics post-injury is of crucial importance in the regeneration field. We monitored CXCR4 transcript and protein expression in controls and after sciatic nerve crush or cut using RNAscope Technology and immunostaining. CXCR4 protein increased in motor neurons (MNs) in the spinal cord and along the sciatic nerve, accumulating in discrete structures still under investigation. CXCR4 transcripts, detectable in control MNs within the spinal cord, increased after nerve damage, indicating peripheral injury triggers CXCR4 transcription in the neuronal soma. Notably, CXCR4 transcripts are also located in the nerve, both in the axon and SCs, increasing in number at the site of damage. This suggests that CXCR4 mRNA is trafficked to the periphery and made available for local translation, through which the receptor can be expressed when axon regrowth is required. Through Puromycilation assay, we visualized active local protein synthesis in injured sciatic nerve axons. We are currently investigating whether SCs could be a viable source of transcripts for the regenerating nerve.

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