BOOK OF ABSTRACTS

BrayN

ROME 2022

28•29•30 SEPTEMBER

AUDITORIUM SERAPHICUM
Via del Serafico 1, Rome

5th BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

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STEERING COMMITTEE

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Antonio Uccelli	IRCCS San Martino Hospital, Genoa (Italy)

INVITED SPEAKERS

Marco Cambiaghi	Dep. Neurosciences, Biomedicine and Movement Sciences, University of Verona (Italy)
Laura Cancedda	Italian Institute of Technology - IIT, Genova (Italy)
Rafael Fernández-Chacón	Instituto de Biomedicina de Sevilla (IBiS) (Spain)
Giacomo Koch	Santa Lucia IRCCS/Università di Ferrara, Ferrara (Italy)
Konstantinos Meletis	Karolinska Institutet / Department of Neuroscience, Stockholm (Sweden)
Rodrigo Quian Quiroga	Centre for Systems Neuroscience, University of Leicester (UK)
Maria Rescigno	Humanitas University, Humanitas Research Hospital, Milan (Italy)
Dirk Sieger	Centre for Discovery Brain Sciences, University of Edinburgh (UK)
Amanda Sierra Saavedra	Achucarro Basque Center for Neuroscience Fundazioa, Leioa (Spain)
Henrique Veiga-Fernandes	Champalimaud Research Foundation, Lisbon (Portugal)

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Luca De Palma	Rare and Complex Epilepsy Unit, Department of Neuroscience, Bambino Gesù Children’s Hospital IRCCS, Rome (Italy)
Lorenzo Ferri	Department of Biomedical and Neuromotor Sciences, University of Bologna (Italy)

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Dear Young Neuroscientists,

The BraYn Association and the BraYn Conference team warmly welcome you to the **5th Brainstorming Research Assembly for Young Neuroscientists**, the BraYn conference.

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

Nearly **500 delegates** attended the BraYn 2021 conference. They included experienced senior researchers, attending as mentors and contributors, and eight invited speakers, including the Nobel Prize winner Prof. Thomas Südhof.

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

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

We are looking forward to welcoming you at the 5th BraYn conference!

The BraYn Staff

BRAYN SCIENTIFIC SESSIONS

NEUROIMAGING

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

NEUROINFLAMMATION

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

NEUROPHYSIOLOGY & NEURAL PLASTICITY

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

NEURO-ONCOLOGY

Neuro-oncology is an emerging field of investigation that studies nervous system tumors. As many of them can cause severe nervous system damage, neuro-oncology represents a trending research area in neuroscience, which may identify the molecular mechanisms involved in tumor pathogenesis. This would ultimately lead to the development of novel therapeutic approaches for the treatment of life-threatening diseases such as glioma, and medulloblastoma. These topics will be discussed in depth during the NO session.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS

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

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.

CLINICAL NEUROSCIENCE

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

28 SEPTEMBER • Day 1

10:00 Registration

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

BRAYN STARTING GRANT SESSION

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

11:30 **Loredana Leggio** (Starting Grant 2021 Winner)
Identification of bioactive molecules responsible for the neuroprotection of astrocyte-derived EVs.

11:45 **Eveljn Scarian** (Starting Grant 2021 Winner)
Brain organoids RNA-seq analysis for the study of sALS pathogenesis.

12:00 Lecture | **Marco Cambiaghi**
Non-invasive brain stimulation: an old tool into the hands of modern translational research.
(Chairwoman: M. Medelin)

12:30 Lunch box with Poster session 1

PARALLEL SESSION (13:00-13:30)

• For scheduled groups only (max 20 persons) •

Alexion Practical Workshop

Francesco Saccà • *Can we prevent damage for relapsing NMOSD patients?*

SESSION 1 • EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS • ORAL COMMUNICATIONS

(curated by Young Epilepsy Section-Italy, YES-Italy, ILAE)

Chairpersons: G. Balagura, S. Balestrini, M. Rasile, A. Rutsch

14:15 Lecture | **Rafael Fernández-Chacón**
Homeostatic reprograming of GABAergic neurons upon presynaptic dysfunction.
(Chairwoman: G. Balagura)

14:45 **Luca Fusar Bassini** • *A genome-wide atlas of poison exons for antisense oligonucleotide therapeutics in the Central Nervous System.*

15:00 **Antonella Lauri** • *Mutations in the new disease-causing gene ARF3 have disruptive consequences on Golgi integrity and brain development.*

15:15 **Gianmarco Pallavicini** • *Patients derived organoids show differences in DNA damage accumulations in neural progenitors leading microcephaly syndrome.*

15:30 **Maryam Khastkhodaei Ardakani** • *Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment.*

SESSION 2 • NEUROINFLAMMATION • ORAL COMMUNICATIONS

Chairpersons: S. Angiari, I. Prada, A. Musella, M. Tiberi

16:00 Lecture | **Henrique Veiga-Fernandes**
Neuroimmune interactions in health and disease.
(Chairman: S. Angiari)

16:30 **Mikolaj Opielka** • *The pH-sensing receptor TDAG8 modulates inflammatory signalling and maturation of oligodendrocytes.*

16:45 **Francesca Fagiani** • *Modelling chronic neuroinflammation in Multiple Sclerosis using patient-derived 3D BrainSpheres and single-cell transcriptomics.*

17:00 **BraYn Educational Symposium • Beckman Coulter ▶ Valerio Chiurchiù**
Another break in the brain wall: from tissue dissociation to identification and immunophenotyping of resident and infiltrated immune cells.
(Chairmen: V.A. Baldassarro, M. Rasile)

17:15 **Francesca Montarolo, Federica Azzolini** • *The role of MICROGLIA in MS: from micro to macro different point of view.*

17:40 Coffee Station

18:00 **Matteo Bizzotto** • *Interplay between microglial receptor TREM2 and maternal immune challenges in schizophrenia onset.*

18:15 **Jessica Garau** • *DNA methylation profiling of patients with Aicardi-Goutières Syndrome carrying the identical p.A177T RNASEH2B mutation but showing heterogeneous phenotypes.*

18:30 **Marta Bottero** • *Anti-NG2 autoantibodies as prognostic biomarker in persons with multiple sclerosis.*

18:45 Closing remarks

29 SEPTEMBER • Day 2

SESSION 3 • NEUROPHYSIOLOGY & NEURAL PLASTICITY • ORAL COMMUNICATIONS

Chairwomen: E. Boda, R.C. Paolicelli, G. Calabrese, M. Di Domenico

9:00 Lecture | **Laura Cancedda**
Treating neurodevelopmental disorders: the road is long and winding, but we need to try.
(Chairwoman: I. Prada)

9:30 **Ori Roethler** • *Cooperation between two experience-regulated enhancers maintains visual processing by controlling E/I ratio in VIP interneurons.*

9:45 **Valeria de Rosa** • *D-Aspartate treatment attenuates myelin damage and stimulates myelin repair.*

10:00 **BraYn Educational Symposium • BGI ▶ Xin Yi**
Sequencing strategy in neuroscience : From RNAseq to Spatial Transcriptomics.
(Chairpersons: S. Amoretti, P. Pacifico, L. Porta)

10:15 **BraYn Educational Symposium • Femtonics ▶ Balázs Rózsa**
High-Speed 3D Acousto-Optical Network and Dendritic Imaging in Behaving Mice Revealed that Brain Activity is Organized Locally in Spatio-Temporal Clusters of Neuronal Functional Assemblies.
(Chairpersons: S. Amoretti, P. Pacifico, L. Porta)

10:30 Coffee Break

11:00 **Claudia Cristiano** • *Effect of maternal butyrate supplement on autistic-like behavior and synaptic plasticity deficits in mice offspring.*

11:15 **Fanny S. Martineau** • *Microglia contribution to neuronal network remodeling after paralysis onset.*

11:30 Lecture | **Maria Rescigno**
The microbiota in gut-brain vascular axis.
(Chairman: V. Chiurchiù)

12:00 Lunch box with Poster session 2

PARALLEL SESSION (12:30-13:00)
• For scheduled groups only (max 20 persons) •

Alexion Practical Workshop
Fiammetta Vanoli • *Can we regain control of daily life for gMG patients?*

SESSION 4 • NEURO-ONCOLOGY • ORAL COMMUNICATIONS

Chairpersons: G. D'Alessandro, E. Vannini, G. Pallavicini, M. Conenna

14:00 Lecture | **Dirk Sieger**
Understanding the role of microglia during brain tumour initiation.
(Chairwomen: G. D'Alessandro, E. Vannini)

14:30 **Antonino Cucinotta** • *Blocking the Hedgehog-dependent tumor growth by a new selective Endoplasmic Reticulum Aminopeptidase 1 inhibitor.*

14:45 **Maria Velasco-Estevez** • *Mechanoreception in glioma: an insight into the role of Piezo1 in GBM progression and cancer stem cells.*

15:00 **BraYn Educational Symposium • Euroclone ▶ Diego Muzzini**
Deciphering the Complex Biology of Brain Tumors with Single Cell and Spatial Technologies.
(Chairpersons: G. D'Arrigo, G. Pallavicini)

15:15 **Alessandro Mormino** • *Histone-deacetylase 8 drives the immune response and the growth of glioma.*

15:30 **Francesca Viale** • *Design of implantable hydrogel for glioblastoma treatment.*

15:45 Coffee Break

SESSION 5 • NEUROIMAGING • ORAL COMMUNICATIONS

Chairpersons: F. Di Lorenzo, S. Schiavi, S. Ruinet

16:30 Lecture | **Rodrigo Quian Quiroga**
What makes us human?
(Chairman: G. Ferrara)

17:00 **Pietro Bontempi** • *Investigating the feasibility of assessing magnetization transfer properties of distinct white-matter connections.*

17:15 **BraYn Educational Symposium • Fujifilm Visualsonics ▶ Philippe Trochet**
Multimodal and Multiscale In-Vivo Imaging of Cerebral Hemodynamic.
(Chairmen: P. Lippiello, G. Sansevero)

17:30 **BraYn Educational Symposium • Siemens Healthineers ▶ Gian Franco Piredda, Domenico Zacà**
Probing myelin content of the human brain with MR relaxometry.
(Chairmen: P. Lippiello, G. Sansevero)

17:45 **Francesco Tazza** • *Differentiating MS lesions with or without paramagnetic rim with advanced MRI.*

18:00 **Andrea Termine** • *Development of a Frontotemporal dementia computer-aided diagnostic tool using a Dense Convolutional Neural Network on 3D brain scans and explainable artificial intelligence methods.*

18:15 Closing remarks

30 SEPTEMBER • Day 3

SESSION 6 • NEURODEGENERATION • ORAL COMMUNICATIONS

Chairpersons: G. Nardo, B. Bettegazzi, M. Medelin, C. Natale

9:00 Lecture | **Amanda Sierra**
Not just corpse removal: how microglial phagocytosis maintains brain tissue homeostasis.
(Chairwoman: R.C. Paolicelli)

9:30 **Elisa Pagliari** • *Optimization of AAV9 gene therapy for Spinal Muscular Atrophy with Respiratory Distress type 1 using in vivo disease model.*

9:45 **Elena Abati** • *Combined RNA interference and gene replacement therapy targeting MFN2 for the treatment of Charcot-Marie-Tooth type 2A.*

10:00 **Delia Gagliardi** • *Exploiting three-dimensional in vitro models to identify early neuronal vulnerability and test therapeutic strategies in amyotrophic lateral sclerosis.*

10:15 **BraYn Educational Symposium • Miltenyi Biotec ▶ Beatrice Formicola**
Beyond the boundaries of Neuroscience with 3D microscopy: Application and methods with the Light-Sheet Ultramicroscope Blaze.
(Chairmen: L. Cuffaro, S. Negro)

10:30 Coffee Break

11:00 **Giulia Lunghi** • *New insights into the effects of SARS-COV-2 infection on nervous system: alteration of dopamine metabolism in IPSCs-derived dopaminergic neurons.*

11:15 **Francesca Natale** • *Aberrant Protein Palmitoylation: a novel therapeutic target in Alzheimer's disease.*

11:30 **Alessandro Matera** • *Role of SHIP1 as a modulator of microglial function.*

11:45 **BraYn Educational Symposium • Perkin Elmer ▶ Francesca Malerba**
Looking for a needle in a haystack: how to detect a biological drug against its natural background. The case of painless NGF.
(Chairmen: P. Lippiello, G. Sansevero)

12:00 Lunch box with Poster Session 3

SESSION 7 • CLINICAL NEUROSCIENCE • ORAL COMMUNICATIONS

Chairmen: M. Tartaglia, L. Cuffaro, G. Abbadessa

14:00 Lecture | **Giacomo Koch**
Non-Invasive brain stimulation in neurodegenerative diseases: clinical implications.
(Chairman: F. Di Lorenzo)

Neuroimmunology (multiple sclerosis)

14:30 **Margherita Maria Ravanelli** • *A humanized model of blood brain barrier to investigate immune cells infiltration in Multiple Sclerosis: toward a personalized medicine approach.*

14:40 **Francesca De Vito** • *The emerging role of microRNAs in experimental and clinical multiple sclerosis: implications for inflammation-driven synaptic dysfunctions and disease course.*

14:50 **Gianmarco Bellucci** • *Deciphering Multiple Sclerosis endophenotypes through Mendelian disorders: a network-based approach.*

Neurodegenerative (movement disorders, MND)

15:00 **Cecilia Mei** • *New insight for Riboflavin Transporter Deficiency (RTD) Syndrome: gene therapy as a new therapeutic strategy for RTD patients.*

15:10 **Petra Šoštarić** • *Central Effects of Botulinum Toxin Type A in Motor Nervous System of the Rat.*

Neuro-oncology

15:20 **Marta Ibáñez Navarro** • *Driving CARs on a highway to cure pediatric CNS malignant tumors.*

15:30 **Eugenia Guida** • *Cytotoxic activity of small molecule inhibitors on patient-derived glioblastoma cells.*

Inherited Leukoencephalopathies

15:40 **Ingrid Battistella** • *Human iPSC-based cellular systems to model Autosomal dominant leukodystrophy.*

SESSION 8 • Curated by Karolinska Institutet • ORAL COMMUNICATIONS

Chairmen: K. Ampatzis, G. Ferrara

16:00 Lecture | **Konstantinos Meletis**
Organization and function of circuits that control motivated behaviors.
(Chairman: G. Ferrara)

16:30 **Emanuela Santini** • *Dysregulated brain protein synthesis in autism spectrum disorders.*

16:45 **Daniel De Castro Medeiros** • *Studying sleep-related disturbances in a mouse model of Parkinson's disease.*

17:00 **Irene Pallucchi** • *Transformation of an early-established motor circuit during maturation in zebrafish.*

17:15 Questions & Answers

17:30 Closing remarks • BraYn Awards (Best Oral and Poster Presentation and BraYn Starting Grant)

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Mutations in the new disease-causing gene ARF3 have disruptive consequences on Golgi integrity and brain development

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Patients derived organoids show differences in DNA damage accumulations in neural progenitors leading microcephaly syndrome

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Interplay between microglial receptor TREM2 and maternal immune challenges in schizophrenia onset

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DNA methylation profiling of patients with Aicardi-Goutières Syndrome carrying the identical p.A177T RNASEH2B mutation but showing heterogeneous phenotypes

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EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS (p. 61-67)

EBN01 | Self-Organizing 3D Human Organoids to dissect the role of Choroid Plexus (ChP) in cortical layer patterning during brain development • Vanessa Aragona

EBN02 | Glial Fibrillary Acid Protein correlates with the phenotype in adult patients with Tuberous Sclerosis Complex • Valeria Manzini

EBN03 | Somatosensory processing deficits and altered connectivity in Cntnap2 -/- and Shank3b−/− mouse models of autism spectrum disorder. • Luigi Balasco

EBN04 | Prenatal exposure to poly I:C induces tissue-specific expression of several ERVs and related genes, and immune effectors in cortex, hippocampus, and blood samples from C57BL/6 mice • Martina Giudice

EBN05 | The effect of a Ketogenic Diet on the host Microbiota, the Immune System and the CNS • Andrina Rutsch

EBN06 | Gene expression profiling in trigeminal ganglia from Cntnap2 -/- and Shank3b -/- mouse models of autism spectrum disorder • Alessandra Ciancone Chama

EBN07 | The role of bdnf in epilepsy: evidence from a pharmacological zebrafish model of disease • Carmine Merola

NEUROINFLAMMATION (p. 68-79)

NI01 | Novel synthetic thyroid hormone receptor beta ligands to regulate oligodendrocyte precursor cells differentiation in demyelinating diseases • Giuseppe Alastra

NI02 | Effects of MAGL inhibitor on striatal neuroinflammation and synaptic dysfunction in experimental multiple sclerosis • Livia Guadalupi

NI03 | Approaching behavior and its synaptic and transcriptomic signatures in medial prefrontal cortex pyramidal neurons: the involvement of excitatory neurotransmission and immune system • Anna Panuccio

NI04 | Luteolin treatment ameliorates brain development and behavioral performance in a mouse model of CDKL5 deficiency disorder • Marianna Tassinari

NI05 | Antioxidant and anti-inflammatory role of grapefruit IntegroPectin • Chiara Valenza

NI06 | Possible implications of the kynurenine pathway in the pathogenesis of Amiotrophic Lateral Sclerosis • Giusy Laudati

NI07 | Possible role of the sympathetic nervous system in the definition of glioblastoma immune microenvironment • Erika Ricci

NI08 | RvD1 modulates maturation of monocyte-derived dendritic cells in Multiple Sclerosis • Federico Fazio

NI09 | Understanding the role of microglial extracellular vesicles in neuroinflammation spreading: an in vitro study • Francesca De Chirico

NI10 | Systemic inflammation upregulates the expression of oxysterol 7α,25OHC-synthesising enzymes in the blood-brain barrier • Fionä Caratis

NI11 | Specialized pro-resolving mediator RvD1 reduces neuroinflammation in a transgenic rat model of Parkinson's disease • Marta Tiberi

NI12 | Evaluation of D-loop methylation level and mtDNA copy number in Aicardi-Goutières patients • Francesca Dragoni

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NEUROPHYSIOLOGY & NEURAL PLASTICITY (p. 80-87)

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NP02 | Cortical Rewiring Following Peripheral Injection of Botulinum Neurotoxin Type A • Alexia Tiberi

NP03 | Assessing the contribution of altered cholinergic signaling in ASD social deficits • Alice Tartacca

NP04 | Antioxidants counteract the plastic effect of physical exercise in the adult primary visual cortex • Irene Di Marco

NP05 | Loss of MCT4 in microglia results in altered brain development and anxiety-like behavior • Katia Monsorno

NP06 | Neuroligin 2 regulates synaptic and network activity of hippocampal CA3 neurons during development • Gianfranco Porcheddu

NP07 | SynActive (SA) toolbox potentiality to study the role of microglia in spine potentiation • Marina Di Domenico

NP08 | Impact of intermittent fasting on neural plasticity and peripheral-cerebral metabolism in a mouse model of diet induced obesity (DIO) • Andrea Tognozzi

NEURO-ONCOLOGY (p. 88-92)

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NO02 | Rearrangements of peritumoral tissue that take place during glioma progression • Elisa De Santis

NO03 | An innovative technique to anticipate the diagnosis of glioblastoma: analysis of extracellular vesicles in liquid biopsies. • Mariassunta De Luca

NO04 | The role of Short Chain Fatty Acids in the modulation of glioma cell growth • Francesco Marrocco

NO05 | Drug-loaded MMP2-activable -liposomes as promising strategy for glioblastoma treatment • Milena Mattioli

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NIM02 | Artificial Intelligence for Alzheimer's Disease: a data-centric approach to 3D MRI Deep Learning classification • Carlo Fabrizio

NIM03 | Neuroprotective effects of Montelukast treatment in a rat model of Huntington-like neurotoxicity: a PET covariance study • Margherita Tassan Mazzocco

NIM04 | Ultrasound multiparametric imaging of neuroinflammation • Solène Ruinet

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ND02 | Raman Spectroscopy as a powerful instrument for the Parkinson's disease managing • Luana Forleo

ND03 | Dietary intervention on brain aging in vivo and ex-vivo • Letizia Brogi

ND04 | Towards unveiling the nexus between axonal granules and polysomes in neurological disorders • Fabio Lauria

ND05 | Brain-ageing modulators in human blood as novel therapeutic targets of Alzheimer's Disease • Federica Anastasi

ND06 | Epigenetic and transcriptional dysregulation in NSCs and OPCs proliferation defects of AGC1 deficiency • Eleonora Poeta

ND07 | Nucleoporin 153 deficiency in adult neural stem cells defines a pathological protein-network signature and defective neurogenesis in a mouse model of AD • Alessia Bertozzi

ND08 | Molecular and metabolic pathways underlying the in vivo anti-amyloidogenic action of 12A12, a cleavage-specific tau antibody targeting the 20-22kDa toxic peptide. • Valentina Latina

ND09 | Modeling of nigro-striatal circuits through the generation of human 3D organoids • Manuela Magni

ND10 | Effects of the homeobox gene Dbx2 on astrocyte function and on their cross talk with neural stem cells. • Sara D'Angelo

ND11 | Neuroprotective effect of a novel metabotropic glutamate receptor 3 positive allosteric modulator in in vitro model of Parkinson's disease • Miriana Scordino

ND13 | Effects of gH625-liposome-PACAP in an in vitro fluid model of Parkinson's disease • Teresa Barra

ND14 | Hydrogen peroxide: a new player in peripheral neve regeneration • Samuele Negro

ND15 | Characterization of spinal cord organoids derived from sALS patients • Matteo Bordoni

ND16 | The loss of frataxin impairs microglia homeostatic functions in Friedreich's ataxia • Martina Milani

ND17 | The Serum Response Factor (SRF) regulates motoneuron vulnerability in ALS through the regulation of autophagy flux • Natalie Yashoda Dikwella

ND18 | Chronic administration of palmitoylethanolamide counteracts cognitive decline in Tg2576 Mice • Davide Decandia

ND19 | Iron-fed microglia: an in vitro system to model microglial phenotype in vitro and test new therapy in neurodegenerative diseases? • Giulia Cutugno

CLINICAL NEUROSCIENCE (p. 116-119)

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CN02 | Towards developing a mass spectrometry assay to identify post-translational modifications of deoxycytidine kinase possibly relevant to the response to cladribine • Federico Carlini

CN03 | The function of GPR183/7α,25OHC signalling in the brain microvessels and multiple sclerosis. • Aleksandra Rutkowska

CN04 | Multi-dimensional genome-wide analysis reveals robust pre-symptomatic defects in translation in two SMA mouse models • Martina Paganin

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EBN09 | Healthy life-style approaches to attain disease modification in acquired epilepsies • Valentina Kebede

EBN10 | Effect of Type 5 Phosphodiesterase (Pde5) deletion on neurogenesis • Serena Fuda

EBN11 | Somatic mutations and epileptic seizures originating from the contralateral hemisphere: two possible pathogenetic mechanisms and personalized pharmacological approaches • Cristiana Pelorosso

EBN12 | Dissecting the role of HCN1 in Developmental and Epileptic Encephalopathy (DEE) by exploiting patient-specific models of cerebral cortex development • Giulia Demenego

EBN13 | Nr2f1 haploinsufficiency affects immature granule neurons morphology and leads to an altered activation of neuronal ensembles within the adult mouse hippocampus • Eleonora Dallorto

EBN14 | Oligophrenin-1 (OPHN1): a novel sumo target in synaptic function and dysfunction • Cristina Guglielmetti

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NI14 | The spinal cord plasticity: regionalization and time-course of neurovascular events following peripheral nerve injury • Ivana Allocca

NI15 | Comparison of brain damages between male and female in a model of encephalopathy of prematurity : study of a sexual dimorphism • Jennifer HUA

NI16 | The promoter methylation status, mRNA expression and production of TNFα, IL6 and IL10 in Multiple Sclerosis patients • Lisa Aielli

NI17 | Neuroinflammation in Fabry's disease: a new insight into a multisystemic disease • Francesca Massenzio

NI18 | Time-dependent modifications in glia cells, macrophages and extracellular matrix supporting glioblastoma progression. • Raffaella Cirillo

NI19 | N-acetyl L-cysteine counteracts cerebellar inflammation and autism-like behaviours in mice lacking the Cntnap2 gene • Enrica Cerilli

NI20 | MiR-142-3p is a critical modulator of TNF-mediated neuronal toxicity in multiple sclerosis • Sara Balletta

NI21 | Stavudine "interferes" via alpha-7nAChR to inhibit NLRP3 in (LPS+Amyloid-beta) stimulated PBMC of AD Patients • Francesca La Rosa

NI22 | Characterization of astrocyte reactivity in a model of encephalopathy of prematurity • Ariane Heydari Olya

NI23 | Microglia-derived Extracellular Vesicles are involved in synaptic pruning in vitro • Giulia D'Arrigo

NI24 | Pro-resolving lipid mediator neuroprotectin D1 ameliorates chronic experimental autoimmune encephalomyelitis by modulating macrophage plasticity and polarization • Alessandro Matteocci

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NP12 | Alterations of cholesterol metabolism in experimental models of Rett syndrome • Cecilia Cabasino

NP13 | Botulinum neurotoxin as a tool to study the plasticity of motor axon terminals • Stefano Amoretti

NP14 | Mothers and sons: how bisphenols target brain and behaviors • Brigitta Bonaldo

NP15 | Sexually dimorphic organizational role of estrogen receptors on different neuroendocrine systems controlling metabolism and reproduction • Marilena Marraudino

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NO07 | Lactate Metabolism and YAP/TAZ tumorigenic effect in Glioblastoma • Eleonora Curzi

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NO09 | Cullin3/REN^{KCTD11} and SALL4/HDAC1 interplay promotes Hedgehog-dependent medulloblastoma through GLI1 deacetylation • Ludovica Lospinoso Severini

NO10 | Glioblastoma Tunneling Nanotubes as potential targets for nanomedicines • Giulia Sierri

NO11 | Addressing the significance of tumor-released microvesicles in glioblastoma aggressiveness and invasion • Valentino Ribecco

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NIM06 | Relationship between fatigue, disability, and reserve in patients with MS: a cross-sectional and longitudinal analysis • Alessandra Scaravilli

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NIM08 | SANDIAMICO: an open-source toolbox for Soma And Neurite Density Imaging (SANDI) with AMICO • Mario Alberto Ocampo Pineda

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ND21 | Tau aggregation affects glutamatergic genes expression • Arianna Scarlatti

ND22 | Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease by moving at the axon surface • Martina Gabrielli

ND23 | The synergistic role of SMN and eIF3e in ribosome heterogeneity and the impact of their loss in Spinal Muscular Atrophy • Deborah Donzel

ND24 | A preliminary in vitro study to assess the stressor effect on Amyotrophic Lateral Sclerosis onset and progression • Daniela Maria Rasà

ND25 | Multifunctional liposomes increase synaptic transmission strength in mouse cortical neurons • Giulia Terribile

ND26 | Co-ultramicronized Palmitoylethanolamide/Luteolin prevents alteration in astrocyte-oligodendrocyte crosstalk relevant for myelination in an in vitro model of β-amyloid toxicity • Marta Valenza

ND27 | Zebrafish as a model for Alexander disease • Deianira Bellitto

ND28 | Mitochondrial SMN1-anticorrelated genes as potential targets for Spinal Muscular Atrophy therapy • Anna Caretto

ND29 | The chrOMICles of ALS spinal cord organoids - OMIC characterization of patient-derived spinal cord organoids to unravel new therapeutic targets in C9ORF72 form of Amyotrophic Lateral Sclerosis • Noemi Galli

ND30 | Towards understanding the role of translational heterogeneity in SMA disease. • Gaurav Sharma

ND31 | Cognitive frailty and oxygen-ozone therapy: differential expressed genes as predictive biological markers of response/improvement to treatment. • Chiara D'Amelio

ND32 | A new intrabody based optogenetic tool to degrade the aggregation prone proteins • Angela Bitonti

ND33 | The neuropathology of the SARS-CoV-2: an autoptic COVID-19 "biobanking" of brain specimens for future translational biomedical research • Giuseppina Amadoro

ND34 | Transcriptome-phenotype relationship in unmutated sporadic ALS patients highlights phenotype-specific gene expression patterns • Maria Garofalo

ND35 | The possible role of cholesterol metabolism in the onset and progression of Huntington's disease • Monica Favagrossa

ND36 | Mitochondrial alterations in subjects with idiopathic REM sleep disorders as a predictive biomarker for conversion to Parkinson's disease • Gerardo Ongari

ND37 | RNA Expression Profiling in Lymphoblastoid Cell Lines from Mutated and Non-Mutated Amyotrophic Lateral Sclerosis Patients • Rosalinda Di Gerlando

ND38 | Contingent intramuscular boosting of P2X7 axis improves motor function in transgenic ALS mice • Paola Fabbrizio

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CN06 | Disease Modifying Therapy specifically impacts on microRNAs expression profiling in Relapsing-Remitting Multiple Sclerosis • Leonardo Malimpensa

CN07 | Stathmin-2 in Spinal Muscular Atrophy (SMA): assessing molecular and therapeutic role in SMA human and murine models • Paolo Manzini

CN08 | Oxygen-Ozone Therapy and Cognitive Frailty: a non-pharmacological approach to potentially resolve immune and inflammatory dysfunctions • Miriam Ciani

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EBN16 | Remodulation of Rac1 GTPase pathway in cytoskeletal related Intellectual Disabilities • Carla Liaci

EBN17 | A new role of NBS1 in the regulation of primary cilium • Mariaconcetta Augusto

EBN18 | 3D Human Cortical Organoids to investigate developmental and epileptic encephalopathy • Monica Tambalo

EBN19 | In vivo functional validation of new disease-genes and variants impairing trafficking and cytoskeleton dynamics as underlying cause of undiagnosed neurodevelopmental diseases • Giulia Fasano

EBN20 | Towards an in vitro model for therapeutic opportunities in Lafora disease • Gabriele Trentini

EBN21 | A new role of Nijmegen Breakage Syndrome gene in Neuronal development • Damiana Battaglini

EBN22 | Expression of a secretable, cell-penetrating CDKL5 protein enhances the efficacy of AAV vector-mediated gene therapy for CDKL5 deficiency disorder • Manuela Loi

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NI26 | The role of SK channels and the vagus nerve in turning on AgRP neurons in experimental autoimmune encephalomyelitis • Eleonora Cornacchia

NI27 | Nerve Growth Factor influences microglial activity in vivo via TrkA receptors • Giulia Borgonovo

NI28 | Dendritic cells educated through exposure to specialized pro-resolving mediators acquire a tolerogenic phenotype. • Marta Bottero

NI29 | Altered expression of specific HSPs in the spinal cord in an animal model of rheumatoid arthritis • Malak Fouani

NI30 | Counteract the outer Blood-Retinal Barrier breakdown targeting ocular inflammation to delay vision loss in Retinitis Pigmentosa. • Beatrice Di Marco

NI31 | Influence of the sympathetic nervous system on the thymus: β3-adrenergic receptor-expressing stromal cells as sentinels of the thymic function • Maria Cristina Mariani

NI32 | Nutritional overload promotes inflammatory synaptic damage and disease course worsening in clinical and experimental multiple sclerosis • Silvia Caioli

NI33 | Evaluation of early aging following perinatal inflammation-driven encephalopathy of prematurity in a mouse model • David Guenoun”

NI34 | Targeting the brain 5-HT7 receptor to prevent hypomyelination in a rodent model of perinatal white matter injuries • Cindy Bokobza

NI35 | Specialized pro-resolving lipid mediator neuroprotectin D1 attenuates motor disability by reducing synaptotoxic a • Diego Fresegna

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NP18 | Olfactory stimulation reverses anxiety and depression-like behaviours induced by acute and chronic stress. • Marco Rinaudo

NP19 | Inhibitor of the excitation-contraction coupling machinery act as enhancer of Botulinum Neurotoxin type A pharmacological activity • Marika Tonellato

NP20 | The effects of anesthetics on glycogen concentration in microwave-fixed brain samples • Maria Fernanda Veloz Castillo

NP21 | Tetanus Toxin Injections into the Rat Motor Cortex and Striatum Impair the Narrow Beam Walking Performance • Patrik Meglić

NP22 | Role of group I metabotropic receptors in the synaptic alterations in the dorsal striatum of theR451C-Nlgn3 mouse model of autism • Martina Montanari

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NO13 | Cancer-neuronal crosstalk in glioblastoma • Chiara Saulle

NO14 | Reduction of lipoprotein receptors levels synergistically potentiates the anti-tumour activity of Givinostat on human glioblastoma cancer cells • Lorenzo Taiarol

NO15 | CTX-CNF1 treatment boosts the immune system • Elisabetta Mori

NO16 | The role of the cytoskeleton regulator inverted formin INF2 in medulloblastoma tumorigenesis • Marilisa Conenna

NO17 | Molecular changes underlying decay of sensory responses and enhanced seizure propensity in peritumoral neurons • Marta Scalera

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NIM10 | In vivo evaluation of dentato-thalamo-cortical tract integrity in friedreich ataxia using diffusion MRI • Mario Tranfa

NIM11 | Examination of whole-brain structural and functional connectivity in Fabry Disease • Ilaria Gabusi

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ND39 | Emerging roles of SLITRK family members in αSyn- p.A53T mediated synaptic dysfunction • Elissavet-Kalliopi Akrioti

ND40 | The functional coupling between the NaV1.6 voltage-gated channel and the Na+/Ca2+ exchanger 3 promotes an endoplasmic reticulum Ca2+ refilling in a transgenic model of Alzheimer's disease • Ilaria Piccialli

ND41 | Sleep fragmentation accelerate dementia in transgenic 5xFAD AD mice model inducing astrogliosis and affecting glymphatic system. • Valeria Vasciaveo

ND42 | Histone Deacetylase inhibition in Retinitis Pigmentosa rescues cone cells • Noemi Orsini

ND43 | The role of astrocytes in β-Amyloid- and magnetite nanoparticles-induced neurotoxicity • Veronica D'Ezio

ND44 | Compensatory myogenesis and acetylcholine receptor clustering delay symptoms onset and progression in SOD1 mutant mice • Cassandra Margotta

ND45 | Therapeutic potential of nanoformulations in a zebrafish model of retinal degeneration • Giorgia Giuseppetti

ND46 | Single-cell transcriptomic comparison of human microglia in Alzheimer's disease and Multiple Sclerosis • Edoardo Pedrini

ND47 | n-3 PUFA improves psychological well-being during menopausal transition. • Sefano Sacchetti

ND48 | Microglia-released extracellular vesicles to slow down the aging process in relation to the gender • Arianna Rinaldi

ND49 | Biophotonics platforms for the characterization of multifunctional nanoliposomes for Alzheimer's disease and Glioblastoma • Valentina Mangolini

ND50 | Counteracting alpha-synuclein aggregation: a novel role for GM1 oligosaccharide • Maria Fazzari

ND51 | Trafficking of the glutamate transporter is impaired in LRRK2-related Parkinson's disease • Ludovica Iovino

ND52 | The role of Nrf2-mediated System xc- activation in HIV-1 Tat-induced neurotoxicity • Ludovica Carpinelli

ND53 | Mitochondrial dysfunctions in Spinal Muscular Atrophy: mitochondrial aconitase as a potential biomarker of the disease • Gianna Pavarino

ND54 | Niclosamide ameliorates disease progression in a model of amyotrophic lateral sclerosis • Ilaria Della Valle

ND55 | Defective protein O-GlcNAcylation in Parkinson's disease patients brain and blood cells • Michele Mario Gennari

ND56 | Eye as a mirror of brain neurodegeneration: retinal characterization of neuroinflammatory and neurodegenerative aspects in a mouse model of NGF deprivation • Lucia Buccarello

ND57 | Characterization of the early cognitive, emotional, motor, and behavioral features of a mouse model of Parkinson's disease. • Francesca Balsamo

ND58 | Possible roles of amyloid-β in microglia-mediated synapse remodeling • Kyllian Ginggen

CLINICAL NEUROSCIENCE (p. 238-240)

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CN10 | Generation of isogenic control of TBCD mutated induced pluripotent stem cells using CRISPR/Cas9 gene editing • Federica Benigni

CN11 | Centrin 2: A Novel Marker of Mature and Neoplastic Human Astrocytes • Elisa Degl'Innocenti


COMMUNI
ORAL
CATIONS

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS 28th SEPTEMBER • 14:45

A genome-wide atlas of poison exons for antisense oligonucleotide therapeutics in the Central Nervous System

Luca Fusar Bassini [1] - Boxun Zhao [1] - Timothy Yu [1]

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Poison exons (PEs) are naturally occurring alternative exons that contain a premature termination codon. More than 20% of isoforms in the human transcriptome contain PEs, which direct the carrier transcripts to nonsense-mediated decay (NMD). Splice-switching antisense oligonucleotides (ASOs) are short, chemically modified RNAs that can block the inclusion of naturally occurring PEs by modulating RNA splicing, thereby up-regulating productive mRNA and protein levels. Recently, such strategy has been deployed in ASO development to treat diseases caused by mutations in haploinsufficient genes (Lim, 2020). However, our knowledge of where PEs reside in the genome is still limited, as it is challenging to detect such NMD-sensitive transcripts with low abundance in total RNA samples. Here, we identify thousands of novel PEs and generate a genome-wide atlas of gene targets for therapeutic ASO development for Mendelian diseases. To capture previously undetectable PE-containing transcripts, we use a variety of strategies to inhibit NMD in patient-derived fibroblasts, neurons, astrocytes, and retinal pigmented epithelium cells, followed by deep stranded RNA sequencing. We propose a gene annotation-agnostic pipeline for genome-wide, tissue-specific identification of PEs. So far, our pipeline has discovered >4,000 novel PEs and confirmed >4000 known PEs, while missing <100 high-confidence PEs from previous studies. We experimentally validate 30 PEs using RT-PCR, 22 out of which in haploinsufficient genes or oncogenes. We are developing splice-switching ASOs for PEs within genes relevant to Central Nervous System monogenic diseases. In the pilot exploration for therapeutic targets in MAPK8IP3-Related Neurodevelopmental Disorder, we identify 8 ASOs targeting a PE that can up-regulate MAPK8IP3 expression in fibroblasts. Our study delineates a rich set of interventional targets for therapeutic ASO development for several Mendelian disorders.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS 28th SEPTEMBER • 15:00

Mutations in the new disease-causing gene ARF3 have disruptive consequences on Golgi integrity and brain development

Giulia Fasano [(1)] - Valentina Muto [(1)] - Francesca Clementina Radio [(1)] - Martina Venditti [(1)] - Alban Ziegler [(2)] - Giovanni Chillemi [(3)] - Bruno Dallapiccola [(1)] - **Antonella Lauri** [(1)] - Marco Tartaglia [(1)]

Ospedale Pediatrico Bambino Gesù, IRCCS, Genetics and Rare Diseases Research Division, Roma, Italy (1) - CHU d'Angers, Département de Génétique, Angers, France (2) - University of Tuscia, Department for Innovation in Biological Agro-food and Forest systems (DIBAF), Viterbo, Italy (3)

Rare diseases affect more than 400 million people worldwide. Most of these conditions are characterized by highly disabling malformations of cortical development (MCD). Yet, despite the recent increase in disease-genes/variants discovery, heterogenous MCD remain without treatment due to poor knowledge of the underlying mechanisms. Here we employed an integrated functional genomics pipeline comprising human exome sequencing, in silico, in vitro and in vivo cell/developmental biology analysis in zebrafish to tackle a previously unidentified disease showing variable degrees of MCD, i.e. microcephaly, cortical atrophy associated with skeletal anomalies. We identified de novo missense variants affecting ARF3, a far neglected member of small GTPases of the RAS superfamily involved in Golgi-trafficking, as causative of the disease and provide first insights into ARF3 activity throughout vertebrate embryogenesis. In silico and biochemical investigations demonstrated that microcephaly-causing ARF3 mutations affect highly conserved residues regulating the catalytic activity of the protein participating in GTP binding. Experiments in fish embryos corroborated this finding and proved disruptive consequences of aberrant ARF3 on trans-Golgi integrity. Comparable in vitro results substantiated the pathophysiological role the newly discovered ARF3 mutations, leading to various patterns of Golgi dysfunction, as an underlying mechanism of this new form of Golgipathy. Our zebrafish models further validated the occurrence of a severe microcephalic trait caused by the severe mutations. The data showed a fundamental perturbation of precursor cells proliferation in the developing forebrain as well as planar cell polarity (PCP)-dependent cell processes establishing the body plan axes, resembling a known effect caused by dominant ARF1. In conclusion, utilizing an integrated multi-level analysis (genomics, in silico, in vitro and in vivo), our work 1. provides molecular classification for disease stratification, 2.offers a mechanistic knowledge of a previously unrecognized neurodevelopmental disorder and 3. document an obligate dependence on proper ARF3 function for Golgi homeostasis and early developmental processes.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS 28th SEPTEMBER • 15:15

Patients derived organoids show differences in DNA damage accumulations in neural progenitors leading microcephaly syndrome

Gianmarco Pallavicini [1] - Amanda Moccia [2] - Roberta Parolisi [1] - Giorgia Iegiani [1] - Martina Lorenzati [1] - Fiorella Balzac [3] - Emilia Turco [3] - Enrica Boda [1] - Annalisa Buffo [1] - Stephanie Bielas [2] - Ferdinando Di Cunto [1]

Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Department of Neuroscience "Rita Levi Montalcini",, Torino, Italy (1) - The University of Michigan Medical School, Department of Human Genetics, Ann Arbor, MI, United States (2) - Molecular Biotechnology Center, University of Turin, 4. Department of Molecular Biotechnology and Health Sciences, Torino, Italy (3)

In primary hereditary microcephaly (MCPH), brain volume reduction is the main clinical phenotype, associated with conserved brain architecture and mild to moderate intellectual disability. Mutations in citron (CIT), leading to loss or inactivation of the citron kinase protein (CITK), cause primary microcephaly in humans and rodents. This disorder is associated with cytokinesis failure and apoptosis in neural progenitors. It has therefore been postulated that the apoptosis observed after CITK loss is a consequence of impaired cytokinesis. However, studies performed in many different models indicate that cytokinesis failure leads more frequently to cell cycle arrest than apoptosis, suggesting that another fundamental event must occur. Using CIT ko and kinase inactive mice models compared to fore brain organoids derived from CIT mutated patients iPSCs, we found that CITK inactivation induces DNA damage accumulation and chromosomal instability in human and mouse neural progenitors. Moreover, recruitment of RAD51 to DNA damage foci is compromised by CITK loss or inactivation indicating that CITK is involved in homologous recombination. Despite same molecular lesion in ko and kinase inactive mutations in neural progenitors, different amount of damage in generates syndrome with less severity compared to ko. This suggests that there is a soil of damage to induce a precise apoptosis in neural progenitors that can represent a common thread between unrelated microcephaly syndromes.

EPILEPSY, BRAIN DEVELOPMENT & NEUROGENETICS 28th SEPTEMBER • 15:30

Rescuing neural cell survival and maturation in a primary autosomal recessive microcephaly-17 (MCPH17) mouse model: effects of the postnatal N-acetyl cysteine treatment

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Microcephaly 17 (MCPH17) is a rare neurodevelopmental disorder caused by mutations in the CIT gene, which encodes for the Citron Kinase (CIT-K) protein involved in DNA repair and cytoskeletal dynamics. Patients show reduced brain volume, simplified gyrification, intellectual disability, motor deficits, epilepsy, and early mortality. Cit-k KO mice recapitulate the human MCPH17 phenotype and shows epilepsy, ataxia and early lethality, DNA damage and reactive oxygen species (ROS) accumulation, apoptosis and maturation defects in neuronal and glial progenitors, and microglia increase. With the aim to identify pharmacological treatments that can reduce the cellular damage accumulation and improve the functional and histopathological phenotype of Cit-k KO mice, we performed a chronic treatment during the first 2 postnatal weeks with the antioxidant drug N-acetylcysteine (NAC), which is already FDA-/EMA-approved and can pass the blood brain barrier. NAC treatment reduces brain ROS levels and slightly increases Cit-k KO mouse life span. Nevertheless, treated mice show a significant improvement in motor performances and reduction in myoclonus. Major neuroanatomical defects and reduction of cortical interneurons persisted in the treated Cit-k KO mice. Yet, cortical oligodendrocyte progenitors and astrocytes significantly increased in numbers, while microglia density and morphology were largely normalized by NAC treatment. Interestingly, deposition of perineuronal nets around cortical interneurons was also significantly rescued by NAC treatment, suggesting the promotion of interneuron maturation. In the periphery, NAC promotes the maturation of the neuromuscular junctions, possibly underlying part of the rescue of Cit-k KO mouse motor phenotype. Patch-clamp recordings and in vivo calcium imaging analyses in the cerebral cortex are ongoing to unveil the functional bases of NAC effects. Our data suggest NAC postnatal treatment may be beneficial for the treatment of MCPH17.

NEUROINFLAMMATION 28th SEPTEMBER • 16:30

The pH-sensing receptor TDAG8 modulates inflammatory signalling and maturation of oligodendrocytes

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Acidosis is one of the hallmarks of demyelinating central nervous system (CNS) lesions in multiple sclerosis (MS). Response to acidic pH is mediated by a family of G protein-coupled proton-sensing receptors including OGR1, GPR4, and TDAG8. These receptors remain inactive at alkaline pH, while at acidic pH ~6.5 they are maximally activated. Their recently discovered functions include regulation of inflammation and immune responses, modulation of hypoxic/ischemic environment and tumorigenesis. In particular, TDAG8, which is highly expressed in the immune cells, is a negative regulator of inflammation. Its immunomodulatory effects depend mainly on Gαs signalling and cyclic AMP accumulation. Moreover, genome-wide association studies identified TDAG8 locus to be associated with several autoimmune diseases including MS. Notably, we found that TDAG8 is upregulated in demyelinating plaques and the peri-plaque regions and downregulated in the white matter of MS patients. In the animal model of MS, the experimental autoimmune encephalomyelitis, TDAG8-deficient mice develop an exacerbated course of the disease. Interestingly, we found that pH-sensing receptors are in disequilibrium in TDAG8 knockout mice. In the absence of TDAG8, the CNS expression levels of OGR1 and GPR4 are upregulated, thus changing the balance towards pro-inflammatory signalling. We also demonstrated that TDAG8-mediated signalling is involved in MO3.13 oligodendrocyte migration and maturation. In acidic pH, oligodendrocytes upregulate TDAG8 and cease to mature and differentiate. Moreover, treatment of MO3.13 oligodendrocytes with TDAG8 agonist, tetrahydropalmatine, decreased the levels of pro-inflammatory cytokines after challenge with bacterial lipopolysaccharide. Together these findings elucidate the role of TDAG8 in oligodendrocyte biology and indicate it might play a role in the pathophysiology of MS.

NEUROINFLAMMATION 28th SEPTEMBER • 16:45

Modelling chronic neuroinflammation in Multiple Sclerosis using patient-derived 3D BrainSpheres and single-cell transcriptomics

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Multiple sclerosis (MS) is the most common chronic inflammatory, demyelinating and neurodegenerative disease of the central nervous system (CNS) in young adults. Its pathological hallmark is the formation of demyelinating lesions, associated with neuro-axonal damage in the brain and spinal cord. Limited access to human oligodendrocytes (OL) represents a major limit in understanding OL pathology in MS. Here, we generated 3D BrainSpheres (BS) consisting of mature neurons, astrocytes, and OL and co-cultured them with hiPSC-derived microglia to dissect neuroinflammatory signaling in MS. In particular, hiPSC lines derived from 2 MS patients and 2 healthy donors were used to generate neural precursors (NPCs) containing a SOX10-enhanced green fluorescent protein cassette under a doxycycline-inducible promoter to foster the differentiation of mature OL. First, 3D BS were differentiation and co-cultured with iPSC-derived microglia, whose differentiation was assessed by RT-qPCR, flow cytometry, immunofluorescence and electron microscopy. After 8 weeks of differentiation, BS were stimulated with 10% MS patient-cerebrospinal fluid (CSF) for 24 hours and the transcriptome of CNS cells was profiled by single cell RNA-sequencing. We confirmed in 3D cultures that SOX10 overexpression in human NPCs promoted the differentiation of OL's resembling the primary humans. Ultrastructure analysis revealed a variety of differentiated and mature cell types in the BS, including functional myelinating OL. Thus, we established a reproducible protocol to obtain human 3D BS, displaying the diversity of CNS cells, as assessed by RT-qPCR and immunostaining. Exposure to CSF induced a marked alteration of intracellular signaling pathways related to inflammatory and oxidative stress response, paving the way for future investigations dissecting the underlying molecular mechanisms. Thus, we implemented BS as a valuable 3D model for modelling chronic inflammation in MS and drug discovery effort.

NEUROINFLAMMATION 28th SEPTEMBER • 18:00

Interplay between microglial receptor TREM2 and maternal immune challenges in schizophrenia onset

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Schizophrenia (SZ) is a neurodevelopmental disorder and its onset in the offspring has been associated to viral infections in the mother during pregnancy. Microglia play major roles during brain development and express the Triggering Receptor Expressed on Myeloid cells 2 (TREM2), which, through the interaction with its adaptor TYROBP, is involved in phagocytosis and synapses elimination. The receptor is also released in a soluble form (sTREM2), whose levels change during neuroinflammation. Here, we aim at understanding the interplay between environmental factors such as viral infections in pregnant dams and microglial TREM2 receptor on the development of SZ. Our results showed that treatment of murine microglia with the viral analog polyriboinosinic-polyribocytidilic acid (PolyIC) resulted in Trem2 and Tyrobp mRNA and sTREM2 decrease. This was paralleled by reduced CD68 expression and synaptosomes engulfment by microglia. In vivo, PolyIC injection determined Trem2 mRNA downregulation in the hippocampus of adult female mice 6 hours after treatment. Next, we evaluated the effects of PolyIC treatment on the offspring derived from PolyIC injected dams. PolyIC offspring analyzed at post-natal day (P) 18 displayed decreased excitatory synapses and defective microglial phagocytosis in the hippocampus. Flow cytometry and immunohistochemistry analyses revealed that TREM2 protein was decreased in the offspring at P18, thus showing a long-lasting effect of PolyIC treatment. Since IFNβ has been described to impact synapses amount and microglia phagocytosis, we tested the hypothesis of its involvement in PolyIC-mediated effects. Ifnb transcript, IFN-stimulated genes, and soluble IFNβ levels were increased in PolyIC-treated microglia in vitro and in the hippocampus and plasma of female mice. Our data show that the pathways downstream TREM2 and IFNβ could mediate the cellular and molecular phenotype observed in PolyIC offspring and might be new promising targets in SZ.

NEUROINFLAMMATION 28th SEPTEMBER • 18:15

DNA methylation profiling of patients with Aicardi-Goutières Syndrome carrying the identical p.A177T RNASEH2B mutation but showing heterogeneous phenotypes

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Aicardi-Goutières Syndrome (AGS) is a rare genetically mediated pediatric inflammatory disease characterized by overexpression of interferon-stimulated genes (ISGs) and cerebral abnormalities. Patients with mutations in some AGS-related genes (TREX1, RNASEH2A, RNASEH2B, SAMHD1) may exhibit variable clinical phenotypes although carrying the same mutation. Currently, the severity of the disease can only be assessed through clinical evaluation and no biomarkers are available to predict disease severity or progression. The RNASEH2B p.A177T mutation is the most common variant observed in our cohort of AGS patients. It is associated with variable clinical phenotypes that range from "severe" devastating neuro-inflammatory disease to "mild" courses with late onset.

To identify molecular signatures that correlate with disease severity, we performed DNA methylation profiling in peripheral blood cells from AGS patients with "mild" or "severe" disease who carry the same RNASEH2B p.A177T mutation and heathy controls.

When compared to controls, AGS patients presented hypomethylation of ISGs and differential methylation patterns in genes involved in neutrophil and platelet activation. Patients with "mild" phenotypes exhibited DMPs in genes involved in DNA damage and repair, whereas patients with "severe" phenotypes had different methylation profiles in genes involved in cell fate commitment and organ development. We also found hypomethylated positions in two key ISGs (IFI44L, RSAD2) which associated with gene overexpression in patients with "severe" when compared to "mild" AGS phenotypes (qRT-PCR). Based on this observation, a "reduced" interferon score, consisting of IFI44L and RSAD2, may aid in discriminating "mild" from "severe" phenotypes in AGS patients carrying the RNASEH2B p.A177T mutation.

Taken together, this project delivered predictive biomarker candidates that may allow evaluation of disease severity and prediction of progression to guide therapeutic decisions in AGS.

NEUROINFLAMMATION 28th SEPTEMBER • 18:30

Anti-NG2 autoantibodies as prognostic biomarker in persons with multiple sclerosis

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Nerve glial antigen 2 (NG2) is a marker of oligodendrocyte progenitor cells (OPC) and pericytes, and is also expressed by murine immune cells like dendritic cells, T cells, and macrophages. OPCs are precursors of oligodendrocytes while pericytes are essential components of the neurovascular unit. In multiple sclerosis (MS), an inflammatory demyelinating disease of the central nervous system (CNS), remyelination occurs early in the disease, but fails at later stages. While remyelination failure is not fully understood, OPCs are targets of the disease, affecting their recruitment and/or their differentiation. During neuroinflammation, NG2 is processed by metalloproteases, and its extracellular portion is deposited in the CNS parenchyma. We hypothesized that NG2 could be a target of the immune system, because the soluble peptides of NG2 in the CNS could trigger those response. Accordingly, the aim of this project is to understand if anti-NG2 antibodies (αNG2) are present in the cerebrospinal fluid (CSF) of MS persons and to understand their role. We analysed CSF from 114 MS persons and from 108 persons with other neurological diseases (OND), as controls. We found that αNG2 were present in 32% of the CSF of MS persons (MS+) who also showed a higher disease progression index, suggesting a possible role for αNG2 as prognostic biomarker. Immunofluorescence experiments confirmed that MS+ αNG2 stained OPCs and we identified the laminin G-like domain as containing an epitope recognised by MS+ αNG2. We found that MS+ CSF induced the activation of Caspase-8 upon complement activation and flow cytometry experiments showed that OPC death was increased in OPCs exposed to MS+ CSF as compared to control CSF. In conclusion, we suggest that αNG2 recognizing a specific epitopic region are elevated in a group of MS persons, and that those antibodies might play a role in OPC death or impair their differentiation and should be further studied as a possible adjunct tool for MS prognosis.

NEUROPHYSIOLOGY & NEURAL PLASTICITY 29th SEPTEMBER • 9:30

Cooperation between two experience-regulated enhancers maintains visual processing by controlling E/I ratio in VIP interneurons

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Experience-dependent plasticity of neural circuits is essential for information processing and underlies adaptive behaviors as well as higher cognitive functions, and key questions in neuroscience concern the underlying molecular and cellular mechanisms. Experience-regulated non-coding regulatory regions of the genome - e.g. promoters and enhancers - are thought to control the function and plasticity of neural circuits by regulating the experience-dependent transcription of genes that, in turn, modulate specific cells and synapses. However, it remains untested whether such genomic sites can indeed control the experience-induced transcription of such genes and whether these genomic mechanisms thereby control experience-dependent modulation of cells and neural circuits. We address this gap-in-knowledge by focusing on the secreted growth factor IGF1 that is expressed and activity-induced in VIP interneurons (INs) in the adult visual cortex. Using cell-type-specific Chip-Seq and newly generated mouse alleles, we identify two experience-induced enhancers upstream of the Igf1 locus and demonstrate in cultured neurons and in visual cortex VIP INs in vivo that these genomic sites selectively and cooperatively control the experience-induced transcription of Igf1 but not its basal (i.e. non-experience-regulated) transcription. Using intersectional genetics for acute cell-type-specific knockout of these enhancers in combination with patch-clamp electrophysiology in acute brain slices, we further demonstrate that these enhancers control the E/I-ratio in VIP INs. Finally, we performed calcium imaging in the visual cortex of awake behaving mice to demonstrate that these enhancers control the activity of VIP INs and that this, in turn, controls visual processing in vivo. Taken together, these experiments demonstrate that enhancer-mediated experience-induced transcription of a single gene can control E/I-ratio in single neurons and that this is required for maintaining proper information processing in neural circuits.

NEUROPHYSIOLOGY & NEURAL PLASTICITY 29th SEPTEMBER • 9:45

D-Aspartate treatment attenuates myelin damage and stimulates myelin repair

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Glutamate signaling may orchestrate oligodendrocyte precursor cell(OPC) development and myelin regeneration through the activation of glutamate receptors at OPC-neuron synapses. Recently, D-Aminoacids are emerging as molecules with important roles in brain.Among them,D-Aspartate exerts modulatory actions at glutamatergic synapses.Chronic administration of D-Aspartate has been proposed as therapeutic treatment in diseases related to myelin dysfunction and NMDA receptors hypofunction,including schizophrenia and cognitive deficits. Here,we investigated the effects of D-Asp both in vitro,during OPC differentiation and myelination, and in vivo,in mice fed with the copper chelator cuprizone,a model of myelin damage and repair. We found that 100µM D-Aspartate exposure accelerated developmental myelination in cerebellar organotypic slices and stimulated progenitor differentiation into myelin-producing oligodendrocytes. Behavioural testing,confocal and electron microscopy analyses demonstrated that oral administration of 20mMD-Aspartate solution during in vivo remyelination improved motor coordination,accelerated myelin recovery,and significantly increased the number of small-diameter myelinated axons. Chronically administered during demyelination, D-Aspartate also attenuated myelin loss and inflammation.Functional studies demonstrated that D-Aspartate boosting effects on OPC differentiation involved an orchestrated stimulation of calcium signaling pathways that are consequent to a cooperative activation of glutamate transporters,AMPA and NMDA receptors and NCX3 exchanger. In fact, while blocking NMDA or NCX3 significantly prevented D-Aspartate-induced [Ca2+]i oscillations, blocking AMPA receptors and glutamate transporters prevented both the initial and oscillatory [Ca2+]i response as well as D-Aspartate-induced inward currents in OPC. Our findings suggest that exogenous D-Aspartate treatment might produce beneficial effects during demyelination and remyelination processes.

NEUROPHYSIOLOGY & NEURAL PLASTICITY 29th SEPTEMBER • 11:00

Effect of maternal butyrate supplement on autistic-like behavior and synaptic plasticity deficits in mice offspring

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Several studies have demonstrated a relationship between alteration of maternal gut microbiota and increased risk of neurodevelopmental disorders in offspring, including autism spectrum disorders (ASD). Among the microbiota-derived metabolites, butyrate (BUT) is a short-chain fatty acid (SCFA) produced in the colon by bacterial fermentation of dietary fibers that in addition to its local effects, has neuroactive properties influencing neurological and behavioral processes. Indeed, BUT attenuates social deficits in an ASD mouse model and its levels are low in ASD subjects. However, the idea of compensating such metabolic dysfunction at a very early stage of disease via maternal treatment has not been enough explored and much less is known about the cellular mechanisms on the brain physiology and behavior in ASD.
For our study we used an inbred BTBR T+Itpr3tf/J (BTBR) mouse strain and we treated dams with BUT from mating to weaning. We analyse behavioral and synaptic plasticity deficits in the offspring during juvenile and adult life, focusing on the cerebellum.
Our results show that BUT treatment of BTBR dams prevents the social deficit and partly reduces the repetitive behavior in the offspring and prevents the hypertrophy of the cerebellar molecular and granular layers in the BTBR offspring compared to untreated mice. This effect was accompanied by a rescue of Purkinje cells (PC) firing and long-term synaptic plasticity deficits involving the parallel fiber-PC synapse.
In conclusion, our results show for the first time how the early treatment with a gut microbiota metabolite, such as BUT, prevents the development of ASD in mice offspring, support the hypothesis that the gut-brain axis plays an important role in the pathogenesis of ASD.

NEUROPHYSIOLOGY & NEURAL PLASTICITY 29th SEPTEMBER • 11:15

Microglia contribution to neuronal network remodeling after paralysis onset

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Microglia are major mediators of experience-dependent synapse plasticity. Through this process, they contribute to neuronal network remodeling following sensory deprivation. Such neuronal network reorganization largely occurs in the brain after paralysis, both from the disability itself and from the physiotherapy that often follows. To improve existing therapies, it is crucial to understand properly how the brain rearranges itself during these events. However, little is known about the cellular and molecular events that underlie this process. Microglia contribution, especially, remains unknown. Here, we are investigating this question using a mouse model of unilateral hind limb paralysis. A single injection of botulinum toxin A was used to induce chemical denervation of the right calf muscles, therefore triggering a transient painless paralysis. Motor functions were evaluated through rotarod and tail suspension tests, revealing impairment as early as 1 day post-injection (dpi) and persisting at 3 and 7 dpi. After ensuring paralysis, we sought to identify brain areas affected by the motor deprivation. We used the immediate early gene cFOS as a proxy for neuronal activation and identified a decrease in the somatomotor cortex associated with a smaller microglia density. Additional analysis of synapses is currently ongoing. Finally, physiotherapy was mimicked by training animals on the rotarod every 2 days. Physical training significantly improved the motor performance of paralyzed mice, with functional recovery observed by 7dpi. However, when being fed with a PLX3397-containing diet inducing microglia-depletion, paralyzed mice took much longer to compensate for their poor performance, reaching control levels only by 11 dpi. Further investigation of the cellular and molecular mechanisms are currently ongoing but this evidence already points to a crucial role for microglia in developing coping strategies following paralysis onset and therefore facilitating recovery.

NEURO-ONCOLOGY 29th SEPTEMBER • 14:30

Blocking the Hedgehog-dependent tumor growth by a new selective Endoplasmic Reticulum Aminopeptidase 1 inhibitor

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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, such as medulloblastoma (MB), the most common brain malignancy in childhood wich shows a high drug-resistance to current therapies. Therefore, is extremely overriding to understand the molecular mechanisms that regulate Hh pathway and to develop new therapeutic strategies acting through the Hh pathway regulation.

Recently, we identified Endoplasmic Reticulum Aminopeptidase 1 (ERAP1), a key player of the immune response, as a new positive regulator of the Hh pathway and essential in promoting stability of GLI1, the final effector of the pathway. Hence, ERAP1 stands as promising therapeutic target for Hh-driven tumors. 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 ERAP1 catalytic domain. The specificity and efficacy of selected molecules have been evaluated by an antigen presentation assay in HeLA. Kb and among them, we identify compound N1, an alkaloid, as a potent and non-toxic inhibitor of ERAP1. We demonstrate that this compound, blocking ERAP1 activity, significantly reduces stability of GLI1, thus counteracting Hh signaling. Further, we show that N1 impairs self-renewal ability and clonogenicity of tumor-derived MB stem-like cells and suppresses MB growth in vitro and in vivo. Our finding strongly indicates N1 as a good candidate for further preclinical studies in the treatment of Hh-dependent tumors.

NEURO-ONCOLOGY 29th SEPTEMBER • 14:45

Mechanoreception in glioma: an insight into the role of Piezo1 in GBM progression and cancer stem cells

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Glioblastoma multiforme (GBM) is the most aggressive brain tumour in adults, affecting 2-3 per 100.000 adults/year. Current treatments include surgery, radiotherapy and chemotherapy; however, prognosis is not optimistic, with survival rate of 14-18months and only 10% of patients living up to 5years after diagnosis. This is partly due to the existence of glioma 'cancer stem cells' (CSC), a subtype of tumour-initiating cells with stem cell-like properties and resistant to conventional treatments, being the cause of most relapses. Historically, researchers have focused on the biochemical and genetic aspects of cancer. But in the last decade, the importance of mechanoreception has been evident. It is known that the microenvironment of tumours is very stiff, and that tumour cells overexpress mechanoreceptor proteins able to respond to these changes. Amongst them we can find Piezo1, a calcium channel described in 2010 that is implicated in multiple cellular processes such as migration or apoptosis, and seems to be altered in various cancers. In this work, we explore the role of Piezo1 in GBM, as we hypothesize that Piezo1 contributes to the progression and malignancy of GBM by formation of CSC. Here, we have observed that chemical activation of Piezo1 in IPSC led to upregulation of stem marker Oct3/4. Likewise, in colony formation assay using human stem cells grown in methylcellulose, we observed that activation of Piezo1 prevented differentiation, while inhibition had the opposite effect, suggesting that Piezo1 has an effect on stem-like phenotype maintenance. Furthermore, we have modified U251 cell line to have Piezo1-KO and Piezo1-overexpressing cells (via CRISPR-Cas9 and CRISPR-SAM, respectively) and analysed their viability, colony formation capacity, migration and cell cycle arrest. Finally, we are developing a Tg.Piezo1/GFAP-cre mouse model to explore the effects of Piezo1 overexpression in glial cells in vivo in terms of neuroinflammation and tumour development.

NEURO-ONCOLOGY 29th SEPTEMBER • 15:15

Histone-deacetylase 8 drives the immune response and the growth of glioma

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Many epigenetic modifications occur in glioma, in particular the histone-deacetylase class proteins play a pivotal role in glioma development, driving the proliferation rate and the invasiveness of tumor cells, and modulating the tumor microenvironment. In this study, we evaluated the role of the histone deacetylase HDAC8 in the regulation of the immune response in glioma and tumor growth. We found that inhibition of HDAC8 by the specific inhibitor PCI-34051 reduces tumor volume in glioma mouse models. We reported that HDAC8 modulates the viability and the migration of human and murine glioma cells. Interestingly, HDAC8 inhibition increases the acetylation of alpha-tubulin, suggesting this epigenetic modification controls glioma migration. Furthermore, we identify HDAC8 as a key molecule that supports a poorly immunogenic tumor microenvironment, modulating microglial phenotype and regulating the gene transcription of NKG2D ligands that trigger the Natural Killer cell-mediated cytotoxicity of tumor cells. Altogether, these results identify HDAC8 as a key actor in glioma growth and tumor microenvironment, and pave the way to a better knowledge of the molecular mechanisms of immune escape in glioma.

NEURO-ONCOLOGY 29th SEPTEMBER • 15:30

Design of implantable hydrogel for glioblastoma treatment

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Glioblastoma multiforme (GBM) is the most common and aggressive tumor of the central nervous system and with a very high risk of recurrence. The current standard care involves resection of the primary tumor mass, followed by radiation and chemotherapy. In the last years, many efforts were made to improve the delivery of anti-glioma drugs to the brain, particularly to cross the blood-brain barrier (BBB). Trying to overcome this issue, we are developing a protein-based hydrogel with the following characteristics: biodegradability, biocompatibility, possibility of direct implantation in the brain cavity/tumour and ability to control the release of anti-tumor drugs, free or embedded in nanoparticles. We have prepared solutions at 18-20% (w/v) of soy isolated protein (SPI) dissolved in phosphate buffer useful to generate a stable hydrogel without the need of external crosslinking agents. Characterizing them, we found that hydrogel gain weight because of swelling (+20% of weight/h) and that they undergo to hydrolytic degradation (-25% of SPI in 72 hours). To evaluate the capacity of SPI-based hydrogel to entrap and release drug-loaded carriers, liposomes have been used as a model. The results showed that after 72 hours the 18% SPI hydrogel released the 48% of entrapped liposomes, while the 20% SPI hydrogel the 56%. Additionally, drug-loaded liposomes released by hydrogels retain their ability to affect the cell viability of a GBM in vitro cellular model, assessed by MTT assay. These preliminary results support SPI hydrogels as a valuable candidate to be tested in a more complex biological system.

NEUROIMAGING 29th SEPTEMBER • 17:00

Investigating the feasibility of assessing magnetization transfer properties of distinct white-matter connections

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Magnetization Transfer Ratio (MTR) maps can be associated to the myelin content of the tissue: the higher the MTR the higher the myelin content. However, in white matter regions where multiple fiber population (i.e. bundles) can cross the same voxel, the MTR value is voxel specific rather than bundle specific. We propose a method that allows for the assessment of bundle-specific MTR by combining a co-encoded diffusion and MT weighted sequence with Convex Optimization Modeling for Microstructure Informed Tractography (COMMIT), a framework that allows estimation of bundle-specific tissue properties. Four healthy subjects (HS) were imaged with a T1w sequence and a novel MT-prepared diffusion-weighted (DW) sequence (MTon). An identical DW sequence, without MT-preparation, was also acquired (MToff). T1 images were segmented in 85 grey matter regions with FreeSurfer and registered to the DW data. A probabilistic tractogram was reconstructed from MToff data and then the COMMIT model has been fitted to Mtoff and Mton data separately. Two connectomes, for the Mtoff and Mton data, have been calculated by grouping streamlines connecting the same region pair. An MTR weighted connectome has then been calculated with element-wise operation on the two connectomes (MTR=(MToff–MTon)/ MToff) thus allowing to calculate a bundle specific MTR value. The proposed method was compared to tractometry which, for each streamline in a specific bundle, averages the MTR values along the streamlines path. In all the four HS, in some representative bundles that belong to the left motor network, the MTR values estimated with COMMIT are higher for the bundles connecting the left precentral gyri (L-PrCG) with the medulla (which is a heavily myelinated bundle), than those that connect the L-PrCG with the left subcortical nuclei. In contrast, the tractometry approach appears flat. By applying COMMIT to an innovative dual-encoded MT-dMRI weighted sequence it is possible to measure bundle-specific MTR.

NEUROIMAGING 29th SEPTEMBER • 17:45

Differentiating MS lesions with or without paramagnetic rim with advanced MRI

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Introduction: In MS, paramagnetic rim lesions (PRLs) are thought to reflect chronic active inflammation mediated by microglia which may lead to a progressive neuronal damage and peripheral iron accumulation. PRLs microstructural characterization by advanced MRI techniques may help to clarify their role in MS pathophysiology. Objectives: To investigate if there are differences in microstructure between PRLs and no-PRL MS lesions detectable via diffusion MRI and/or quantitative susceptibility mapping (QSM). Methods:78 RRMS patients were prospectively enrolled. WM lesions were stratified as PRLs and no-PRLs by visual inspection on GRE-phase images and QSM maps. Both PRLs and no-PRLs were further subdivided in two groups: FLAIR hyperintense/T1 isointense (isoT1) and FLAIR hyperintense / T1 hypointense (hypoT1) lesions. Within the lesions groups, differences in microstructure were studied with diffusion tensor imaging (DTI) and neurite orientation dispersion and density imaging (NODDI) while intensity of paramagnetic signal was extrapolated from QSM. All measures were compared with Kruskal-Wallis and then Mann-Whitney Test accounting for age, sex, and lesion volume. Results: out of 2819 lesions identified, 125 (4.4%) were PRLs. While all PRLs resulted hypoT1, 432 (15.3%) no-PRLs were isoT1 and 2262 (80.2%) were hypoT1. All DTI and NODDI parameters except for NODDI-isotropic volume fraction were significantly different between PRLs and isoT1 no-PRLs, and between no-PRLs hypoT1 and isoT1 ($p<0.001$ for all parameters). Statistically significant lower FA (p:0.005) and higher MD (p:0.034) and RD (p:0.005) together with higher ICVF (p:0.013) were found in PRLs compared to hypoT1 no-PRLs. Paramagnetic signal was significantly higher in PRLs than in both hypoT1 and isoT1 no-PRLs (p: <0.001 for both groups). Conclusions: Microstructural analysis with DTI and NODDI and paramagnetic signal quantification with QSM were able to distinguish PRLs from no-PRLs. Particularly, PRLs showed higher degree of axonal damage and increased paramagnetic signal in comparison not only with isoT1 but also with hypoT1-noPRL. Therefore, PRLs seemed to show a more destructive behaviour which may contribute to explain their association with disability accrual in MS.

NEUROIMAGING 29th SEPTEMBER • 18:00

Development of a Frontotemporal dementia computer-aided diagnostic tool using a Dense Convolutional Neural Network on 3D brain scans and explainable artificial intelligence methods

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Despite Artificial Intelligence (AI) being a leading technology in biomedical research, real-life implementation of AI-based Computer-Aided Diagnosis (CAD) tools into the clinical setting is still facing obstacles. In particular, CAD tools lack standardization practices, leading to poorly reproducible results. This heterogeneity in development is frequently associated with unexplainable results, as Deep Learning (DL) is often considered a “black box” AI technology. Here we present the development of an easily reproducible and fully explainable CAD tool using the Clinica and MONAI frameworks and the Explainable AI methods (XAI). In particular, a Deep Learning (DL) convolutional neural network was trained to detect Frontotemporal Dementia (FTD) on 3D neuroimages from the NIFD database to ensure reproducibility. The DL pipeline includes the preprocessing and the augmenting steps of the 3D images, as well as hold-out Cross-Validation. The DL Convolutional Neural Network (CNN) achieved a performance comparable to other FTD classification approaches, yielding .80 accuracy (95% confidence intervals: .64, .91), 1 sensitivity, .6 specificity, an F1-score of .83 and an AUC of .86 while maintaining full replicability. XAI methods were applied to understand AI diagnostic behavior and to identify regions of the images where the CNN misbehaves. Specifically, Attention maps highlighted that the CNN decision was driven by hallmarking brain areas for FTD and helped us to understand how to improve FTD detection. AI-based CAD tools should be developed with the goal of standardizing pipelines, as varying pre-processing and training methods, along with the absence of model behavior explanations, negatively impact regulators’ attitude towards CAD. The adoption of common best practices for neuroimaging data analysis is a step toward fast evaluation of efficacy and safety of CAD, and may accelerate the adoption of AI products in the healthcare system.

NEURODEGENERATION 30th SEPTEMBER • 9:30

Optimization of AAV9 gene therapy for Spinal Muscular Atrophy with Respiratory Distress type 1 using in vivo disease model

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Spinal muscular atrophy with respiratory distress type 1 (SMARD1) is a rare autosomal recessive motoneuron disease with infantile onset. It is caused by mutations in the immunoglobulin mu-binding protein 2 (IGHMBP2) gene, which lead to a deficient amount of the encoded protein. The main clinical symptoms are distal muscular atrophy and diaphragmatic palsy. In this work, we compared the efficiency of two AAV9-IGHMBP2 vectors, different for promoters, by administering them intracerebroventricularly in presymptomatic SMARD1 mice model at postnatal day 1 (p1); the selected best construct was then tested in already symptomatic mice at p7 by systemic subcutaneous injection, to define the therapeutic window and the best route of administration. Expression analysis at p20 on mice treated during the pre- and symptomatic phase of the disease demonstrated a significant increase in the IGHMBP2 protein expression level and resulted in an extended survival time, higher body weight, and improvement in motor behaviours. In particular, p1 treated mice showed an increased innervation of the neuromuscular junctions, recovery of muscles fibers' diameter, and an increased number of motoneurons in the spinal cords associated with reduced gliosis. To support the translatability of the therapy, we confirmed the lack of a significant alteration of the toxicity biomarkers after the treatments, demonstrating thus the efficacy of gene therapy for SMARD1 in vivo model with a lack of relevant toxic effects. In addition, the preliminary results of the same analysis performed on delayed mice cohort, treated systemically with the selected best construct, showed a similar outcome. The results obtained so far have contributed to paving the way for the first Phase I/IIa gene therapy clinical study for SMARD1 started in December 2021 at Nationwide Children's Hospital, Columbus, Ohio, in parallel, defining the therapeutic window and the choicest administration route to optimize gene therapy strategy.

NEURODEGENERATION 30th SEPTEMBER • 9:45

Combined RNA interference and gene replacement therapy targeting MFN2 for the treatment of Charcot-Marie-Tooth type 2A

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Introduction: Mitofusin-2 (MFN2) is an outer mitochondrial membrane protein essential for mitochondrial networking in most cells. Autosomal dominant mutations in the MFN2 gene cause Charcot-Marie-Tooth type 2A disease (CMT2A), a severe and disabling sensory-motor neuropathy that impacts the entire nervous system. Here we propose a novel potential therapeutic approach combining RNA interference (RNAi) and gene therapy, whereby mutant and wild-type MFN2 mRNA are inhibited by RNA interference (RNAi), while the wild-type protein is restored by overexpressing cDNA encoding functional MFN2 modified to be resistant to RNAi.
Methods: After obtaining induced pluripotent stem cells (iPSCs) from somatic cells of CMT2A patients, we targeted the MFN2 mutant allele with specific short hairpin RNAs (shRNAs) and simultaneously introduced a mutagenized MFN2 gene resistant to shRNA activity and encoding the native protein. We then differentiated iPSCs into spinal motor neurons (MNs) and analyzed the sub-cellular parameters previously found to be altered in CMT2A in vitro model to assess the impact of our therapy. We then evaluated this strategy in vivo in the MitoCharc1 CMT2A transgenic mouse model after cerebrospinal fluid (CSF) delivery of the constructs into newborn mice using adeno-associated virus 9 (AAV9).
Results: This approach significantly rescues the CMT2A MN phenotype in vitro, stabilizing the altered axonal mitochondrial distribution and correcting abnormal mitophagic processes. This strategy also allows proper MFN2 molecular correction in CMT2A MitoCharc1 mice. Conclusions: Overall, our results led to a significant level of rescue of disease phenotype in CMT2A MNs, suggesting that RNAi/gene therapy combined approach might represent a promising therapeutic strategy for the broad spectrum of human diseases associated with MFN2 mutations.

NEURODEGENERATION 30th SEPTEMBER • 10:00

Exploiting three-dimensional in vitro models to identify early neuronal vulnerability and test therapeutic strategies in amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder affecting motor neurons. Development of ALS therapeutics is hampered by incomplete knowledge of pathogenic mechanisms and lack of reliable disease models. C9ORF72, whose hexanucleotide repeat expansion (HRE) represents the main genetic cause of ALS, has been postulated to play a role in neurodevelopment. To investigate whether early developmental vulnerability in ALS could result in late onset neurodegeneration, we will exploit 3D patient-specific in vitro models of central nervous system (CNS).

We generated induced Pluripotent Stem Cell (iPSC)-derived brain (BrOs) and spinal cord (ScOs) organoids of C9ORF72-ALS patients and isogenic controls, using a free-floating, 3D-culture method, based on aggregation in embryo bodies, embedment in matrigel, and agitation in spinning bioreactor. Organoids were characterized by immunohistochemistry, Western-blot, and transcriptomics analysis. Further, to assess the presence of a neural activity, we performed calcium imaging. Finally, we treated BrOs with an antisense oligonucleotide targeting C9ORF72-HRE.

BrOs and ScOs expressed pluripotency markers and mature neuronal markers in early and late stages, respectively. ALS organoids presented higher rate of cell death and a lower degree of maturity compared to isogenic controls. C9ORF72-ALS organoids recapitulated disease hallmarks, like TDP-43 cytoplasmic mislocalization, and displayed a disruption of key cellular processes like DNA damage response and axonal elongation. Functional studies showed an increased calcium influx in C9ORF72-ALS BrOs and an increased susceptibility to glutamate stimulation in C9-ALS ScOs, compared to isogenic controls, suggesting neuronal overexcitability in ALS.

Patient-specific iPSC-derived 3D CNS models reproduce at different time points the maturation of neural and glial cells, resembling physiologic human neurodevelopment. BrOs and ScOs are valuable tools for disease modeling since they improve the characterization of C9ORF72-ALS pathology, dissecting specific disease hallmarks, and provide the opportunity to test therapeutic strategies.

NEURODEGENERATION 30th SEPTEMBER • 11:00

New insights into the effects of SARS-CoV-2 infection on nervous system: alteration of dopamine metabolism in iPSCs-derived dopaminergic neurons

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Increasing evidence related to the onset of neurological symptoms is emerging from a high proportion of patients affected by COVID-19 pathology, suggesting the possible neuroinvasiveness of SARS-CoV-2. Recent studies show that an increasing number of patients, even with mild COVID-19, experiences symptoms even weeks or months after the infection. These symptoms comprise a wide range of neurological conditions such as memory and cognitive dysfunction, brain fog, headaches, insomnia, balance and speech issues, anxiety, and depression.
These premises suggest that SARS-CoV-2 infection is not restricted to the respiratory system but reaches also the central nervous system. Particularly, in light of the COVID-19-related symptomatology, it has been hypothesized that SARS-CoV-2 might affect dopaminergic neurons. However, no scientific evidence has been produced so far.
To investigate this aspect, human iPSCs were differentiated into dopaminergic neurons and infected with three different SARS-CoV-2 variants (EU, Delta and Omicron). The infection with EU and Delta variants, but not with Omicron, was responsible for a reduced intracellular content and extracellular release of dopamine. Moreover, neurons infected with EU and Delta SARS-CoV-2 were characterized by a reduced protein levels of Tyrosine hydroxylase together with a reduced mRNA expression of DOPA-decarboxylase and dopamine transporter, and an increase in VMAT2 transporter. In addition, the infected neurons displayed the onset of neurodegeneration, demonstrated by the reduction in MAP2 and TAU content. Finally, we found an intense activation of antiviral intracellular innate response and an increase in neuronal stress markers.
Taken together these preliminary observations let us to speculate that neurons are affected by SARS-CoV-2 infection, with particular consequences on the dopamine production and metabolism, explaining some of the neurological symptoms developed upon SARS-CoV-2 infection.

NEURODEGENERATION 30th SEPTEMBER • 11:15

Aberrant Protein Palmitoylation: a novel therapeutic target in Alzheimer's disease

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Metabolic alterations may play a critical role in Alzheimer's disease (AD) pathogenesis and progression. Our previous findings highlighted how brain insulin resistance (BIR) leads to memory impairment by impinging on protein palmitoylation, a posttranslational modification regulating neuronal protein localization and synaptic function. To begin, we analyzed 3xTg-AD mice brains and found high levels of AKT, GSK3β and IRS-1 proteins phosphorylation, which is recognized as a molecular hallmark of BIR. Then, we analyzed the levels of palmitoylation of different proteins involved in synaptic function and plasticity in the hippocampus of 9-month-old 3xTg-AD mice through an acyl-biotin exchange assay. We found hyper-palmitoylation of several proteins compared to wild-type controls. After, we tested the effect of chronic intranasal administration of the palmitoylation inhibitor 2-bromopalmitate on both male and female 3xTg-AD mice by performing behavioral (novel object recognition and object displacement tests), electrophysiological (long-term potentiation, LTP), and molecular analyses (ELISA, immunofluorescence, western blot). 2-bromopalmitate delayed the onset of memory deficits and significantly enhanced cognitive performances in 6-, 9- and 12-month-old mice. Accordingly, electrophysiological analyses on hippocampal brain slices from 2-bromopalmitate-treated 3xTg-AD mice revealed greater LTP at CA3-CA1 synapses (LTP slope: 137.55 ± 10.82% vs 225.7 ± 9.71%). In addition, 2-bromopalmitate reduced Aβ deposition in the hippocampus of both males and females (-45%/-60%, respectively). Taken together, our data suggest that aberrant palmitoylation plays a critical role in the onset and progression of AD. This study also represents the first preclinical study on the effects of 2-bromopalmitate on AD-related cognitive decline.

NEURODEGENERATION 30th SEPTEMBER • 11:30

Role of SHIP1 as a modulator of microglial function

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Microglia, the innate immune cells of the central nervous system, play crucial roles in brain development, plasticity and repair. GWAS studies reveal that hundreds of genetic variants associated with neurodegeneration are found in genes expressed in microglia. However, the exact function of these genes, and their roles during brain development, is poorly studied. Here, we investigated how the SH-2 containing inositol 5' polyphosphatase 1 (SHIP1) influences key microglial properties. SHIP1, encoded by Inpp5d, is responsible for the dephosphorylation of phosphatidylinositol (3,4,5)-trisphosphate (PIP3) to PI(3,4)P2, involved in actin remodeling and phagocytosis. It is predominantly expressed by microglia, and it is upregulated in the proximity of Alzheimer's plaques. Protein assessment in the wild-type mouse brain revealed high expression at postnatal day 7 (P7) and progressive decrease with aging, supporting a role for SHIP1 in the early postnatal brain. Thus, we induced microglial specific conditional KO (cKO) at P3-P4, to examine consequent microglial dysfunction and potential effects on brain development. Combining confocal microscopy and 3D reconstruction, we found that microglia lacking SHIP1 are smaller in size and less complex than controls. Proteomic analysis revealed a significant increase in C1q, a well-known eat-me signal, in the hippocampus of cKO mice, which we found to be associated with decreased post-synaptic markers, PSD95 and Gephyrin. cKO mice also displayed reduced levels of myelin basic protein (MBP) and decreased number of Olig2+ cells, indicating alterations in myelination. Our in vitro data support an aberrant phagocytic phenotype of SHIP1 KO microglia, which engulfed and degraded higher amount of amyloid beta and synaptosomal cargoes. Overall, this study shows that microglial SHIP1 is required for proper brain development, suggesting that risk variants in this gene might contribute to neurodegeneration by providing early susceptibility.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 14:30

A humanized model of blood brain barrier to investigate immune cells infiltration in Multiple Sclerosis: toward a personalized medicine approach

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Multiple Sclerosis (MS) is an inflammatory demyelinating disorder of the central nervous system (CNS) with autoimmune origin, characterized by the infiltration into the brain of autoreactive immune cells coming from the periphery. Leukocytes migrate across the blood-brain barrier (BBB) which is composed of highly specialized cerebral endothelial cells surrounded by pericytes and astrocytic endfeet, forming the neurovascular unit (NVU). We generated a human NVU in vitro model by co-culturing, on opposite sides of a matrix-coated permeable membrane, human astrocytes and endothelial cells. Specifically, as a source of primary endothelial cells, circulating endothelial colony forming cells (ECFCs) were isolated from the peripheral blood of MS patients and healthy subjects, while primary human astrocytes were purified from the peripheral area of surgical samples of patients undergoing cerebral tumor resection. By comparing, in vitro, the frequency of appearance of colonies of ECFCs in healthy controls, naïve and treated patients we observed a significant increase in the number of colonies in naïve patients compared to both controls and subjects under pharmacological treatment. To characterize ECFCs' phenotype a multicolor flow cytometry panel was set up, confirming that they lack the expression of the lineage markers CD45 and CD14, while they express endothelial epitopes. Human CD3+ T cells transmigration across different in vitro prototypes of BBB evidenced that astrocytes presence during the transmigration assay can influence the polarization of CD4+ T cells to Th1 and Th17 cells. Moreover, preliminary results highlighted a significantly greater transmigration capacity of T cells isolated from MS patients compared to healthy subjects.
Our personalized NVU model demonstrated to be an interesting tool to perform T cells transmigration studies that will allow us to investigate the T cell signature and the phenotype of cells that are able to cross the BBB in MS patients.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 14:40

The emerging role of microRNAs in experimental and clinical multiple sclerosis: implications for inflammation-driven synaptic dysfunctions and disease course

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MicroRNAs (miRs) are post-transcriptional regulators of gene expression, which have recently come up as pleiotropic determinants in the crosstalk between central nervous system and immune system.

We investigated their role in the course of multiple sclerosis (MS) especially linked to the inflammatory synaptopathy, a crucial hallmark of the disease. Specifically, we screened, by qPCR and Bio-plex system, 24 selected miRs and 27 inflammation-related proteins circulating cerebrospinal fluid (CSF) in a large cohort of MS patients, and we correlated them with clinical, cognitive and transcranial magnetic stimulation parameters assessed at the diagnosis (T0) and after follow-up periods (Tf). Multiple statistical and bioinformatics analyses were also combined with preclinical studies on MOG35-55 EAE model and transgenic mice.

We identified let-7b-5p and miR-142-3p as two main miRs with opposite functions in neuroinflammation and MS prognosis. Let-7b-5p was a potential protective factor for MS course, with anti-inflammatory and neuroprotective properties from the earliest stages of the disease. Moreover, CSF let-7b-5p levels were reduced in progressive MS and negatively correlated with disease severity at T0 and Tf. On the contrary, miR-142-3p emerged as an adverse biomarker of the synaptopathy-driven disease progression and a promising tool for identifying personalized therapies. Indeed, we demonstrated in MS and in EAE that miR-142-3p was an essential effector of interleukin-1beta-induced synaptic alterations and low miR-142-3p levels associated with a more effective response to dimethyl fumarate, an established MS treatment.

Our results lay the basis for an important advance in MS diagnosis and prognosis related to synaptopathy-driven detrimental outcomes, with possible implication in the therapeutic decision-making strategy.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 14:50

Deciphering Multiple Sclerosis endophenotypes through Mendelian disorders: a network-based approach

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Growing evidence indicates that complex diseases constitute phenotypical continuums with monogenic disorders. Cross-matching "simple" diseases, endophenotypes of multifactorial disorders and their risk variants from GWAS could facilitate the understanding of shared physiopathology and relative biomarkers. Furthermore, analysis of functionally related genes and their products' interactome offers a basis to drug targets identification for both conditions (i.e., the Mendelian disease and the phenotypically-matched endophenotype of the complex disease).
Here we apply such principles through reworking the latest GWAS for Multiple Sclerosis (MS), a common dysimmune and neurodegenerative disease of the central nervous system displaying extreme clinical heterogeneity.
We define an MS-Mendelian molecular network, on which MS intermediate phenotypes and their matched monogenic disorders are unraveled by bioinformatic approaches (including a genetic enrichment pipeline, biological pathway analysis and protein-protein interactions).
Starting from the MS genetic architecture, we describe the enrichment and molecular subnetworks of primary disorders of the visual system, neurodegenerative ataxias, axonopathies, metabolic disorders and immune deficiencies.
Network-based drug targeting algorithms were finally employed to prioritize cross-phenotype molecules, such as tyrosine kinase inhibitors, and phenotype-specific drugs, such as promethazine for neurodegenerative processes, bithionol for optic dysfunctions, zinc-based compounds for ataxias.
Our results underscore the existence of shared pathophysiologies between MS and phenotypically-affine rare diseases. The MS-Mendelian network may boost future investigations in endophenotype-specific biomarkers and possible combinatorial therapies. Also, this work support smarter clinical trial designs enrolling subgroups of people with MS and matched Mendelian diseases, searching for effective, quickly actionable and shareable cures.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 15:00

New insight for Riboflavin Transporter Deficiency (RTD) Syndrome: gene therapy as a new therapeutic strategy for RTD patients

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RTD is a rare childhood-onset disorder caused by mutations in SLC52A3 and SLC52A2 genes, encoding the riboflavin (RF) transporters RFT2 and RFT3, respectively. Since RF is a precursor of flavin mononucleotide and flavin adenine dinucleotide, the reduction of its intracellular availability compromises several vital processes. Even if empirical studies reported clinical improvement with administration of large dose of RF, it can't be considered as an effective cure because some patients do not benefit from RF supplementation. The main goal of this project is to explore a new therapeutic approach using gene therapy with adeno-associated viral vector serotype 9 (AAV9) carrying human codon-optimized SLC52A2 cDNA (AAV9-SLC52A2) to rescue the RTD neural phenotype.

Induced pluripotent stem cells (iPSCs) derived from the skin fibroblasts of healthy subjects and RTD patients with mutation in SLC52A2 gene were successfully differentiated into motor neurons (MNs). In order to establish the best experimental conditions to obtain the maximal rate of infection, we pretreated the MNs with several concentrations of sialidase, in combination with multiple multiplicity of infection of the AAV9-SLC52A2 vector. After fixing and staining the MNs for β III-tubulin, we confirmed the successful infection of the MNs by immunofluorescence analyses and we found the best efficiency of infection for the MNs. In addition, we examined the neurites length of infected and uninfected RTD MNs against the normal control MNs and we observed an increase in neurites length. Collectively, our results indicate that AAV9-SLC52A2 vector generates promising rescue in derived MNs from RTD patients, which warrants further in vitro as well as in vivo studies to develop the gene therapy as a potential treatment.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 15:10

Central Effects of Botulinum Toxin Type A in Motor Nervous System of the Rat

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Botulinum toxin type A (BoNT-A) is a potent neurotoxin with anticholinergic effect. It is a standard therapy in various movement disorders, presumably due to action on local neuromuscular terminals. However, observations in clinics and recent experimental data, points to the possible central effects. The aim was to examine the contribution of the transcytosis-dependent central toxin action on the long term muscular function recovery in rats, as well as tetanus neurotoxin (TeNT) evoked spastic paralysis after peripheral application. Rats were bilaterally injected with BoNT-A into the gastrocnemius muscle (2 U/kg) or sciatic nerve (5 U/kg). To stop the toxin central transcytosis, BoNT-A-neutralizing antitoxin was intrathecally (i.t.) administered after 24 hours. After recovery from flaccid paralysis, TeNT was intramuscularly (i.m.) injected to animals on day 62. In different motor tests (gait ability score, digit abduction score, rota-rod, beam walking and swimming performance), i.t. antitoxin significantly accelerated the flaccid paralysis and motor performance recovery. TeNT-evoked increase in muscle tone was reduced by BoNT-A dependently on its central effect. However, the H-reflex, when corrected for reduced muscle size or reduced compound muscle action potential (CMAP), was not affected by the toxin treatment, suggestive of the lack of the toxin's direct effect on monosynaptic reflex. The toxin enzymatic activity examined by cleaved synaptosomal-associated protein 25 (cSNAP-25) immunohistochemistry, was still present in neuromuscular junctions and spinal cord. cSNAP-25, presence in second order spinal cord cholinergic neurons, depended on the toxin's central transcytosis. Conclusion:Long term motor effects of BoNT-A both on normal motor performance (day 1-62), as well as the spastic paralysis (days 62-78), are influenced by the toxin's ongoing central action mediated by retrograde transport and transcytosis. These data suggest that clinically relevant beneficial effect of BoNT-A result from toxin's combined peripheral and central effects.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 15:20

Driving CARs on a highway to cure pediatric CNS malignant tumors

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Pediatric Central Nervous System (CNS) malignant tumors are the most common solid tumors and the leading cause of cancer-related mortality in children. Although current treatments have resulted in prolonged free survival rates (60-70%), in those patients with relapse or metastases the prognosis is very poor. In addition, the effects of surgery, chemo and radiotherapy in the developing brain of children may involve irreversible neurological effects, underlying the urgent need to find more specific and less toxic treatments. In this regard, T cells expressing a Chimeric Antigen Receptor (CAR T) are a promising therapeutic strategy to treat brain tumors. The interaction between NKG2D receptor, expressed in Natural Killer (NK) cells and their ligands (NKG2DL), overexpressed in tumor cells, are essential for NK cell anti-tumor immunosurveillance. Additionally, NK cells can exert Antibody-Dependent-Cell-Cytotoxicity (ADCC) on antibody coated tumor cells through CD16 receptor. However, the use of NK cells in the clinical setting have some limitations derived from their poor in vivo expansion and lack of memory, among others. In an aim to overcome these limitations, we have engineered T cells with three different CAR constructs: NKG2D, CD16 and NKG2D-CD16 and tested their ability to target CNS pediatric tumor cells in vitro, either alone or in combination with Dinutuximab, a therapeutic IgG1 targeting GD2. We found NKG2D CAR T cells were highly cytotoxic against different CNS tumors cell lines in 2D and 3D cultures. CD16 CAR and NKG2D-CD16 CAR T cells showed an antibody dose-dependent anti-tumor activity when combined with Dinutuximab. Importantly, cytotoxicity of NKG2D-CD16 CAR T cells outperformed that exerted by CD16 CAR T cells, suggesting a potential synergistic effect. In conclusion, our preliminary results show that NKG2D, CD16 and NKG2D-CD16 CAR T cells target CNS tumor cells in vitro and could be a novel therapeutic approach to treat these tumors.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 15:30

Cytotoxic activity of small molecule inhibitors on patient-derived glioblastoma cells

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Gliomas are the most common tumors of central nervous system and among them glioblastoma (GBM), the most aggressive, occurs in 49% of the cases.
Somatic mutations in GBM involve proliferation, survival, angiogenesis, and invasion pathways, converging on EGFR, PDGFR, PTEN, RB, TP53, CDKN2A genes.
To personalize the pharmacological treatment of patients undergoing GBM surgery and to accelerate the identification of the molecular pathway/s controlling cancer growth, we isolated primary cell cultures from surgery samples and evaluated the cytotoxic effect of small molecules currently undergoing clinical trials. Regardless of the mutational landscape of the tumor and MGMT status, we utilized temozolomide as the standard chemotherapeutic agent along with 1) selected small molecules from a library of natural molecules and 2) pathway inhibitors currently tested for the therapy of other solid tumors. As a natural small molecule, we selected Sempervirine, that we previously identified as an RNA polymerase I inhibitor blocking germ cell cancer growth. As pathway inhibitors, we identified Ipatasertib, a high affinity AKT inhibitor and Regorafenib, an inhibitor of tyrosine kinase receptors. Human glioblastoma cell lines (LN18, U87MG, A172, T98G) were used in parallel to validate molecule effects and to verify pathway inhibition.
Experiments on GBM cell lines demonstrated that Temozolomide (25mg/ml) and 10mM of Sempervirine, Ipatasertib or Regorafenib differentially inhibited cell proliferation up to 50% after 72h of treatment. Experiments on patient-derived GBM cells demonstrated that Temozolomide (25mg/ml) sensitivity corresponded to the MGMT methylation status and was ineffective in 50% of the samples (MGMT unmethylated). 5 mM of Sempervirine, Ipatasertib and Regorafenib were able to inhibit cell proliferation of to100% after 72h of treatment.
This study attempts to evaluate the efficacy of targeted therapies in vitro to translate their use in vivo.

CLINICAL NEUROSCIENCE 30th SEPTEMBER • 15:40

Human iPSC-based cellular systems to model Autosomal dominant leukodystrophy

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

POSTER SESSION

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EBN01 | Self-Organizing 3D Human Organoids to dissect the role of Choroid Plexus (ChP) in cortical layer patterning during brain development

Vanessa Aragona [1] - Monica Tambalo [2] - Gabriele Di Napoli [2] - Marlene Khin [3] - Simona Lodato [2]

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The development of the human cerebral cortex relies on spatially and temporally coordinated molecular signals, finely tuned to generate an extraordinary diversity of neuronal and non-neuronal subtypes. Since early stages, the developing brain, including the cerebral cortex, is exposed to the cerebrospinal fluid (CSF) that fills the ventricles. The choroid plexus (ChP), the main source of CSF, is a highly organized tissue of specialized ependymal and epithelial cells with an inner core of fenestrated endothelial cells surrounded by connective tissue. ChP structural characteristics explain its role as blood-CSF barrier (B-CSF), and in regulating neurogenesis and brain development. CSF composition, resulting from barrier permeability, contributes to the developing brain homeostasis, and its imbalance has been associated to autism spectrum disorders (ASD) and comorbid neurodevelopmental disorders, especially epilepsy. Currently, due to technical and ethical limitations, the mechanisms of secretion, barrier function and homeostatic regulation are still poorly understood in the developing cortex. We aim to improve physiologically relevant three-dimensional (3D) models to evaluate how the ChP-CSF system changes can affect the maturation of neural circuits during critical periods of brain development. Here, we used a human pluripotent stem cell (hPSC)-based approach to simultaneously generate human ChP and cortical organoids in an integrated system representative of the complex interplay between vascular and neural compartments. Identity establishment of both ChP and cortical tissue can be determined at multiple levels and compared with human fetal brain tissues. The resulting 3D organoid cultures will be used as screening platform to assess the effects from the exposure to different compounds on B-CSF permeability and CSF composition, and to identify, in this human-like brain milieu, the components that trigger maturation into highly functional neuronal circuits.

EBN02 | Glial Fibrillary Acid Protein correlates with the phenotype in adult patients with Tuberous Sclerosis Complex

Valeria Manzini [1] - **Biagio Orlando** [2] - Alessandra Morano [2] - Emanuele Cerulli Irelli [2] - Enrico Michele Salamone [2] - Maria Sole Borioni [2] - Anna Teresa Giallonardo [2] - Elisa Moliterni [3] - Sandra Giustini [4] - Ivan Arisi [5] - Chiara D'Amelio [1] - Mara D'Onofrio [1] - Caterina Veroni [6] - Paola Piscopo [6] - Carlo Di Bonaventura [2]

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Tuberous Sclerosis Complex (TSC) is a rare dominant autosomal, neurocutaneous syndrome related to the hyperactivation of the mammalian Target of Rapamicine (mTOR) pathway, caused by mutation in one of the two genes, TSC1 or TSC2 (encoding for Hamartin and Tuberin). The mTOR pathway contribute to tau dysregulation, a process linked to the neurodegeneration and neuroinflammation and involved in the broad spectrum of clinical TSC phenotype. Among TSC neurological manifestations, epilepsy, intellectual disability and psychiatric/behavioral disorders are of paramount importance. The aim of the study was to investigate correlations between biomarkers of neurodegeneration and neuroinflammation and TSC anatomo-clinical features. We hypothesised that molecular biomarkers reflecting inflammatory and neurodegenerative processes (Neurofibrillary Light Chain (NfL), Glial Fibrillary Acid Protein (GFAP), Aβ40, Aβ42, t-Tau and p-181 tau) could be differentially represented in peripheral blood. Clinical and radiological features have been collected by reviewing clinical charts and brain MRI scans. We investigated plasma samples derived from 31 TSC patients versus 38 healthy controls, using Single Molecule Assay (Simoa™) technique and identified TSC1 and TSC2 mutation carriers and non-mutation identified (NMI). GFAP levels were increased in TSC patients, both in TSC1 and TSC2 mutation carriers. On the contrary, NMI showed a decreasing trend of GFA, which reached the significance when it was compared with TSC2 one. Plasma levels of NfL, Aβ40, Aβ42 and both total and p-181 Tau forms showed no-statistically significant differences. Interestingly, t-Tau levels became significant when we differentially analysed the mutation carriers. Higher levels of GFAP were strongly associated with neurological symptoms and epileptic spasms. Our study documented a significant increase of GFAP levels in TSC adult patients, which appeared to be correlated with the severity of the neurological phenotype.

EBN03 | Somatosensory processing deficits and altered connectivity in Cntnap2 -/- and Shank3b−/− mouse models of autism spectrum disorder

Luigi Balasco [1] - Marco Pagani [2] - Luca Pangrazzi [1] - Gabriele Chelini [1] - Francesca Viscido [1] - Alessandra Ciancone Chama [1] - Evgenia Shlosman [1] - Lorenzo Mattioni [3] - Alberto Galbusera [2] - Giuliano Iurilli [2] - Giovanni Provenzano [3] - Alessandro Gozzi [2] - Yuri Bozzi [1]

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Abnormal tactile response is considered an integral feature of Autism Spectrum Disorders (ASDs), and hypo-responsiveness to tactile stimuli is often associated with the severity of ASDs core symptoms. Mutations in the human CNTNAP2 and SHANK3 genes result in cortical dysplasia-focal epilepsy syndrome (CDFE) and Phelan-McDermid syndrome (PMS) respectively, two syndromic forms of autism. Likewise, Cntnap2-/- and Shank3b-/- mice show deficits relevant to core symptoms of human ASDs. Sensory abnormalities have been described in mice lacking ASD-associated genes. However, the neural underpinnings of these somatosensory abnormalities are still poorly understood. Here we investigated, in Cntnap2-/- and Shank3b-/- mice, the neural substrates of whisker-mediated responses, a key component of rodents' interaction with the surrounding environment. When compared to their controls, both Cntnap2-/- and Shank3b-/- mice displayed impaired whisker-dependent discrimination in the textured novel object recognition test (tNORT). Additionally, Shank3b-/- but not Cntnap2-/- mice showed a marked behavioural hypo-responsiveness to repetitive whisker stimulation in the whisker nuisance (WN) test. Notably, while Cntnap2-/- mice displayed increased c-fos mRNA induction within primary somatosensory cortex (S1) following whisker stimulation, Shank3b-/- mice showed a significantly reduced activation of S1. Moreover, when tested in a resting-state fMRI paradigm, Cntnap2-/- mice showed focal hyper-connectivity within the S1, while reduced S1-hippocampal connectivity was found in Shank3b-/- mice. Together, these findings suggest that impaired neuronal activation and dysfunctional connectivity within S1 might underlie hypo-reactivity to whisker-dependent cues in Cntnap2-/- and Shank3b-/- mice, highlighting a potentially generalizable form of dysfunctional somatosensory processing in ASD.

EBN04 | Prenatal exposure to poly I:C induces tissue-specific expression of several ERVs and related genes, and immune effectors in cortex, hippocampus, and blood samples from C57BL/6 mice

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Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder resulting from complex interactions among genetic, environmental, and epigenetic factors. Human Endogenous retroviruses (HERVs) are relics of ancestral germline infections by exogenous retroviruses, stably integrated into the host cellular DNA, which comprise about 8% of genome in human and over 10% in mice. HERVs deregulation has been associated with many complex human diseases, such as neurological and psychiatric disorders. Our previous study on two preclinical mouse models of ASD showed an altered expression of several ERVs and cytokines, in embryos, blood and brain samples at different post-natal ages, supporting the potential involvement of ERVs and immune response in the pathophysiology of ASD. Aim of this work was to study the effect of prenatal exposure to viral mimetic analogous to a double-stranded RNA (Poly I:C) on the expression of several ERVs families, ERV-related genes, immune effectors, and marker of damage to the central nervous system (CNS), in different tissues of adult mice. C57BL/6J pregnant female mice were treated with a single injection of Poly I:C or saline solution at gestational day 12.5. Behavioural evaluation of the offspring and tissue collection (cortex, hippocampus, and blood samples) were done on post-natal day 60. The analysis of the expression of several ERVs and related genes, proinflammatory and regulatory cytokines, toll-like receptors, and markers of CNS damage by quantitative Real-Time PCR analysis, showed that Poly I:C exposure results in tissue-specific deregulation of ERVs and inflammatory and regulatory cytokines, in parallel to the appearance of an autism-like phenotype in offspring. This supports the hypothesis of an interaction between HERVs activity and altered inflammatory response and their involvement in the biological mechanism underlying ASD.

EBN05 | The effect of a Ketogenic Diet on the host Microbiota, the Immune System and the CNS

Andrina Rutsch [1] - Johan Björn Kantsjö [1] - David Ferreira Francisco [2] - Marco Kreuzer [2] - Pamela Nicholson [3] - Francesca Ronchi [1]

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The intestinal microbiota plays a fundamental role in host protection, metabolism and in the function of host organs, including the central nervous system (CNS). Changes in the microbiota composition have been reported in several neurological disorders. The CNS and the intestine interact through a bidirectional network called gut-brain axis, in which microbial metabolites are importantly involved, as well the immune system and nervous structures like the enteric nervous system and the vagus nerve. The microbiota is highly influenced by the dietary habits of the host. Ketogenic diet (KD) ameliorates conditions in metabolic and neurological diseases, however, the mechanisms behind this are not well known. Our aim is to understand the effects of KD on the function of the gut microbiota and subsequently the CNS under healthy and neurological disease conditions. We performed 16S rRNA sequencing and metatranscriptomics on the intestinal content of mice fed KD or a control diet, colonized with an undefined - (specific-pathogen free, SPF) or with a defined, microbiota (sDMDMm). KD increased the Firmicutes/Bacteroidetes ratio and induced important metabolic changes in Clostridia. To investigate the effect of KD on the immune- and nervous system, we analyzed intestinal- and brain immune cells by flow cytometry and the brain cells by spatial transcriptomics of mice fed KD or control diet in germ-free (GF) or SPF condition. In the brain, several genes were affected upon KD and most of them in a microbiota-dependent way. In general, KD impacts genes involved in neuronal development and regeneration in several brain regions. Additionally, KD induced a decrease in γδT cells in the brain of SPF mice compared to control groups. We are currently investigating if bacterial metabolites are responsible for the effects we observed in a KD-microbiota-dependent way. Overall, we are studying new mechanisms of action of diet and microbiota on immune cells and on brain function.

EBN06 | Gene expression profiling in trigeminal ganglia from Cntnap2 -/- and Shank3b -/- mouse models of autism spectrum disorder

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Tactile sensory deficits are commonly found in individuals diagnosed with autism spectrum disorder (ASD). For this reason, considerable research is currently dedicated to understanding the underlying basis of these disturbances, utilizing ASD animal models such as Shank3b-/- and Cntnap2-/- mice. Notwithstanding the existing body of work, a focus on the whisker system, which constitutes the dominant somatosensory pathway in mice, is lacking. The present study seeks to fill this knowledge gap by characterizing gene expression profiles in the trigeminal ganglia (TG) of Shank3b-/- and Cntnap2-/- mice. TG receive direct innervation from the whiskers, and therefore represent a crucial area for somatosensory input processing. mRNA expression of ASD markers within the TG of Shank3b-/- and Cntnap2-/- adult and juvenile mice relative to age-matched controls was analyzed using qRT-PCR. Results show a differential expression of key molecular markers in the TG, such as markers for inhibitory and excitatory neurotransmission, as well as neuroinflammatory molecules. Both knockout mice lines exhibit a dysregulation in Gad1 and Gfap gene expression throughout development. Therefore, it can be concluded that ASD Shank3b and Cntnap2 mutations influence the somatosensory whisker system resulting in altered gene expression even at the level of first order sensory neurons. Such results suggest early dysregulation in synaptic signaling and neuroinflammation pathways, both of which have been strongly implicated in the neuropathology of ASD. These findings are crucial for promoting the development of novel peripherally targeted treatments for tactile sensory deficits exhibited in such neurodevelopmental disorders.

EBN07 | The role of bdnf in epilepsy: evidence from a pharmacological zebrafish model of disease

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Brain-derived neurotrophic factor (BDNF) is a key molecule in neurons survival, growth and differentiation during the development of the central nervous system (CNS), and recent studies have also documented its ability to modify CNS structure and function in adulthood. It is thus not surprising that BDNF has been linked to several neurological diseases, including epilepsy, and it has been reported that seizure activity increases BDNF expression and protein and that modulation of BDNF signal transduction inhibits the development of the epileptic state (Binder et al., 2001 Trends Neurosci. 2001 Jan;24(1):47-53). However, the exact role of BDNF in the etiology of epilepsy disease still need to be clarified. In order to acquire such information and comply with 3Rs principles on animal experimentation, we here report new data using zebrafish at early-life stages, before independent feeding, as a valuable animal model to monitor seizure-like behaviors as well as molecular mechanisms associated to altered phenotypic outcomes. Zebrafish larvae have been exposed to pentylenetetrazole (PTZ) developing changes in locomotor behavior within a few minutes after the treatment (approximatively after 5-10 minutes), thus complying with an epileptic like-behavior. Moreover, zebrafish larvae exposed to PTZ had an increase of approximately 5-fold in bdnf gene expression compared to the negative controls and, preliminary data, also showed a consistent decreased trend in DNA methylation at gene promoter. Our results confirmed the effectiveness of the PTZ zebrafish model of epilepsy and provide new evidence on the role of bdnf gene regulation in epileptogenesis.

NI01 | Novel synthetic thyroid hormone receptor beta ligands to regulate oligodendrocyte precursor cells differentiation in demyelinating diseases

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Oligodendrocyte precursor cells (OPCs) are the cells responsible for the myelin formation and repair in the central nervous system (CNS). Among molecular regulators of the OPC in situ maturation toward myelinating oligodendrocytes (OLs), thyroid hormones (THs) play a key role as ligands of TH receptors (TRα and TRβ). In particular, TH is responsible for OPC cell cycle exit and regulation of gene expression related to mature OLs. Thus, pharmacological research is looking for novel TR ligands to avoid TH related systemic side effects, that seems to be mainly mediated by TRα. The aim of this study is to investigate the effect of two novels synthetic TRβ agonists, TG68 and IS25, on OPC cultures. We used the neural stem cell derived OPCs, in which we already demonstrated the role of natural TR ligand to induce the physiological differentiation process. Cultures were exposed to different concentrations of the new synthetic ligands (1, 10, 50 µM), compared to TH, and TRβ ligand reference GC1, to evaluate the percentage of OPCs (NG2-IR) pre-myelinating (CNPase-IR) and myelinating (MBP-IR) OLs as analyzed by cell-based High Content Screening. Among the two tested molecules, TG68 resulted efficient to induce differentiation as the natural ligand. Since the OPC differentiation block due to inflammatory cytokines is recognized as the main cause of the remyelination failure in Multiple Sclerosis (MS) and other neurological conditions characterized by severe inflammation, we tested the ability of TG68 to overcome this differentiation impairment. We exposed the OPCs to a cytokine mix (TGFβ1, TNFα, IL1β, IL6, IL17 and IFNg) identified in the experimental autoimmune encephalomyelitis, the animal model of MS. We proved that the exposure to TG68 restores the differentiation capability of OPCs. The design of novel TRβ agonists will provide new therapeutic strategies to overcome the inflammation induced remyelination failure in MS and other CNS diseases that are still uncurable.

NI02 | Effects of MAGL inhibitor on striatal neuroinflammation and synaptic dysfunction in experimental multiple sclerosis

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Multiple Sclerosis (MS) is an inflammatory neurodegenerative disorder in which the neuronal compartment is affected since the early stages of the disease. Data from MS patients and the MS mouse model, experimental autoimmune encephalomyelitis (EAE), have underscored a harmful but potentially reversible inflammatory synaptopathy in several brain area and a significant alteration of the endocannabinoid system (ECS). Studies from the EAE model have shed a light on the biological effects of endocannabinoids (eCBs) -anandamide (AEA) and 2-arachidonoylglycerol (2AG)- and their receptors (CB1R, CB2R and TRPV). Of note, recent evidence showed that the inhibition of monoacylglycerol lipase (MAGL), the key hydrolytic enzyme responsible for 2-AG inactivation, can exert a beneficial effect on EAE disease, but the mechanism is still unclear. Here, we took advantage of a reversible MAGL inhibitor (MAGLi) to investigate for the first time its effects on motor disability, neuroinflammation and synaptopathy in EAE mice. Our data clearly indicate beneficial effects of MAGLi treatment in both ex vivo and in vivo conditions in EAE mice. We observed that MAGLi treatment is able reduce the clinical disability of the mice. Electrophysiological recordings revealed a recovery of the spontaneous glutamatergic current frequency in the striatum of EAE mice in association with an effective enzymatic MAGL inhibition and increased 2AG levels. Moreover, we observed by immunofluorescence analysis a significant reduction of striatal microgliosis. In particular, we characterized the effects of MAGLi on microglia activation phenotype by a detailed morphological study, using the Sholl and Skeleton analysis. Overall, we demonstrated that an up-regulation of the endocannabinoid tone induced by MAGL inhibition is potentially involved in the recovery of both inflammatory status and glutamatergic alterations mediated by CB1 receptor occupancy in EAE mice.

NI03 | Approaching behavior and its synaptic and transcriptomic signatures in medial prefrontal cortex pyramidal neurons: the involvement of excitatory neurotransmission and immune system

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Approaching (AP) and avoiding (AV) tendencies are basic behavioral aptitudes in responding to rewarding and aversive stimuli, and their balancing (BA) tendency is critical for successful adaptation to the environment. The AP tendency is associated to novelty seeking and it has important evolutionary value in the identification of new sources of reward. However, the AP tendency exposes individuals to potential risks, increasing the predisposition to externalizing behaviors, such as attention deficit and hyperactivity disorder, addiction, and eating disorders. In this framework, even if the medial prefrontal cortex (mPFC) is a crucial hub that supports AP behavior by sustaining attention towards relevant and novel stimuli, its specific synaptic and transcriptomic signatures have not yet been identified. In this research, we used an experimental model of individual differences permitting the selection of a subpopulation of mice that spontaneously responded with AP or BA behaviors toward conflicting emotional stimuli, and expressed yellow fluorescent protein (YFP) in pyramidal neurons of mPFC. Patch-clamp electrophysiological recordings showed that mPFC pyramidal neurons of AP mice had a significantly higher frequency of spontaneous excitatory post-synaptic currents when compared with BA mice. YFP-expressing pyramidal neurons from mPFC of AP and BA mice have been sorted to purify cell-specific RNA for a transcriptome-wide analyses. The omic results showed differential gene expression between AP and BA mice in the pathways associated to the regulation of immune system. Namely, AP mice were characterized by a gene overexpression for immune system response pathways and a significant change in cell number and activation of specific peripheral and central immune cells such as CD3+ T lymphocytes and microglia. Overall, our findings suggest that in the mPFC both the increased excitatory neurotransmission and the altered immune response are crucial underpinnings of AP tendency.

NI04 | Luteolin treatment ameliorates brain development and behavioral performance in a mouse model of CDKL5 deficiency disorder

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disease caused by mutations in the X-linked CDKL5 gene. The consequent misexpression of the CDKL5 protein in the nervous system leads to a severe phenotype characterized by early-onset epilepsy, intellectual disability, and autistic features. To date, no therapies are available for CDD. Evidence in animal models of CDD, that recapitulate various features of the disease, has shown that absence of CDKL5 negatively affects neuronal survival and dendritic outgrowth; however, knowledge of the substrates underlying these alterations is still limited. Recently, we found increased microglial activation in the brain of a knockout mouse model of CDD (Cdkl5 KO), suggesting that a neuroinflammatory state, known to be involved in brain maturation and neuronal dysfunctions, may contribute to the pathophysiology of CDD. The present study aimed to evaluate the possible beneficial effect of microglia inhibition by luteolin - a naturally occurring polyphenolic flavonoid – on brain development and behaviour in Cdkl5 KO mice. Here we show that the administration of luteolin to symptomatic heterozygous Cdkl5 KO female mice, by restoring microglia alteration, increased hippocampal neurogenesis, and improved or even restored dendritic length and branching of hippocampal and cortical pyramidal neurons, and spine maturation. Notably, neuroanatomical changes induced by the anti-inflammatory treatment with luteolin were associated with an improvement in behavioural performance in Cdkl5 KO mice. In conclusion, our findings show that microglia over-activation contribute to neuronal defects underlying behavioral deficits in Cdkl5 KO mice and that treatments aimed at counteracting the neuroinflammatory process should represent an adjuvant therapeutic strategy to ameliorate CDD patients' quality of life.

NI05 | Antioxidant and anti-inflammatory role of grapefruit IntegroPectin

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Oxidative stress, one of the major mechanisms involved in neurodegenerative diseases, alters numerous cellular processes such as mitochondrial functionality, DNA repair, and cell signaling being propagating cellular injury that leads to neurodegeneration. Recently, a new pectin, called IntegroPectin, particularly rich in citrus absorbed flavonoids and terpenes, was extracted from citrus processing waste via hydrodynamic cavitation in water only. This sustainable extraction method is exceptional in highly preserving the rhamnogalacturonan RG-I region, which is essential in the structure and function of the pectin. Tested on neuronal and microglial cells, grapefruit IntegroPectin proved to be effective in protecting different cells from apoptosis after exposure to oxidizing agents, reducing the amount of intracellular reactive oxygen species (ROS) and activating intracellular signaling cascades involved in cell protection. Preliminary results also suggest that IntegroPectin may modulate inflammatory phenomena. These data, alongside the absence of toxicity of this new pectic biomolecule, suggest a potential therapeutic role of grapefruit IntegroPectin. Though preliminary, these results support experimentation on preclinical models of neurodegenerative diseases, widely known as complex pathologies marked by extensive phenomena of oxidative stress and inflammation.

NI06 | Possible implications of the kynurenine pathway in the pathogenesis of Amiotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease, whose pathogenic mechanisms are still largely unknown; detrimental neuroinflammation, driven by activated resident microglial and infiltrating leukocytes, appears as one of the major features of this pathology. Tryptophan (Trp) catabolism, triggered by the enzymes indoleamine 2,3-dioxygenase 1 and 2 (IDO1 and IDO2, respectively) in immune cells, represents a well-known mechanism of modulation of the immune response, mainly involved in the maintenance of immune homeostasis. High concentrations of quinolinic acid (QUIN), a tryptophan-derived metabolite along the kynurenine pathway, endowed with neurotoxic and neuroinflammatory properties, have been detected in cerebrospinal fluid, as well as in the neuronal and microglial cells of ALS patients. This evidence suggests that an alteration of tryptophan metabolism could represent one of the mechanisms underlying onset and/or progression of ALS. Indeed, we investigated whether alterations of this pathway could be linked to ALS pathophysiology. Interestingly, our data carried out in a in a model of ALS, the G93A-SOD1 mouse, showed a strong modulation of Trp metabolism. In particular, IDO1 and IDO2 enzymes were down-regulated in terms of mRNA and protein expression in the spinal cord (SC) of late symptomatic G93A-SOD1 mice. Moreover, QUIN levels of expression were higher in the serum of asymptomatic G93A-SOD1 mice compared to age-matched WT. These data support the involvement of Trp in the pathophysiology of ALS and pave the way for the identification of druggable targets for the development of innovative therapeutic interventions in ALS. Future experiments will be carried on in double transgenic mice for SOD1 and IDO1 and for SOD1 and IDO2, derived by breeding male transgenic G93A-hSOD1(+/-) mice with IDO1(-/-) or IDO2(-/-) deficient females, in order to unravel the specific contribution of each of the two Trp-metabolizing enzymes in ALS progression.

NI07 | Possible role of the sympathetic nervous system in the definition of glioblastoma immune microenvironment

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Glioblastoma (GBM) is a brain tumor associated with neuroinflammation. Both low-grade and high-grade tumors are surrounded by activated microglia and contain infiltrating immune cells. However, the immune microenvironment changes during progression from low-grade to high-grade GBM tumors. In fact, T lymphocytes predominate in low-grade tumors, while immunosuppressive macrophages are the main immune population infiltrating high-grade tumors. Brain inflammation alters the function of the sympathetic nervous system (SNS), which regulates the generation of immune cells in the bone marrow (BM) and thymus. It is hypothesized that intrinsic changes in tumor gene expression, together with changes in immune cell generation due to altered SNS transmission in the BM and thymus, may contribute to shaping the immune microenvironment during tumor progression. To define the tumor-intrinsic changes associated with its progression, we performed single-cell RNA sequencing (sc-RNAseq) analysis of murine GBM cells isolated from low- or high-grade tumors. Among the 11 clusters of GBM cells identified, those characterized by high HLA expression were overrepresented in high-grade tumors, suggesting that the reduced frequency of tumor-infiltrating lymphocytes in these tumors was due to mechanisms extrinsic to the cells. We then evaluated the generation of immune cells in the BM and thymus of mice with low- or high-grade tumors by multiparametric analysis with flow cytometry. We observed increased generation of B lymphocytes and T lymphocytes in mice with low-grade tumors, but not in those with high-grade tumors, in which the SNS neurotransmitter norepinephrine (NE) and the frequency of hematopoietic stem cells in the BM were altered. Our data suggest that mechanisms extrinsic to the tumor, possibly mediated by the SNS, alter lymphocyte generation in the BM and thymus and may help define a different immune microenvironment in low- and high-grade GBM.

NI08 | RvD1 modulates maturation of monocyte-derived dendritic cells in Multiple Sclerosis

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Chronic inflammation is a key pathological hallmark of multiple sclerosis (MS) and suggests that resolution of inflammation, orchestrated by specialized pro-resolving lipid mediators (SPMs), is impaired. Recent studies from our group have shown that resolvin D1 (RvD1), one of the most studied SPMs, is significantly decreased in plasma of MS patients and reduces monocyte activation and migration into the CNS. Here, through high dimensional flow cytometry and qRT-PCR, we characterized the expression of its receptors ALX/FPR2 and GPR32 in the main immune cell populations of peripheral blood (i.e. CD14+ monocytes, CD66b+ granulocytes, CD4+ and CD8+ T-lymphocytes, CD19+ B-lymphocytes, and CD56+ NK cells) and we observed that monocytes showed the highest expression of these receptors. For both receptors the signal intensity was ranked as follows: monocytes > granulocytes > B-lymphocytes > T-lymphocytes. When comparing ALX/FPR2 and GPR32 expression in monocytes of relapsing-remitting MS patients (RR-MS), GPR32 was significantly reduced compared to healthy donors. Furthermore, monocytes from RR-MS patients were differentiated into immature dendritic cells (immDC) and then into mature (matDC) in presence of TNF-a/IFN-g, and we found that GPR32 expression was significantly altered between immDC and matDC and according to the disease clinical form. Hence, we investigated the impact of RvD1 administration during DC maturation in RR-MS patients and we observed that this pro-resolving lipid reduced the expression of maturation markers CD80, CD83, CD86 and HLA-DR in matDC obtained from remitting but not from relapsing MS patients. This is the first study that shows the immunomodulatory effects of specific SPMs on DCs in MS, with an ability to block their maturation and activation and keep them in an immature state upon inflammation and suggests that resolvins might be used as potential agents to reprogram immune cells into a less pro-inflammatory and more pro-resolving phenotype.

NI09 | Understanding the role of microglial extracellular vesicles in neuroinflammation spreading: an in vitro study

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Neuroinflammation is a crucial mechanism that commonly underlies the majority of the neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's disease and Amyotrophic Lateral Sclerosis. Microglia, the immune cells of the brain, play a critical role in the inflammatory condition following the onset of neuropathology. In fact, it was shown that microglia display the M2 anti-inflammatory phenotype at the early stages of the disease, switching to the M1 classically activated subtype as the disease progresses. The shift of microglial phenotype from M2 to M1 phenotype could be related to a change in the protein and/or microRNAs content in extracellular vesicles (EVs) involved in intercellular communication. This suggests that the spreading of neuroinflammation could be mediated by the release of vesicles in the extracellular environment and, therefore, by the effect that the content of these vesicles has on surveying microglia and other cell types.
To evaluate whether activation could be transmitted among microglial cells, activation has been pharmacologically induced in a microglial murine cell line (N9) by using LPS towards a M1 phenotype or ATP towards M2. Then, non-activated microglia has been treated with the media conditioned by differentially activated microglia or the isolated vesicles.
Furthermore, we investigated the expression profiles of microRNAs, identified as regulators of microglial activation; in particular, miRNA-155, miRNA-124, miRNA-34a, miRNA-125b that are known to be dysregulated in different pathological states. We focused on miRNA-34a contribution in neuroinflammation spreading and we tried to downregulate its expression using cleaving sequences of anti-mir34a DNAzyme delivered by DNA nanostructures.
Given that evidence, the role of EVs miRNAs released by microglia deserves to be deeply investigated both as potential therapeutic targets and as biomarkers for neurodegenerative diseases.

NI10 | Systemic inflammation upregulates the expression of oxysterol 7α,25OHC-synthesising enzymes in the blood-brain barrier

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The Epstein-Barr virus-induced gene 2 (EBI2), alongside its most potent ligand the oxysterol 7α,25OHC, is involved in several neuroinflammatory and neurodegenerative disorders and plays a key role in modulating innate immunity. Oxysterol 7α,25OHC is synthesised from cholesterol with the enzymes CH25H and CYP7B1 and degraded with HSD3B7. EBI2 activated by 7α,25OHC coordinates immune cell positioning in the secondary lymphoid tissues, enabling proper humoral and cellular immune responses. This coordinated lymphocyte positioning is possible with a tightly regulated concentration gradient of 7α,25OHC in the secondary lymphoid tissue formed by CH25H, CYP7B1 and HSD3B7 expressing cells. Lipopolysaccharide (LPS) modulates the expression of EBI2, 7α,25OHC, CH25H, CYP7B1 and HSD3B7 enzymes in vitro and in vivo. Notably, the concentration of 7α,25OHC and CH25H increases in the brain in the early phases of the experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis, leading to an enhancement of EBI2-expressing lymphocytes infiltration in the central nervous system. Here, we induced systemic inflammation in mice with a single peripheral high-dose injection of LPS and analysed the expression of pro-inflammatory cytokines, blood-brain barrier (BBB)-forming proteins, as well as the expression of EBI2 and 7α,25OHC-related enzymes in the brain. LPS injection increased the expression of pro-inflammatory cytokines, IL-6 and IL-1β, indicating neuroinflammation after peripheral immune challenge. Tight junction protein, occludin, adhesion protein, N-cadherin, as well as EBI2 were downregulated in the brain, while the 7α,25OHC-related enzymes were upregulated in the BBB after LPS challenge. Taken together, the data indicate the blood-brain barrier as a source of oxysterol-synthesising enzymes after systemic inflammation further implicating the EBI2/oxysterol signalling in neuroinflammatory diseases.

NI11 | Specialized pro-resolving mediator RvD1 reduces neuroinflammation in a transgenic rat model of Parkinson's disease

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The neuroinflammatory processes in Parkinson's disease (PD) are usually associated with activation of the immune system caused by a growing aggregation of α-synuclein (α-Syn) in central nervous system. The active immune response in brain of PD patients leads to infiltration of lymphocytes, production of cytokines and microgliosis, these features could be a consequence of failure to resolve inflammation, a process mediated by a superfamily of endogenous lipids termed specialized pro-resolvin mediators (SPMs). A previous study from our group has shown that precocious treatment with resolvin D1 (RvD1) prevents the onset of PD by attenuating immune response in a rat model of PD. Herein, we explored the long-term effect of RvD1 in α-Syn rats by treating them with intraperitoneal injections twice a week, starting at early stage of the disease (2 months old) until the symptomatic phase (12 months old). Hence, we assessed motor deficit evaluated through Rotarod test and the infiltration of the main CD45+ leukocyte cell populations (i.e. CD3+ T-cells, CD45RA+ B-cells, CD161+ NK-cells and CD45/CD11bhigh macrophages) within substantia nigra and striatum by flow cytometry. We found that α-Syn rats showed a higher degree of nigral and striatal infiltration of all cell subsets compared to age-matched wild-type rats and that RvD1 treatment not only ameliorated motor deficits but also reduced their infiltration in both anatomical regions. Furthermore, although the percentage of CD45lowCD11b+ microglial cells remained unchanged between the different experimental groups, we observed that microglia of α-Syn rats shifted from a pro-inflammatory M1-like to a pro-resolving/anti-inflammatory M2-like immunophenotype upon RvD1 treatment, in terms of modulation of their respective M1 (CD68, CD86, MHC-II) and M2 (CD206, TREM2) markers. These results suggest that RvD1 is able to delay disease progression by blunting neuroinflammation and inducing a microglia-driven pro-resolving response.

NI12 | Evaluation of D-loop methylation level and mtDNA copy number in Aicardi-Goutières patients

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Aicardi-Goutières Syndrome (AGS) is a pediatric rare disorder that affects the brain, the immune system and the skin. Mutations in 9 AGS genes lead to an accumulation of endogenous nucleic acids (NAs) which are recognized as foreign NAs of viral origin by the organism triggering an abnormal Interferon-alpha (IFN-α) mediated immune response. Mitochondrial dysfunction may lead to the release of mtDNA and trigger immunological pathways with the production of IFN-α. Alterations in methylation levels of the mitochondrial displacement loop (D-loop) region, which governs mtDNA replication, were recently discovered in other neurological disorders, i.e. Alzheimer's disease and amyotrophic lateral sclerosis. Up to now, nothing is known about methylation levels in the D-loop area in AGS patients.

The purpose of this study was to look at D-loop methylation levels and mtDNA copy number in AGS patients and healthy controls' peripheral blood. In peripheral blood cells from 25 AGS patients and 22 age- and sex-matched controls, pyrosequencing analysis of D-loop methylation levels and quantitative measurement of mtDNA copy number were performed.

D-loop methylation levels were considerably greater in AGS patients compared to controls, with the RNASEH2B A177T mutation driving the difference. In addition, the number of copies of mtDNA was much higher in AGS patients, with the RNASEH2B mutated patients accounting for the majority of the variance. In total samples, controls, and AGS patients, there was a positive correlation between mtDNA copy number and D-Loop methylation levels. Furthermore, a strong positive correlation was found between D-Loop methylation and the age of subjects in the controls and between the number of copies of mtDNA in AGS patients and the age of samples, too.

These data imply that D-loop methylation and mitochondrial replication are closely related, and that changes of methylation pattern could be used as compensatory strategy for the mitochondrial dysfunction.

NP01 | The role of glial cells in the adaptive and maladaptive response to acute stress: evidence from a preclinical model

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Each individual reacts differently to stress. When the response is physiological, it promotes adaptive plasticity; when it is excessive or unregulated, it induces maladaptive harmful effects. Considering the innumerable homeostatic functions in which glial cells are implicated, their involvement in the response to chronic stress has been already established. Little evidence is instead available regarding a possible role of these cells in the response to an acute stress. To fill this gap, and to explore the presence of glial factors determining the adaptive/maladaptive trajectory, Sprague-Dawley rats were exposed to a footshock stress (FS). Rat baseline sucrose intake was monitored for 5 weeks before stress and after FS. Animals with a decrease in sucrose intake <10% after FS respect to baseline consumption were considered resilient (RES) and animals with a variation >25% compared to baseline consumption were considered vulnerable (VUL). Through PCR, immunofluorescence and western blot analyses, we investigated the morphofunctional alterations affecting astrocytes, microglia and neurons 24 and 48 hours after FS in the prefrontal cortex of non-stressed, RES and VUL animals. Results obtained showed that, in VUL animals, FS exposure triggers a proinflammatory response guided by glial cells reactivity, reducing the expression of neurotrophic factors and impairing neuronal integrity. RES animals showed a better ability to metabolize glutamate. This study establishes, for the first time, the involvement of glial cells in the response to acute stress. More interestingly, our data reveal that astrocytes and microglia respond differently to acute stress in VUL and RES animals, potentially designating these cells as a target for personalized medicine.

NP02 | Cortical Rewiring Following Peripheral Injection of Botulinum Neurotoxin Type A

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Botulinum neurotoxin type A1 (BoNT/A1) is a bacterial metalloprotease that can cleave SNAP-25 (Synaptosomal-Associated Protein, 25kDa), thus inhibiting synaptic vesicle fusion and ultimately blocking neuronal activity. BoNT/A1 action is potent, specific, long-lasting, yet reversible. These features underlie its wide use in human therapy for treating neurological conditions characterized by neuronal hyperactivity. Interestingly, evidence suggests that a fraction of BoNT/A1 can undergo long-distance axonal transport, possibly mediating a direct effect on central circuits. Recently, fMRI studies on human patients with dystonia have indeed demonstrated wide and long-lasting changes in the cortical activity after BoNT/A1 injection, at timescales that are not compatible with the peripheral blockade at the level of the neuromuscular junction (NMJ). Here, we assessed whether and how BoNT/A1 peripheral injections can influence motor cortical areas, affecting the morpho-functional physiology of pyramidal cortical neurons connected with BoNT/A1-affected central nuclei. To visualize dendritic spines, three-month-old Thy1-GFP mice, expressing GFP in layer V pyramidal neurons, were injected with BoNT/A1 (5 U/kg) in the whisker pad. Ex vivo dendritic spine analysis revealed a striking decrease in spine density in cortical motor areas 30 days after BoNT/A1 injection, while whisker paralysis lasted only around 10 days. Moreover, we observed an increase in stubby spines, known to be an immature spine type that could either be new or in the process of being eliminated. To understand the mechanism underlying spine loss, we then measured spine dynamics longitudinally in awake mice using two-photon microscopy. Imaging of apical dendrites in the motor cortex before and after BoNT/A1 injection revealed a decrease in spine density already 15 days after the peripheral insult, confirming our ex vivo data. Moreover, we observed an increase in spine elimination at day 15 after BoNT/A1 injection. Overall, our data reveal profound morphological changes in cortical neurons after intramuscular BoNT/A1 injection, which persist longer than the peripheral effect at the NMJ. Our hypothesis is that cortical spine remodeling plays a key role in the therapeutic action of BoNT/A1 in neuropathologies and strongly contributes to the long-lasting benefits observed in patients.

NP03 | Assessing the contribution of altered cholinergic signaling in ASD social deficits

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Cholinergic (ChAT) neurons in the brain control several cognitive functions such as attention, learning and memory. These neurons are mainly localized in subcortical regions including the medial septum/diagonal band of Broca complex (MSDB). Our laboratory has previously shown that inhibition of MSDB ChAT neurons affects social memory, meaning the ability to discriminate between novel and familiar subjects. Alterations in ChAT neurons have been observed in Autism Spectrum Disorders (ASD), neurodevelopmental conditions characterized by social isolation, stereotyped movements and communication challenges. Some forms of ASD are associated with mutations in genes encoding for synaptic proteins including the neuroligin 3 (NLG3). NLG3 is a postsynaptic adhesion molecule that binds its presynaptic partner neurexin and stabilizes both excitatory and inhibitory synapses. Mice lacking NLG3 (NLG3 knockout , NLG3KO) show deficits in social interaction and social memory similar to those observed in autistic patients, thus NLG3KO mice are considered a valid ASD animal model. Here, we propose to study whether cholinergic dysfunction accounts for social memory deficits observed in NLG3KO mice. Preliminary data showed that NLG3KO mice had a reduced number of MSDB ChAT neurons as compared to control littermates. Furthermore, patch clamp recordings from ChAT neurons revealed an altered synaptic transmission. Ultimately, conditional suppression of Nlg3 expression in MSDB ChAT neurons, using a microRNA-based viral strategy (mi-RNA-Nlg3), induced an impairment in social memory, similarly to what observed in NLG3KO mice. Patch clamp recordings from MSDB ChAT neurons will clarify whether social memory deficits are associated to synaptic dysfunction in mice carrying mi-RNA-Nlg3. Moreover, the rescue of Nlg3 expression in MSDB ChAT neurons of NLG3KO mice will corroborate the evidence that a cholinergic dysfunction may cause social memory deficits in ASD.

NP04 | Antioxidants counteract the plastic effect of physical exercise in the adult primary visual cortex

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Physical activity is known to enhance adult visual cortical plasticity, both in human subjects and animal models. While physical activity activates mitochondrial oxidative metabolism and leads to a production of reactive oxygen species, it remains unknown whether this process is involved in the potentiation of plasticity elicited at the visual cortical level. Here, we investigated whether modulation of oxidative stress through a dietary intervention with antioxidants (vitamins E and C) interferes with the impact of physical exercise on visual cortex plasticity in adult rats. We found that antioxidant supplementation past the end of the closure of the critical period blocked ocular dominance plasticity in response to monocular deprivation induced by physical activity in adult rats. Serum IGF-1 levels and cortical IGF-1 signaling were increased in exercised rats, while they were blocked in response to dietary supplementation with vitamins E and C. Thus, physical activity promotes visual cortex plasticity acting through a mithormetic effect that involved dampening of mitochondrial biogenesis and IGF-1 signaling.

NP05 | Loss of MCT4 in microglia results in altered brain development and anxiety-like behavior

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Microglia, the tissue-resident macrophages of the central nervous system, actively participate in brain development by supporting neuronal maturation and refining synaptic connections. Accumulating evidence points towards the involvement of metabolism and differential substrates catabolism in the regulation of immune cells, including microglia. In particular, lactate, which sustains brain energetics and synaptic activity, also dictates responses of peripheral immune cells. However, the physiological role for lactate in modulating microglial function is still largely unexplored. In our study, we found that upon lactate exposure microglia upregulate the expression of the monocarboxylate transporter 4 (MCT4), which is involved in lactate exchange. Exogenously given lactate is readily shuttled into primary microglia and this correlates with an increase in lysosomal acidification. In order to assess the significance of lactate transport in microglia in vivo, we generated and characterized a microglia-specific conditional knock out (cKO) mouse model for MCT4. Two weeks-old cKO mice present alterations in hippocampal microglial density and in CD68+ phagolysosomal structures. This is associated with increased levels of synaptic markers in the hippocampus, altered excitatory post-synaptic currents as well as an enhanced susceptibility to develop kainic acid-induced seizures. Additionally, adult cKO mice display an anxiety-like phenotype. In vitro, primary cKO microglia exhibit reduced uptake of amyloid beta, a well-known microglial cargo, suggesting that the alterations observed in vivo might be at least partially due to an impairment in microglial phagocytic capacity. In summary, this study highlights the importance of microglial MCT4 for brain circuitries development and function. Given the established role of microglia in neuropathology, a mechanistic understanding of lactate-dependent microglial modulation may be relevant for targeting microglia in brain diseases.

NP06 | Neuroligin 2 regulates synaptic and network activity of hippocampal CA3 neurons during development

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Neuroligins (NLG) are postsynaptic cell adhesion proteins that, by binding to their presynaptic counterparts neurexins, regulate and stabilize synaptic transmission. The neuroligin family comprises four different genes: Nlgn1-4. Of the four, NLG2 is the only protein specifically expressed at inhibitory synapses. In humans, loss of function of this protein is associated to neurodevelopmental disorders such as schizophrenia and autism, as well as to anxiety. In rodents, NLG2 deletion recapitulates the human phenotype and its lack leads to impairment in synaptic GABAergic transmission. The detrimental effect of NLG2 deletion was observed in several brain areas, but little is known about what happens in the CA3 region of NLG2 deficient mice during the early days of life, and how the formation of neural circuits is affected. The CA3 area is important for encoding episodic memory and spatial representation, and also displays organized rhythmic activity. In this context, a primordial form of oscillatory activity called Giant Depolarizing Potentials (GDPs) appears during development. GDPs are generated by the depolarizing and excitatory action of GABA, in synergy with glutamate transmission. This activity is thought to be crucial for synapses maturation and circuit formation. In this study we performed field and whole cell electrophysiological recordings of CA3 pyramidal neurons in acute brain slices obtained from Nlgn2 KO (P3-P10) and littermate wildtype (Wt) mice. Analysis of frequency and amplitude of spontaneous and miniature postsynaptic currents show that GABAergic synaptic transmission is impaired in Nlgn2 KO mice. Coherently, the lack of NLG2 is responsible for a reduction of GDP's frequency compared to Wt mice. Defects in inhibitory synaptic transmission persist in the CA3 of adult mice. Interestingly, glutamatergic transmission is also affected, suggesting an impairment in circuit formation in this region.

NP07 | SynActive (SA) toolbox potentiality to study the role of microglia in spine potentiation

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Encoding and storage of memories in the central nervous system are related to synaptic plasticity, specifically enhancement of synaptic transmission and structural spine changes. The physical substrate of these processes is hypothesized to be the "synaptic engram", an ensemble of synapses whose activity and potentiation is both necessary and sufficient for the establishment and recall of a given memory. Dendritic spines hold most excitatory synapses in the brain and continuously remodel their shape and number to fulfill the demands for acquisition and evoking of memories. On this regard, compelling evidence points to the importance of microglia in spine remodelling, influencing synaptic functionality and plasticity. So far, synaptic activity mapping is limited by the lack of an appropriate tool. To this purpose, the genetically encoded SynActive (SA) toolbox, which allows the expression of a fluorescent reporter at synapses undergoing activity-dependent long-term potentiation (LTP), was exploited in organotypic mouse hippocampal slice cultures, to address whether microglia regulate the mechanism underling synaptic potentiation. We observed that pharmacological removal of microglia from organotypic hippocampal slices prevents the detection of the potentiated SA positive spines elicited by electrical or chemical LTP protocols. Among microglia-neuron signals that may underlie spine potentiation, fractalkine (CX3CL1) with its microglial receptor CX3CR1 is known to modulate synaptic phagocytosis, function and neurotransmission. Notably, our investigations on organotypic slices from CX3CL1 knock-out mice suggest that alteration of CX3CL1-CX3CR1 signaling interferes with spines potentiation. These data provide the first evidence on the critical role of microglia in spine potentiation, adding new insights on the mechanism underlying microglia involvement in learning and memory processes.

NP08 | Impact of intermittent fasting on neural plasticity and peripheral-cerebral metabolism in a mouse model of diet induced obesity (DIO)

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Nowadays, obesity is a worldwide epidemic. Among the complications associated with obesity and metabolic diseases in general, those affecting the central nervous system (CNS) have raised important concerns due to the associated increased risk of neuropsychiatric disorders. Dietary restriction, particularly intermittent fasting (IF), can positively affect metabolism and function of peripheral organs, but it can also improve CNS-specific processes. However, little is known about the effects of IF on brain function, plasticity, lipid profile and turnover in animal models of metabolic disorders. To fill this gap of knowledge, we studied how IF affects CNS metabolism and function in a mouse model of diet-induced obesity (DIO). Adult male mice were fed high fat diet (HFD) for 10 weeks and then divided into different experimental groups depending on dietary regimen for 4 weeks: HFD feeding (HFD), ad libitum balance diet (control chow, CC), IF eating every other day HFD (HFD-IF), IF eating every other day CC (CC-IF). Our results indicate that switching to IF or ad-libitum CC after 10 weeks HFD ameliorated glucose tolerance and decreased body weight in DIO mice. Significant reduction in body fat was observed after CC or IF, but with an increase in brown adipose tissue after CC-IF. Behavioral tests showed more evident changes in CC-IF vs HFD mice. The analysis of gene expression in cortex, hippocampus and liver showed significant changes in plasticity and metabolism related transcripts. Plasma and liver lipidomic analysis showed a reduction in specific lipid species after CC and CC-IF. Thus, our data suggest that CC-IF has the strongest effects on amelioration of body weight and fat composition, lipidomic profiles and behavior.

NO01 | Inter and Intra-tumor Heterogeneity of Pediatric-type Diffuse High-Grade Glioma Revealed by High-Dimensional Single-Cell Proteomics

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Paediatric-type diffuse high-grade gliomas (pDHGG) are aggressive brain tumors, affecting children and young adults. Due to their highly heterogeneous nature, effective treatment have not been developed yet.

To dissect their intra and inter tumor heterogeneity, we exploited the mass cytometry approach that, by using metal-tagged antibodies, allows the simultaneous measurement of more than 40 markers, at single-cell level. By adopting this technique, we characterized 8 primary cell lines derived from H3-wildtype pDHGG (DHGG-WT), Diffuse hemispheric glioma H3G34-mutant (DHG-G34) and Diffuse midline glioma H3K27-altered (DMG-K27) patients. The adopted antibody panel was set to recognize brain and tumor cell antigens, including H3K27M and H3.3G34R variants, and it highlighted important intra- and inter- tumor heterogeneity. Of these, the integrin CD49c was more expressed in the DHGG-WT in respect to the DHG-G34 hemispheric cell lines, which, on the contrary, showed higher level of PDGFRα. Moreover, the H3K27-altered cell lines were characterized by a greater expression of the cancer stem cell marker CD90 while the astroglial differentiation marker GFAP was particularly expressed only in the H3.1K27M subgroup. Interestingly CyTOF data were not always in line with RNSeq data on cells and tumors but CyTOF data was confirmed by immunohistochemistry analysis for GFAP.

The UMAP analysis identified 10 cell clusters, with a minimal overlap between hemispheric and pontine subtypes and with peculiar antigenic profile, whose abundance strongly varied according to the mutational subgroups. For example, while the G34 subgroup was enriched for cluster 1 (CD29/CD63/CD56/PDGRFa), the H3.1K27 was enriched for cluster 3 (CD90/CD63/CD56 and GFAP) and 5 (CD90/CD56).

In conclusion, single-cell mass cytometry reveals a significant tumor heterogeneity at protein level that could contribute to the identification of new clinically biomarkers for pDHGG.

NO02 | Rearrangements of peritumoral tissue that take place during glioma progression

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Glioblastoma (GB) is the most malignant and aggressive form of brain tumor. Although a strong effort in finding new and effective therapeutic strategies, GB remain associated with high morbidity and mortality and the median survival is 12 to 15 months after diagnosis. For a long time, cancer research has mainly focused on understanding the biology of glioma cells and investigating the aberrant pathways that guide tumor onset and progression; however, it has been recently found that the interaction between GB cells and the tumor microenvironment (TME) is crucial in driving tumor growth. Specifically, recent findings highlighted the importance of clarifying the role of peritumoral tissue in GB progression and the need of a more detailed picture on the interactions between tumoral and neural tissue. In this context, our project aims to investigate which are the plastic rearrangement of cortical areas that take place along with GBM progression. Using Thy1-ChR2 glioma-bearing mice and optogenetics, we checked the responsivity of motor cortex at three different time points (i.e.: baseline, before glioma cells injection, 14 and 21 days after tumor implantation). We found that glioma-bearing mice showed a strong remapping of cortical motor areas and an increased threshold required for eliciting a forelimb movement. Immunohistochemical analyses revealed a downregulation of PNNs and of specific inhibitory markers in the peritumoral cortex. These findings demonstrate that the peritumoral tissue undergo through a strong biochemical reorganization along with glioma progression. Increasing our knowledge about these changes will help us to understand which are the mechanisms underlying GBM progression and might be useful to develop new and finally effective therapeutic approaches to counteract this terrible disease.

NO03 | An innovative technique to anticipate the diagnosis of glioblastoma: analysis of extracellular vesicles in liquid biopsies

BACK

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Glioblastoma (GBM) is the most common and malignant primary brain tumor in humans, it has a high level of invasiveness and chemoresistance. Its diagnosis requires neurological, radiological, and histological examinations when the tumor has already reached a critical mass. Current pharmacological treatment is based on temozolomide (TMZ), an alkylating agent, associated with surgical removal. In the last decades, the analysis of liquid biopsies (plasma, urine, CSF) obtained a relevant role in the diagnosis of different diseases, including tumors. They represent a non-invasive technique, permit to collect serial samples and monitor dynamic changes in patients.

All the cells of the body, including brain cells, communicate each other releasing cytokines, growth factors and also extracellular vesicles (EVs), both in physiological and pathological processes. Recently studies suggested that GBM cells release more EVs than healthy cells and EVs increase with the progression of GBM. EVs are composed of bilayer membranes and contains specific lipids, proteins and nucleic acids (such as mRNA, miRNA, ctDNA) that can modulate the functions of recipient cell. EVs can be distinguished on biogenesis, content and size; specifically medium/large (m/lEVs) and small (sEVs) indicate respectively EVs with a diameter above or below 200 nm.

In this study, we used an in vivo glioma model on adult male C57BL6/N mice and we inoculated murine glioma cells (GL261 cell line) in the right striatum. We collected brain tissue and plasma from glioma-bearing mice and control animals at different time points. We separated EVs (both sEVs and m/l EVs) using differential centrifugation. We analyzed the expression of some miRNAs (miR21, miR124, miR222) in both sEVs and m/lEVs in all samples.

The miRNAs in the EVs isolated from the brain could be correlated with the EVs isolated from plasma and we could use EVs and its content as potential prognostic markers in GBM patients anticipating the diagnosis.

NO04 | The role of Short Chain Fatty Acids in the modulation of glioma cell growth

BACK

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Glioblastoma (GBM) is one of the most aggressive tumours of Central Nervous System (CNS) (Ostrom et al., 2014) with few therapeutic treatments. The GBM is a kind of cancer with high frequency of recurrence, and this is also due to the tumour microenvironment that hinders the pro-inflammatory program of the immune cells aimed at counteracting the tumour growth (Xie Q et al., 2014). This suppressive microenvironment extinguishes the activity of immune cells that become part of the tumour mass (approximately 30%) that negatively correlates with survival of the patients (Ooi YC et al., 2014). The infiltrating immune cell panel is composed of leukocytes, such as tumor-infiltrating lymphocytes, Treg and natural killer (NK) cells (Kmiecik J et al., 2014). It has recently emerged that the host microbiota is involved in the immune modulation of several tumors (Gopalakrishnan et al., 2018). Currently, little is known about the effect of the gut-brain axis on immunity involved in brain tumor growth. In our latest work, we highlighted the effect of environmental enrichment (EE) on the composition of the gut microbiota and how short-chain fatty acids (SCFAs) seem to mimic the effects of EE in the brain (Marrocco et al., 2022). These data together with the previous observation that tumor grows less in mice subjected to the EE protocol (Garofalo et al., 2015), prompt us to treat glioma cells in vitro and glioma-bearing mice with a mixture of SCFAs, formate and acetate. Our data show that: (i) SCFAs had a direct effect on glioma proliferation in vitro, both in murine and human glioblastoma cell lines, and in primary human glioblastoma cells; (ii) SCFAs reduced the phagocytic activity of microglia driven by glioma conditioned medium (GCM). In vivo, oral administration of SCFA to glioma-bearing mice reduced the frequency of systemic regulatory T lymphocytes (Tregs), without affecting glioma growth in compared to controls. However, we found a significant extension of animal survival, suggesting that SCFAs may contribute to the beneficial effects driven by the EE microbiota on glioma.

NO05 | Drug-loaded MMP2-activable -liposomes as promising strategy for glioblastoma treatment

BACK

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Glioblastoma (GBM) is still a fatal tumor (overall survival of 14 month). The presence of the Blood Brain Barrier (BBB) represents a major problem for effective therapy. Recently our laboratory provided proof-of-concept for therapeutic potential of a combination strategy based on radiation and adjuvant drug-loaded liposomes (LP) conjugated with an Apolipoprotein E-derived peptide (mApoE), known to facilitate BBB crossing. To improve tumor specificity and efficacy mApoE-LP were implemented by 1) the addition of a metalloproteases-2 (MMP2) activable block (MAB), 2) the encapsulation of Trametinib (TRAM), Pimasertib (PIMA), and Rapalink-1 (RL1), inhibitors of the MEK/ERK and PI3K/AKT/mTOR pathways known to be crucial for GBM Stem Cell (GSC). MAB efficacy was evaluated on patient-derived GSC displaying different MMP2 enzymatic activities by means of calcein-loaded MAB/mApoE-LP. Human endothelial cells, not expressing MMP2, were used to validate the targeted strategy based on MAB cleavage, as well as to assay the cytotoxicity to non-tumoral cells. Results showed a correlation of calcein uptake and MMP2 activity level supporting a MMP2-depended payload release from MAB/mApoE-LIPs and their stability in the absence of MMP2. In addition, augmented calcein internalization was observed in GSC treated with MAB/mApoE-LP compared to mApoE-LP suggesting a probable synergic effect of double compared to single functionalization. GSC incubation (72h) with drug-loaded mApoE-LP caused a dose dependent cell death combined to significant lower phosphorylation levels of ERK and mTOR, and their main target molecules demonstrating that the encapsulation into mApoE-LIPs does not alter inhibitor activity. In conclusion, MEK (TRAM, PIMA) and mTOR (RL1) inhibitors demonstrate to be suitable molecules to affect GSC proliferation and survival and support their encapsulation into mApoE-LP as a promising delivery strategy.

NIM01 | Investigate age-dependent myelin alterations in structural network properties

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Normal brain aging is characterized by different structural alterations: myelin sheaths become less compact, number of axons decrease and become less myelinated. There is also evidence that demyelination contributes to the loss of brain plasticity that occurs during aging. Here, we combined diffusion MRI with myelin volume fraction (MVF) mapping to evaluate the sensitivity of MVF as a marker for age-dependent myelin alterations. We computed whole brain probabilistic tractographies of 85 healthy subjects (46f, 18-69y) and built the connectomes with a grey matter parcellation of 85 regions of interest. To compute myelin-weighted connectomes we used the state-of-the-art tractometry method and new framework called myelin streamline decomposition (MySD), which allow to recover the actual myelin value for each reconstructed fiber. We calculated 3 network metrics: Global Efficiency (information exchange), Modularity (network segregation), and Mean strength (nodal strength). We tested the possible relation between age, age2, and network alterations using sex and white matter volume as confounder factors. We employed the same model to predict these changes using leave-one-out cross-validation. Global efficiency (MySD: Age2 p=0.03, R2=0.61; Tractometry: Age2 p =0.04, R2=0.26) and mean strength (MySD: Age2 p=0.03, R2=0.61; Tractometry: Age2 p =0.04, R2=0.26) appeared to be related with aging: myelination increases up to 35/40 years, then starts to decrease. Despite both methods were sensitive to these changes, data calculated using MySD showed higher adjusted R2. Furthermore, results concerning age prediction showed a considerably lower error for MySD (Global Efficiency: 0.44 MySD, 0.89 Tractometry; Mean Strength: 0.25 MySD, 0.77 Tractometry).
In conclusion, MVF weighted connectomes computed using MySD, appeared to be more sensitive as well as more specific in capturing age-dependent myelin alterations compared to those computed using tractometry only.

NIM02 | Artificial Intelligence for Alzheimer's Disease: a data-centric approach to 3D MRI Deep Learning classification

BACK

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Enhancing diagnostic accuracy on Alzheimer's Disease (AD) and Mild Cognitive Impairment (MCI) is pivotal to foster adequate timely intervention, especially in the early disease stages. Computer-Aided Diagnosis (CAD) tools based on Artificial Intelligence (AI) can make neuroimaging data more profitable with the application of fine-tuned data analysis methods. Since ever, AI application on biomedical data has focused on complex model engineering and data preprocessing methods, hindering results reproducibility. Here we tested a data-centric approach in the development of a CAD tool on an ensemble of Deep Learning (DL) models exploiting multiple imaging modalities. This approach aimed at improving performance using standardized best practices from the Medical Open Network for Artificial Intelligence (MONAI), an open-source framework for AI in healthcare imaging. Here, fine-tuning practices were applied to the data instead of to the model, thus shifting from the classical model-centric approach to the new data-centric approach in AI. A "data boosting" procedure for 3D MRI data was designed and applied, where a baseline train set including 476 curated brain scans was used to train the models for the first time, then the misclassified validation samples were identified. The 20 nearest neighbors for the misclassified validation samples were found and the training set was updated to retrain the models including these new images. This procedure was repeated iteratively until the performance on the validation set could not be further improved. Finally, the performance achieved with this data boosting training procedure was compared with the performance achieved with a classical training procedure using all of the available images. This experiment demonstrates the efficacy of the data boosting procedure when developing an AI-based CAD tool for neurodegenerative diseases from 3D imaging in a standardized framework.

NIM03 | Neuroprotective effects of Montelukast treatment in a rat model of Huntington-like neurotoxicity: a PET covariance study

BACK

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A single intra-striatal administration of quinolinic acid (QA) in rats is sufficient to induce a lesion whose features resemble Huntington's disease. Our aim was to evaluate the effects of the leukotriene receptor antagonist Montelukast (MLK), that previously exhibited neuroprotection in preclinical models of neurodegeneration, on QA-induced neurotoxicity and brain functional connectivity using neuroimaging techniques.
A group of 16 rats underwent Positron Emission Tomography (PET) with 2-Deoxy-2-[18F]-fluoroglucose (FDG) to assess baseline glucose metabolism. Right and left striata of all animals were then injected with QA and vehicle (VEH), respectively. Starting from the day before QA administration, rats were treated with MLK or VEH for 14 days. At 4 months after lesion, FDG PET was repeated. Pairwise correlation coefficients between regional FDG uptake values were calculated to assess brain connectivity. A graph-based analysis was applied to explore the effects of QA and MLK on network measures of connectivity, including node degree and betweenness centrality.
In VEH-treated rats, QA significantly reduced FDG uptake in the lesioned hemisphere, compared to baseline. In animals treated with MLK, FDG was maintained at pre-lesion levels; in particular, QA effect was significantly reduced in ipsi-lesional cortical regions and in the lesioned striatum. Connectivity data showed that in contra-lesional regions as prefrontal cortex, orbitofrontal cortex and midbrain, MLK treatment preserved the changes in connectivity that are present in VEH-treated rats after QA, but was not able to maintain inter-hemisphere associations in the striatum. Finally, MLK counteracted the reduction in node degree and betweenness centrality observed in the lesioned hippocampus of vehicles.
In conclusion, MLK exhibited a neuroprotective effect by preserving regional brain function and connectivity from QA insult, in cortical and subcortical regions, but did not fully prevent striatal damage.

NIM04 | Ultrasound multiparametric imaging of neuroinflammation

BACK

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Background: Neuroinflammation is an inflammatory reaction that takes place in the central nervous system and can be induced by intrinsic (stroke, dysfunction of the immune system…) or extrinsic factors (infection, injury…). When it occurs during the perinatal period, neuroinflammation can impact critical phases of brain development and have long-term consequences on individuals' health (motor impairments, behavioral disorders, cognitive deficits…).
Aim: The aim of this ongoing study is to develop a multiparametric imaging technique based on the sole use of ultrasonic waves in order to better understand the impact of neuroinflammation on brain development. More specifically, three aspects of brain activity will be examined: neurovascular response to task-evoked and spontaneous brain activity, vascular architecture and hemodynamics properties, and cerebral tissue biomechanics.
Methods: This study is based on a mouse model of inflammation, at postnatal days 5 and 30. Two inflammatory molecules are tested: lypopolysaccharide (LPS) and polyinosinic:polycytidylic acid (poly I:C), which reproduce respectively a bacterial and a viral inflammation. A set of combined methods of ultrafast ultrasound imaging is used in the same experimental session: functional ultrasound imaging, to map blood flows in the brain; shear wave elastography, to measure tissue elasticity; and ultrafast ultrasound localization microscopy, to detect cerebral microvessels.
Results: During preliminary experiments, we identified an alteration of the neurovascular coupling during whisker stimulations in a mouse model of inflammation.
Perspectives: The results of this ongoing study should enable us to identify new biomarkers of neuroinflammation both for the screening of inflammatory processes and the follow-up of therapeutic treatment efficacy in the context of drug discovery.

ND01 | GABA signaling and metabolism (dys)regulation in Spinal Muscular Atrophy

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Spinal muscular atrophy (SMA) is a neurodegenerative disease, due to the lack of Survival Motor Neuron (SMN) protein: SMA is characterized by MN degeneration (MND) and muscle atrophy. Moreover, selective degeneration of motor cortex (CRTX) pyramidal neurons was shown in SMA mice compared to controls. Even if SMA genetic causes are well known, many aspects of its pathogenesis remain unclear and the available therapeutic options show many limits, disregarding SMN-independent targets. Intriguingly, neuroprotective effects of GABA-targeting drugs (used for neurological/psychiatric disorders) were reported in SMA, although the mechanisms involved are still elusive. Therefore, we investigated the perturbations in the GABA metabolism and GABAergic-interneuron (IN) functionality in SMAΔ7 mice (a severe SMA murine model) CRTX in the late disease stage (postnatal day 12). By immunofluorescence, we observed a significant reduction of GABAergic signal (-52%) and lower density of GABA+-cells (-20%) in SMAΔ7 motor/somatosensory CRTX compared to WT controls, along with an impaired distribution and significant reduction of GAD67 (enzyme responsible of GABA synthesis) signal (-42%) and GAD67+-cells (-20%). Also, parvalbumin INs (the predominant GABAergic IN subtype) were found significantly reduced in number (-30%), signal (-32%), area and branching in SMAΔ7 cortical areas. Immunoblotting and immunocytochemical analysis in SMAΔ7 further confirmed, respectively, a significant reduction in GAD67 protein levels in the sensorimotor CRTX (-43%) and lower GABA signaling in primary cortical neuron cultures. Overall, the results show a dysregulation of GABAergic synthesis and signaling in SMA mice CRTX, suggesting impaired inhibitory neurotransmission that may contribute to the SMA onset of MND, as a shared key role in other neural diseases. Further studies aimed at fully understanding and pharmacologically rescuing GABA signaling dysregulation will pave the way for new SMA treatments.

ND02 | Raman Spectroscopy as a powerful instrument for the Parkinson's disease managing

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Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder, associated with the inactivation of dopaminergic neurons in the substantia nigra and accumulations of the protein α-synuclein (α-syn) in the remaining neurons. Up to now, no known cure exists and the rehabilitation approaches are based on empirical experience. For this reason, the identification of a global biomarker is mandatory. Saliva is an optimal biofluid, since it contains wide range of biological molecules closely dependent on the pathological state. Moreover, also extracellular vesicles (EVs) have been proposed as new biomarkers and vehicles in the transfer of α-syn. We present the application of Raman Spectroscopy (RS), a fast, sensitive and label-free biophotonics-based technique, for the analysis of entire saliva and blood EVs from people with PD. Spectra of saliva samples from healthy controls (CTRL), people with PD and Alzheimer's disease (AD) were acquired using aluminium as substrate. Principal Component Analysis (PCA) and Linear Discriminant Analysis (LDA) allowed to discriminate between spectra collected from each experimental group and to identify differences compared to CTRL and AD subjects, principally regarding the peaks and bands related to proteins, nucleic acids, saccharides and lipids. Furthermore, drops of EV suspension isolated from blood of people with PD were analysed. Our results demonstrated that blood-derived EVs undergo modifications in people with PD, involving proteins, lipids and saccharides. It is worth noting that the biochemical signature of both saliva and EVs from people with PD were proved to be correlated to their clinical status, providing new hints for the understanding of PD pathophysiology. In conclusion, we believe that the described method has the potentiality to shed light on PD pathological mechanisms and also to be transferred to the clinical setting for both diagnosis of PD and for the evaluation of its rate of progression.

ND03 | Dietary intervention on brain aging in vivo and ex-vivo

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The short-lived annual fish Nothobranchius furzeri has an extremely short life span and accelerated expression of age markers. This model animal is particularly suited for investigating the effects of dietary interventions on longevity and age-related pathologies. ß-glucans are an ingredient of several animal feeds with actions on inflammation and mitochondrial functions. 1,3-1,6 ß-glucans modulate immune system by modifying phagocytic and autophagic activity. Also, 1,3-1.6 ß-glucans have antioxidant, anti-inflammatory, and antineoplastic activity. In this study, we used 1,3-1,6 ß-glucans from the cell wall of Saccharomyces cerevicae. The aim is to observe the effect of 1,3-1,6 ß-glucans at low concentration (comparable with dietary supplement) on expression of age-related markers in Nothobranchius furzeri. Nothobranchius furzeri have been treated with 1,3-1,6 ß -glucans starting from 2 weeks post hatching until 27 weeks post hatching. 1,3-1,6 ß-glucans were included in a commercial feed in two different doses: 12.5 mg/Kg and 125 mg/Kg. 1,3-1,6 ß-glucans induced a statistically significant decrease in lipofuscin accumulation on brain and liver. Moreover, 1,3-1,6 ß-glucans increased autophagy on brain and liver. 1,3-1,6 ß-glucans reduced inflammation on the brain analyzing l-plastin+ cells. This treatment promoted the degradation of protein aggregates increasing the lysosomes. In addition, we created an ex-vivo model from Nothobranchius furzeri to study the direct effects of 1,3-1,6 ß-glucans on brain. In particular, we treated culture brain slices with 8 mg/L of 1,3-1,6 ß-glucans and we observed a statistical increment in autophagy. In conclusion, 1,3-1,6 ß-glucans seem to slow progression some age-related markers and to directly effects on brain. This suggests caution in promoting use of ß-glucans to reduce age-related risk.

ND04 | Towards unveiling the nexus between axonal granules and polysomes in neurological disorders

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Amyotrophic Lateral Sclerosis (ALS) and Front Temporal Dementia (FTD) are neurodegenerative disorders characterized by progressive loss of motor neurons and cognitive impairment. RNA-binding proteins have been identified as major contributors to the development of neurological diseases and are known to modulate RNA synthesis, localization, and translation. However, the cellular mechanisms linking RNA dysregulation to neuropathogeneses remain largely unknown. ALS has been associated to mutations affecting the DNA/RNA-binding protein TDP43. We explored the hypothesis that TDP43 overexpression or mutation causes an imbalance between axonal granule- and polysome-associated RNAs. We developed a tag-free polysomal profiling to identify mRNAs associated to subcellular regions (cell body or axon) and sub-compartments (RNA granules or polysomes) of mouse cortical neurons. Through high-throughput sequencing and dedicated computational pipelines, we investigated translational changes induced by over-expression of TDP43-WT or TDP43-A315T mutant and revealed a loss of balance between free and polysome-engaged RNA in the axon. These results, supported by additional data from axonal puromycilation assay and multiple in vivo validation assays, suggest that the imbalance between granule- and polysome-associated mRNAs is caused by robust degradation of specific RNAs. Massive depletion of TDP43 target RNAs leads to a translational burden on non TDP43 targets due to the increased availability of ribosomes and the hyper-translation of the remaining mRNAs. We investigated this hypothesis in cell lines model of ALS by designing ad hoc constructs that revealed, the presence of a translational burden effect triggered by reduced levels of TDP43 mRNA targets. Our results point to an as yet unexplored translation-centred mechanisms linking TDP43 and ALS pathogenesis and pave the way toward a better understanding of axonal protein synthesis, possibly underlying many neurodegenerative diseases.

ND05 | Brain-ageing modulators in human blood as novel therapeutic targets of Alzheimer's Disease

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Parabiosis experiments in mice identified circulating blood proteins that have a brain 'ageing' or 'rejuvenating' effect and thus have a great potential as Alzheimer Disease (AD) therapeutic targets. Here, we aim to identify circulating blood proteins that modulate brain ageing in humans.
Following a candidate approach, we measured the previously identified mouse 'ageing' (B2M, CCL2, CCL11, CCL19, Haptoglobin, sVCAM1) or 'rejuvenating' (CSF2, TIMP2) proteins in cognitively unimpaired (CU) individuals of the ALFA+ cohort (n=381). Age was significantly associated with higher levels of the 'ageing' proteins CCL11 (β=0.172, p=0.006) and B2M (β=0.194, p=0.002) and with lower levels of the 'rejuvenating' protein CSF2 (β=-0.120, p=0.062). CSF2 was also associated with FDG-PET uptake (AD Meta-ROI composite; β=0.121, p=0.039) in individuals at higher risk of AD. Moreover, in Aβ-negative individuals, higher baseline levels of CCL11 were associated with a higher rate of cognitive decline (3-year follow-up, PACC composite score) specifically in women (β=-0.19, p=0.032).
Following a discovery approach, we then used structural MRI data from CU individuals of the ALFA cohort (n=1414) to calculate the BrainAGE. The DeltaAge (BrainAGE – chronological age) was used to select two extreme biological age phenotypes separated by sex as the 10th (decelerated ageing) and 90th (accelerated ageing) percentiles of DeltaAge. The DeltaAge median of the decelerated ageing group was -6.39 (IQR:1.92), while that of the accelerated ageing group was 5.11 (IQR:1.59). We will use mass-spectrometry based proteomics to identify blood proteins that differ between these extreme groups.
In conclusion, we found that some of the previously identified mice proteins have an age-dependent behaviour in humans (CCL11, B2M, CSF2) and the ageing factor CCL11 is associated with cognitive decline. In the future, we aim to identify new circulating blood factors in humans with an ageing or rejuvenating effect.

ND06 | Epigenetic and transcriptional dysregulation in NSCs and OPCs proliferation defects of AGC1 deficiency

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AGC1 deficiency is an ultra-rare demyelinating disease caused by mutations in the SLC25A12 gene encoding for the mitochondrial aspartate-glutamate carrier isoform 1 (AGC1). Main common pathological features are secondary hypomyelination and altered myelin formation in CNS, most likely due to a reduction of N-acetyl aspartate (NAA) synthesis, but brain cells proliferation deficit is also present. Here we studied whether transcriptional and epigenetic changes correlate with dysregulation of NSCs and OPCs biological mechanisms, and whether this could be counteracted by aminoacid and vitamin supplementations. We observed an altered expression of transcription factors involved in brain precursors proliferation/differentiation, as well as lower global histone acetylation and different histone acetyl transferases (HATs) and histone deacetylates (HDACs) isoforms expression and activities, in both our in vitro AGC1 deficiency models of siAGC1 Oli-neu cells (where a reduction up to 40% of carrier activity was induced by a shRNA) and SVZ-derived AGC1+/- mice neurospheres (vs. controls, respectively). Additionally, treatments with specific HATs and HDACs activity inhibitors have been performed to clarify the molecular roles of these enzymes.
These data let us to suggest histone acetylation defects in brain precursor cells, and consequent transcriptional dysregulation, as a pathogenic mechanism of AGC1-deficiency proliferation/differentiation dysfunction. Therefore, being NAA a source of acetate for histone acetylation, experiments on amino acids and vitamins supplementations directly involved in NAA synthesis are on-going, to compensate the metabolic impairment and the lack of acetyl-CoA with the aim of restoring proliferation/differentiation dysfunction in our in vitro AGC1 deficiency models.

ND07 | Nucleoporin 153 deficiency in adult neural stem cells defines a pathological protein-network signature and defective neurogenesis in a mouse model of AD

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Reduction of adult hippocampal neurogenesis is an early critical event in Alzheimer's disease (AD), leading to progressive memory loss and cognitive decline. The nucleoporin Nup153 has been described as a key regulator of NSC plasticity through gene modulation. Here we investigated the potential role of Nup153 as target to improve neurogenesis in the 3xTg mouse model of AD in vitro and in vivo. By a proteomic based approach, we also explored the Nup153-protein network.

We found that reduced Nup153 levels characterized NSCs from the 3xTg mice (AD-NSCs) and caused inefficient proliferation, migration and differentiation of neuronal precursors that were restored by Nup153 overexpression in vitro. Accordingly, following lentiviral-mediated Nup153 hippocampal delivery in AD mice we found that the number of BrdU/DCX+, BrdU/NCAM+ and BrdU/NeuN+ cells increased at 10 days and 1 month respectively. Further, LV-Nup153-injected AD mice showed an improvement of cognitive performance in comparison with AD control mice at 1 month after virus delivery (MWM test).

A proteomic approach was performed to identify Nup153 interactors in WT- and AD-NSCs potentially implicated in neurogenesis regulation. Gene ontology analysis showed that Nup153-bound proteins in WT-NSCs were involved in RNA metabolism and epigenetic mechanisms. Nup153-bound proteins in AD-NSCs, in addition to RNA-based mechanisms, showed an enrichment of proteins involved in energy processes and mitochondrial function. When these data were analyzed by the KEGG database to find relevant molecular interaction/reaction network in AD-NSCs we find a neurodegenerative signature associated with mitochondrial dysfunction, proteasomal processing, cell cycle and RNA degradation. Our data indicate that Nup153 restoration promotes neurogenesis and cognitive performance. Molecular data suggest that the complex regulatory network orchestrated by Nup153 is based on multiple interactions that are differently regulated in WT and AD-NSCs.

ND08 | Molecular and metabolic pathways underlying the in vivo anti-amyloidogenic action of 12A12, a cleavage-specific tau antibody targeting the 20-22kDa toxic peptide

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The development of antibodies, that selectively remove the neurotoxic target peptides (Ab and tau) from the brain -without interfering with the normal physiology of the neuronal proteins they are derived from- is crucial to ensure an effective and avoid of harmful potential side-effects treatment for Alzheimer's disease (AD). The cleavage-specific, monoclonal Antibody (mAb) 12A12mAb is unique in its ability to selectively bind the neo-epitope generated by cleavage of caspase-3 at D25-(D25(QGGYTMHQDQ) site located at the N-terminal end of the human tau without cross-reaction with the full-length protein. When intravenously injected into 6-month-old Tg2576 mice expressing the human Amyloid Precursor Protein (APP) with the Swedish mutation KM670/671NL, 12A12mAb selectively neutralizes its target, both into hippocampus and retina, leading to a significant improvement of behavioural, electrophysiological, neuropathological and metabolic cerebral-retinal parameters associated with animal's AD phenotype. In the search of the molecular and metabolic mechanisms underlying the neuroprotective action exerted in Tg2576 by 12A12mAb, we found out that 12A12mAb immunization reduces, both into hippocampus and retina, the steady state expression levels of APP and Beta-secretase-1 (BACE-1) along the amyloidogenic route by altering the protein amount of neuron-specific BIN1 and RIN3, two key regulators of endocytic pathways. Besides, retinal and hippocampal alterations of energetic glucose metabolism that are strictly linked with Ab production (lactate production) are mitigated in Tg2576 AD mice by 12A12mAb treatment in concomitance with its local anti-amyloidogenic action. Taken together these findings indicate that the in vivo beneficial action of 12A12mAb involves, both in hippocampus and in retina, the energy-dependent modulation of dynamic convergence of APP and BACE-1 into Rab5-positive early endosomes leading to the Aβ generation.

ND09 | Modeling of nigro-striatal circuits through the generation of human 3D organoids

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Recent advances in stem cell culture techniques led to the development of protocols for in vitro 3D cultures, called organoids, capable of developing organo-typically and exerting organ-specific functions. These complex structures are particularly useful to mimic the embryonic organogenesis. Furthermore, it has been found that by combining two distinct brain regions it is possible to achieve an higher-order thought processes. These assembloids are 3D structures formed from the fusion and functional integration of multiple cell types. And, most important, it has been seen that they mimic the complex cellular interactions from which organs arise in the body. Here, the aim of the project is to generate an assembloid by fusing two distinct brain regions. Specifically, it involves fusing a mesencephalic organoid with a striatal one in order to study the nigro-striatal circuits that are implicated in a rare neurodegenerative disorder called Multiple System Atrophy (MSA). By creating this assembloid using cells derived from patients with this particular disease, it is possible to reproduce the pathology of this disease in vitro.In this study, organoids showed a correct spatial and temporal progression of the genes involved in the development of both mesencephalon and striatum, mimicking the correct neurodevelopment in vivo.

ND10 | Effects of the homeobox gene Dbx2 on astrocyte function and on their cross talk with neural stem cells

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Adult neural stem cells (NSCs) reside in the subventricular zone (SVZ) of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus. These brain areas act as neurogenic niches thanks to a heterogeneous and specialized micro-environment that promotes and sustains adult neurogenesis. During aging, changes to the niche micro-environment progressively reduce NSC ability to generate neurons. Our group identified Dbx2 as a candidate regulator of age-associated neurogenic decline in the mouse SVZ.
Of note, Dbx2 is also expressed in astrocytes and regulates the expression of some genes important for their maturation, but the role of Dbx2 in astrocytes functions remains to be fully elucidated.
Astrocytes are an important component of the NSC niche, contributing to the establishment and maintenance of a permissive neurogenic environment. However, during physiological aging processes, astrocytes undergo age-associated changes that might impair NSC properties and neuron generation. In this study we intend to investigate the role of Dbx2 in the regulation of astrocyte functions.
NSCs derived from the murine adult SVZ were engineered with an inducible expression cassette, allowing for overexpression of Dbx2 by the administration of doxycycline (Dox). NSCs were first differentiated into astrocyte-like cells and then treated with or without Dox for 24h or 48h. We have been carrying out gene expression analyses to define the identity and proprieties of Dbx2-overexpressing astrocytes at molecular level. Furthermore, to assess astrocyte functional properties, we have been collecting conditioned media (CM) from control (-Dox) or Dbx2-overexpressing (+Dox) astrocyte cultures and testing their effects on undifferentiated NSCs. CM collected from astrocytes +Dox inhibits NSC differentiation into neurons when compared with CM -Dox. These preliminary data suggest that increased expression levels of Dbx2 change astrocyte properties, shaping their function toward an anti-neurogenic phenotype.

ND11 | Neuroprotective effect of a novel metabotropic glutamate receptor 3 positive allosteric modulator in in vitro model of Parkinson's disease

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Parkinson disease (PD) is a heterogeneous disorder caused by the necrosis of dopaminergic neurons in the substantia nigra, which leads to disability in motor performance. Due to the fact that PD is characterized by a complex pathophysiology, involving among others oxidative stress and neuroinflammation, there is no cure for PD, and the current pharmacology basically aims to control only the symptoms. Therefore, the development of novel therapies for PD represents a challenge for medical research. In this context, indirect evidences strongly suggest metabotropic glutamate receptors 3 (mGluR3) as good therapeutic candidate for PD. The recent development of positive allosteric modulator (PAM) that selectively activates mGluR3 allowed us an in-depth investigation of the neuroprotective role of these receptors in an in vitro model of PD, represented by SH-SY5Y neuroblast-like cells treated with the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA).

Dose-effect investigation showed that PAM treatment has not toxic effect on cells. Interestingly, when PAM was applied alongside 6-OHDA, it rescued cell viability SH-SY5Y impaired by 6-OHDA treatment. Neuroprotective action of PAM treatment seems to be associated with the modulation of Glial cell line-derived neurotrophic factor (GDNF) expression and its receptors (RET) activation. Indeed, treatment with PAM enhanced both GDNF and RET phosphorylation levels severally impacted by 6-OHDA treatment. Finally, pilot experiments suggested that PAM neuroprotective response might be linked to the activation of the mitogen-activated protein kinase (MAPK) cascade.

These results, although preliminary, support the role of drugs that activate mGluR3 as new disease-modifying and symptomatic agents in PD.

ND13 | Effects of gH625-liposome-PACAP in an in vitro fluid model of Parkinson's disease

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Parkinson's disease (PD) is an age-related disorder. It is characterized by loss of autonomic cognition due to abnormal protein aggregates and consequently to death of dopaminergic neuron (DAn) in the substantia nigra. The causes under the disease are still unclear, so by now successful treatments are not available. We evaluated the neuroprotective effects of functionalized liposome loaded with a neuroprotective molecule (gH625-liposome-PACAP) in an in vitro fluid-dynamic model of PD. The in vitro fluid-dynamic model is made by a bioreactor (Livebox2, LB2) with an upper chamber, where murine endothelial brain cells (bEnd.3) are seeded and a lower chamber with 3D neuroblastoma cells (SH-SY5Y) treated with 1-Methyl-4-phenylpyridinium (MPP+). We evaluated, how PACAP can acts against MPP+ effects. MPP+ is a neurotoxin that indirectly stimulates the production of reactive species of oxygen (ROS) triggering the release of dopamine in the cytosol from synaptic vesicles, causing apoptosis of DAn. Moreover MPP+ affects the mitochondrial complex I causing depletion of adenosine triphosphate (ATP). Pituitary adenylate cyclase activating polypeptide (PACAP) represents a highly effective neuroprotective peptide that prevents DAn degeneration, enhancing DA synthesis. The main drawback of PACAP is its low half-life. To enhance its bioavailability, PACAP was included in gH625-liposome. gH625 is a peptide deriving from the glycoprotein H of the Herpes simplex virus type 1 able to perturb the phospholipid double layer of the membrane without breaks. We evaluated PACAP delivery by functionalized liposomes in LB2 treated or not with MPP+. Results shown the neuroprotective effects of PACAP against MPP+ is enhanced by functionalized liposome drug delivery.

ND14 | Hydrogen peroxide: a new player in peripheral neve regeneration

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Peripheral nerve injuries represent a global health issue with insufficient therapeutic solutions. Despite the peripheral nervous system (PNS) has retained through evolution an intrinsic capability for repair and regeneration, the molecular mechanisms underlying these processes are not completely understood. We recently identified hydrogen peroxide (H_2O_2), produced by stressed mitochondria of injured motor axon terminals, as one of the key mediators for the Schwann cell (SC)-dependent functional recovery of the neuromuscolar junction (NMJ) upon an acute damage induced by α-latrotoxin, a presynaptic neurotoxin. Given that, I test if H_2O_2 triggers motor axon regeneration, which occurs upon sciatic nerve crush, one of the most employed experimental models to study PNS regeneration. I monitored H_2O_2 levels in the sciatic nerve of a living anesthetized mouse before and at different time points after sciatic nerve injury by using H_2O_2-specific probes. Moreover, I performed in vivo imaging of the axonal transport of single endosomes in motor neurons after sciatic nerve crush or H2O2 incubations, in live anaesthetised mice. Labelling of endosomes is achieved using a fluorescently tagged probe: the atoxic binding fragment of tetanus neurotoxin (HCT). I then test if H_2O_2 released by the degenerating neuron, affects the time course of recovery upon injury, and impairs the activation of myelinating axonal SC, tested by activation of the MAPK pathway and cJun transcription factor. With the described experimental systems, I firstly detected H2O2 production in the sciatic nerve after crush. Then, I found that this H2O2 promotes axonal transport of endosomes and acts as a paracrine signal on myelinating SC by inducing the activation of MAPK and c-Jun; mechanisms leading to a faster recovery of nerve functionality upon nerve damage. I believe that the present study will help to define H2O2 as an important signal molecule that activate and support the regenerative capability of PNS.

ND15 | Characterization of spinal cord organoids derived from sALS patients

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Amyotrophic lateral sclerosis (ALS) is a non-cell autonomous disorder as many cell types contribute to motor neurons death. The lack of effective treatments is probably due to the absence of a realistic model that can recapitulate pathogenic mechanisms. Cerebral organoids are pluripotent stem cell-derived self-organizing structures that allow in vitro generation of the tissues. We developed a new method for the generation of spinal cord organoids (SCOs) that can be used for the study of pathogenic mechanisms in ALS. Aim of the work was to characterize a 3D organoid model for the study of ALS pathogenesis. We started from iPSCs obtained from healthy controls and sporadic ALS (sALS) patients. We differentiated iPSCs into neural stem cells (NSCs). We dissociated NCSs using StemPro Accutase and a cell strainer. Then, we plated NSCs on low-attachment plates and we cultured them in floating conditions using an orbital shaker. We differentiated NSCs to generate SCOs. We then characterized cells by phase-contrast and confocal microscopy. We found that SCOs derived from sALS patients were smaller and with irregular morphology compared to healthy controls. Using the GFAP marker, we found that sALS organoids have a thicker glial layer compared to healthy controls. We also found that healthy controls organoids show longer neurites compared to sALS organoids. Finally, we found a diverse composition of cell populations. Indeed, healthy controls organoids show a higher amount of differentiated cells compared to sALS organoids. We investigated cytokines released in culture supernatant of SCOs, and we found several differences between ALS patients and healthy controls organoids. In particular, we reported the upregulation of ApoA1, CD30, EGF, GM-CSF, MMP-9, OPN, ADSF/RETN and the downregulation of Ang-1, HGF, IGFBP3, IL8, MCP-1, VCAM1. In conclusion, our data suggest that SCOs represent a promising tool for the investigation of pathogenic mechanisms of ALS.

ND16 | The loss of frataxin impairs microglia homeostatic functions in Friedreich's ataxia

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Friedreich's ataxia (FRDA) is a rare genetic disorder caused by mutations in the gene frataxin, encoding for a mitochondrial protein involved in iron handling and the biogenesis of iron-sulphur clusters, with consequent progressive nervous system damage. Although the overt manifestations of FRDA in the nervous system are mainly observed in neurons, alterations in non-neuronal cells may contribute to the pathogenesis of the disease, as recently suggested for other neurodegenerative disorders. In FRDA, the involvement of glial cells can be ascribed to direct effects caused by frataxin loss in these cells, eliciting different aberrant mechanisms that can concur to and exacerbate neuron loss. Recent findings obtained in FRDA patients and cellular and animal models of the disease have suggested that neuroinflammation can accompany or even be causative of the neuropathology. Thus, with this project, we aim to demonstrate that frataxin deficiency leads to an impairment of microglia homeostatic functions in FRDA. Our results demonstrate that microglia from the cerebellum of knock-in/knockout (KIKO) FRDA mice display impairment in phagocytic and migratory capabilities and dysregulation in several genes, such as gp91phox, IL-1beta, P2Y12, TREM2, and CX3CR1. Most importantly, frataxin knockdown in primary microglia alters their capacity to support the development and survival of mouse neurons, evidencing a non-cell autonomous detrimental mechanism in FRDA, where frataxin can control microglia activity. These data suggest that microglia targeting could play a valuable role in ameliorating neuronal circuits in FRDA-affected CNS regions, consistently with other neurodegenerative conditions, where the modulation of microglia represents one of the most promising therapeutic strategies.

ND17 | The Serum Response Factor (SRF) regulates motoneuron vulnerability in ALS through the regulation of autophagy flux

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Neuronal activity plays a crucial role in motoneurons vulnerability in amyotrophic lateral sclerosis (ALS). Enhanced motoneurons excitability has been shown to promote neuroprotection while reduced excitability has been shown to accelerate disease progression. However, the molecular basis of neuronal activity impact in ALS has not been found yet. In this study, the impact of the activity-dependent transcription factor Serum Response Factor (SRF) was investigated in vivo and in vitro. Conditional motoneuron-selective SRF ablation in the context of the SOD1(G93A) ALS mouse model caused an early disease onset as revealed by anticipated start of body weight loss, earlier appearance of advanced clinical stages and faster decline in grip-strength. However, overall survival was not affected, indicating a major role of SRF only for some motoneuron subpopulations. At P50, loss of SRF in motoneurons caused a more pronounced neuromuscular junctions' denervation and expanded neuroinflammatory response. Increased vulnerability of motoneurons corresponded to fewer Beclin positive inclusions in histology and reduced induction of autophagy genes assessed in laser-microdissected motoneurons. We complemented these studies assessing autophagy genes induction in HEK cells expressing the C9-Orf72-associated poly Glycine-Alanine (polyGA) polypeptide: the overexpression of SRF resulted in a decreased burden of polyGA inclusions. Live-imaging microscopy with the autophagy sensor P62-GFP-RFP revealed a faster autophagy flux upon overexpression SRF compared to an inactive mutant of SRF. Thus, in vitro data further confirmed a role of SRF in the transcriptional regulation of cellular proteostasis. In conclusion, a novel link between neuronal activity, synaptic input and autophagy imbalance was provided in this project.

ND18 | Chronic administration of palmitoylethanolamide counteracts cognitive decline in Tg2576 Mice

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Palmitoylethanolamide (PEA) has been emerging as a safe and well tolerated analgesic, anti-inflammatory and neuroprotective mediator, acting at several molecular targets in the nervous system. PEA is present in foods, as egg yolk, corn, peanut and soy oil. It is synthesized from lipid components of cellular membranes and can be found in high concentrations in brain tissues. In this study, we evaluated the effects of a chronic (6 months) administration of ultra-micronized PEA on cognitive decline in transgenic Tg2576 (Tg) mice expressing mutant APP. When aged, Tg mice develop accumulation of amyloid peptide and amyloid plaques in the brain, as well as cognitive deficits, representing thus an animal model of AD. PEA administration was performed via a subcutaneous delivery system in Tg mice and wild-type control group (from 6 to 12 months of age). PEA effects on behavior were observed longitudinally in a pre-symptomatic phase (3 months), a mild-symptomatic phase (6.5 months) and a full-symptomatic phase (11-12 months). Behavioral assessment was performed by using the following validated tests: Elevated Plus Maze, Rotarod Test, Y-Maze Spontaneous Alternation Test, Novel Object Recognition Test, Tail Suspension Test and Morris Water Maze. PEA administration restored the novelty recognition memory of Tg mice during the full-symptomatic phase. PEA was able to counteract hippocampal-dependent mnesic deficits, suggesting the therapeutic potential for the early treatment of AD. Further in progress analyses involve histological evaluation of dendritic branching, spine number, amyloid plaques and glial reactivity in the hippocampal CA1. This research is aimed to increase knowledge of the effects of PEA as a safe and low-cost nutraceutical tool useful to improve quality of life in AD.

ND19 | Iron-fed microglia: an in vitro system to model microglial phenotype in vitro and test new therapy in neurodegenerative diseases?

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Neuronal survival and function are highly dependent on microglia, the brain immune cells. In several neurodegenerative diseases microglia display a Disease Associated Microglia (DAM) signature, that may initially protect neurons, but then lose their homeostatic properties, contributing to neuronal loss. Microglia normally lose their protective function during senescence; senescent microglia exhibit cell cycle arrest, impaired metabolism, a Senescence-Associated Secretory Phenotype (SASP) and deficits in phagocytosis. Recently, a subcluster of late-stage DAM has been reported to display increased ferritin and iron content in the human brain. Despite the transcriptional profile of microglia accumulating iron have been defined, whether these cells are senescent and how they impact the brain environment is unknown. Aim of this study was to develop an in vitro model of iron-loaded microglia and to study their function. We explored whether murine microglia chronically (30 days) exposed in vitro to high iron concentration (500 µM) become senescent. Iron-fed microglia were deramified and acquired a senescent-like phenotype, characterized by proliferation arrest, decreased phagocytosis, increased Senescence Associated ß-Galactosidase activity and upregulation of SASP markers, p16 and RPL32, a ribosomal protein overexpressed in human microglia accumulating iron. Biochemical and immunofluorescence analyses showed a decrease in Nicotinamide Adenine Dinucleotide (NAD) content and in the expression of NAD dependent deacetylases Sirtuins 1 and 6, which are downregulated in aged/senescent cells. In preliminary experiments we analyzed the impact on cultured neurons of iron-fed microglia, finding that their secretome is neurotoxic. By next-generation sequencing we are currently investigating whether iron-loaded microglia may recapitulate the transcriptional changes of human DAM accumulating iron, thus offering a useful model to study and modulate the function of senescent microglia.

BACK

CN01 | Increased apoptotic cell death in Riboflavin Transporter Deficiency

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Riboflavin Transporter Deficiency (RTD) is a rare, neurological disorder characterized by hearing loss and sensory ataxia associated with spinal motor neuron (MN) degeneration. The disease is caused by loss of function mutations in SLC52A2/3 genes, respectively encoding riboflavin transporters hRFT2 and hRFT3. As RF is the precursor of the coenzymes FMN and FAD, their abnormally low levels result in defective functionality of flavoproteins, which are involved in cellular bioenergetics and cell survival processes. We previously demonstrated mitochondrial and peroxisomal altered energy metabolism pathways, accompanied by cytoskeletal derangement. As this disorder lacks dependable in vivo models, we took advantage of iPSC technology to recapitulate human neuronal features of RTD. More specifically we studied MNs differentiated from patient-specific iPSCs to perform combined ultrastructural and confocal analyses, aimed at characterizing the pathomechanisms associated to RTD. Patient-specific iPSCs and iPSC-derived MNs have been analysed by Focused Ion Beam/Scanning Electron Microscopy (FIB/SEM) and conventional SEM. RTD cells displayed profound alterations including neurite swellings, typical neurodegeneration hallmarks suggesting impaired intracellular trafficking. Increased apoptosis was observed in RTD cells, confirmed by the presence of vesicles and blebs budding from the cell surface of RTD cells and by activated caspase-3 immunofluorescence and TUNEL assays. Consistent with these results, ultrastructural characterizations revealed aberrant mitochondrial features confirming the persistence of mitochondrial damage after differentiation, suggesting that energy metabolism was impaired. Overall, our work contributes to the knowledge on the multiple cellular features associated to the neuronal phenotype of RTD, supporting a central role played by mitochondrial apoptosis in its pathogenesis, thus indicating potential targets for future therapies.

BACK

CN02 | Towards developing a mass spectrometry assay to identify post-translational modifications of deoxycytidine kinase possibly relevant to the response to cladribine

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Activation of cladribine (2CdA), a drug approved for multiple sclerosis (MS), is driven by a high ratio of deoxycytidine kinase (dCK)/5'nucleotidase. In view of their high dCK content, lymphocytes are preferential targets for 2CdA. We demonstrated that the 2CdA-induced apoptosis in stimulated T cells is correlated with enhanced dCK expression and activity. Phosphorylation of serine (Ser) 74 was shown to be crucial for dCK activity. However, little is known about if and/or how the other 13 phosphorylation sites described to date in dCK amino acid sequence play a role in dCK activity. Our first objective was to assess the differential phosphorylation status of dCK isoforms to understand its possible implication in dCK activity and therefore response to 2CdA treatment. We used Phos-tag™ electrophoresis, which separates proteins according to their phosphorylation status, followed by immunoblotting with a monoclonal anti-dCK antibody. The immunoreactive bands were cut out from the immunoblot and the membrane-bound protein digested with trypsin. This method allows to obtain enriched extracts of phosphorylated dCK isoforms and to reduce, at the same time, the background noise of other proteins. Tryptic digests were analyzed by mass spectrometry and yielded four different dCK peptides. Interestingly, three of these peptides contained the ATP binding site (Gly 28-Thr 36), whereas one encompassed a phosphorylation site at Ser 35). Through in silico modeling of dCK crystalized together with 2CdA (Protein Data Bank code 2ZIA), we hypothesize that Ser 35 is involved in the transfer of a phosphate group from the phosphate donor (ATP) to the substrate (2CdA). None of the peptides obtained from this preliminary experiment contained Ser 74 and other trypsinization protocols are being tested. Further analysis of dCK phosphorylation status and activity in lymphocytes from 2CdA-treated MS patients will help to predict and monitor the impact of 2CdA.

CN03 | The function of GPR183/7α,25OHC signalling in the brain microvessels and multiple sclerosis

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The endogenous ligand for the G protein-coupled receptor GPR183 (aka EBI2) is an oxysterol 7α,25OHC. It is synthesised in vivo from cholesterol via sequential enzymatic activity of CH25H and CYP7B1 and is degraded with HSD3B7. The GPR183 receptor has chemotactic properties and upon binding with 7α,25OHC induces migration of GPR183-expressing cells in vitro and in vivo. We showed before that lipopolysaccharide (LPS)-treated mouse astrocytes release 7α,25OHC in vitro and that the astrocyte/LPS-conditioned media induces migration of macrophages. Others demonstrated that during the early stages of experimental autoimmune encephalomyelitis, the concentration of 7α,25OHC increases in the central nervous system (CNS) thus facilitating brain infiltration by GPR183-expressing immune cells. Most importantly, we and others showed increased expression of GPR183 in infiltrating lymphocytes and glial cells in multiple sclerosis (MS) plaques. Here, we investigate the expression levels of 7α,25OHC-synthesising (CH25H, CYP7B1) and degrading (HSD3B7) enzymes in MS brains and the microvasculature, and challenge the human tri-cell BBB spheroids with cerebrospinal fluid (CSF) or serum from control and MS patients. We then measure the permeability of the spheroid and migration of MS patient CD4+ cells in this model. The data showed that GPR183, CH25H, CYP7B1 and HSD3B7 enzymes are expressed in the human brain white matter and the brain microvasculature. Only the MS patient serum significantly increased the permeability of the spheroids. The spheroid permeability and chemotaxis of patient CD4+ cells in the BBB model were modulated with GPR183 ligands thus indicating GPR183-mediated signalling in CD4+ T cell migration. Taken together, the data indicate that pharmacological modulation of the GPR183/7α,25OHC signalling in the brain microvasculature may limit the entry of encephalitogenic peripheral immune cells into the CNS.

CN04 | Multi-dimensional genome-wide analysis reveals robust pre-symptomatic defects in translation in two SMA mouse models

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Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease representing the most common genetic cause of infant mortality. SMA is caused by deletions or mutations in the Survival Motor Neuron gene (Smn1) which induces reduced levels of the SMN protein. Deficient levels of Survival Motor Neuron protein (SMN) were recently observed to lead to defective translation in primary motor and cortical neurons, and multiple tissues at an early stage of disease in the Taiwanese model of SMA. Even though structural and functional impairments in several tissues and organs are increasingly acknowledged, the impact of SMN loss in ribosome occupancy along mRNAs in diverse tissues, SMA mouse models and disease stages is still unclear. SMN protein levels in physiological conditions vary at different developmental stages and disease models, leading to the hypothesis that these translational defects may vary accordingly. We performed polysomal profiling and a multi-dimensional analysis of translational defects by obtaining and integrating ribosome profiling data in all the above-mentioned conditions. From polysomal profiling we computed the fraction of ribosome in polysomes in brain, spinal cord and liver, at pre, early and late stage of disease in the Taiwanese and Δ7 mouse model of SMA, finding a remarkable decrease of this parameter across these multiple conditions. By ribosome profiling, we identified a set of genes with altered ribosome occupancy in brain, spinal cord and liver, at pre- and early-symptomatic stages in the Taiwanese mouse model. We also found strong translational defects in brain at the pre-symptomatic stage in the Δ7 mouse model, and observed defective biological processes shared with the Taiwanese mouse model. Our results demonstrate that the very strong variations in translation occur in SMA across multiple models at pre-symptomatic stage of disease, suggesting a new scenario in SMA pathogenesis.

POSTER SESSION

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EBN08 | Dissecting the Role of PCDH19 in Clustering Epilepsy by Exploiting Patient-Specific Models of Neurogenesis

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PCDH19-related epilepsy is a rare genetic disease caused by defective function of PCDH19, a calcium-dependent cell-cell adhesion protein of the cadherin superfamily. This disorder is characterized by a heterogeneous phenotypic spectrum, with partial and generalized febrile convulsions that are gradually increasing in frequency. Developmental regression may occur during disease progression. Patients may present with intellectual disability (ID), behavioral problems, motor and language delay, and a low motor tone. In most cases, seizures are resistant to treatment, but their frequency decreases with age, and some patients may even become seizure-free. ID generally persists after seizure remission, making neurological abnormalities the main clinical issue in affected individuals. An effective treatment is lacking. In vitro studies using patient-derived induced pluripotent stem cells (iPSCs) reported accelerated neural differentiation as a major endophenotype associated with PCDH19 mutations. By using this in vitro model system, we show that accelerated in vitro neurogenesis is associated with a defect in the cell division plane at the neural progenitors stage. We also provide evidence that altered PCDH19 function affects proper mitotic spindle orientation. Our findings identify an altered equilibrium between symmetric versus asymmetric cell division as a previously unrecognized mechanism contributing to the pathogenesis of this rare epileptic encephalopathy.

EBN09 | Healthy life-style approaches to attain disease modification in acquired epilepsies

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Healthy life-style was reported to improve neurological outcomes after acute brain injuries. In particular, physical activity might improve neurological deficits and reduce seizures in epilepsy. Our study investigates whether aerobic and regular physical exercise reduces the risk of developing epilepsy, the burden of seizures and neuropathology following a focal brain lesion. C57BL6/N adult male mice were given free access to running wheels in their home cage for 5 weeks before being exposed to intra-amygdala kainate to induce a status epilepticus that leads to epilepsy development. Then, injured mice were allowed to run for 6 additional weeks, a time required for chronic epilepsy development. Control mice were similarly treated with kainate but left in their home cage in the absence of running wheels (sedentary mice). Sham mice were prepared for post-mortem histological analysis. Data show that running mice develop status epilepticus of reduced severity (decreased number of spikes, $p<0.05$ vs sedentary mice). Although, epilepsy incidence and seizure frequency were not modified by running activity, mice developed spontaneous seizures of reduced duration ($p<0.05$) vs sedentary mice. Pyramidal neuron loss in CA1-4 hippocampal sector was similar in running vs sedentary mice whereas GluR2/3-positive hilar mossy cell were protected in running mice ($p<0.05$ vs sedentary mice). Notably, seizure duration negatively correlated with the number of hilar mossy cells ($r=-0.7$, $p<0.05$). Data support the beneficial effects of physical activity to improve pathologic outcomes after an epileptogenic brain insult.

EBN10 | Effect of Type 5 Phosphodiesterase (Pde5) deletion on neurogenesis

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Type 5 phosphodiesterase (PDE5) is an enzyme that specifically controls cGMP levels by breaking the phosphodiester bond, regulating signal transduction triggered by the nitric oxide pathway. Several PDE5 inhibitors, among which sildenafil is the best known, have been developed and used as therapeutic agents to increase cGMP levels and modulate cellular activities. In the brain, PDE5 is expressed in the cytoplasm of pyramidal neurons in cortex and hippocampus, and, within the cerebellum, in Purkinje neurons.
In our lab, we developed a Pde5 knock-out (ko) mouse model, mimicking the effect of a constant Pde5 inhibition. To evaluate the role of Pde5 in mouse brain postnatal development, we focused on studying cortical neurogenesis on wild-type (wt) and ko brain sections obtained from one-month-old mice. Histological analysis of isolated brains revealed a thinning of the hippocampal cortex from ko mice, but we did not observe differences in the migration pattern of neuroblast precursors along the rostral migratory stream. These observations suggested that Pde5 might support adult neurogenesis promoting neuroblasts differentiation towards GABAergic and pyramidal fate. To verify these hypotheses, we performed analysis on neural stem cells (NSCs) isolated from adult Pde5 ko and wt mice, revealing that ko NSCs proliferate and migrate at higher rates compared to wt cells, and they preferentially differentiate towards the neuronal fate. Several other analyses are currently ongoing to better characterize the physiological roles of Pde5 in adult neurogenesis.
Since it has been previously observed that PDE5 inhibition rescues memory impairment in a mouse model of Alzheimer's disease, our model will be useful to better understand the role of PDE5 in controlling postnatal neurogenesis in health and diseases.

EBN11 | Somatic mutations and epileptic seizures originating from the contralateral hemisphere: two possible pathogenetic mechanisms and personalized pharmacological approaches

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In recent years, somatic mosaicism has been demonstrated as a significant cause of neurodevelopmental disorders, and in particular of Focal Cortical Dysplasia (FCD), which is the major cause of drug-resistant epilepsy in children. Among the different types of FCD, Type II FCD, which is the best characterized, is mainly caused by somatic mutations in mTOR pathway genes. Surgery is the only treatment option effective at controlling seizures in FCD patients. As complete as possible resection of the dysplastic tissue influences post-surgery outcome. In some cases, patients undergo multiple surgeries, which may end with a hemispherotomy, due to the persistence of seizures. Although hemispherotomy is effective in about 60–75% of cases, in some patients this surgical procedure resulted in recurrent seizures originating from the contralateral, seemingly normal, hemisphere. Here we propose two possible genetic mechanisms underlying this phenomenon. The first implies a double-hit mechanism involving two pathogenic mutations in different genes of the same pathway. One of the two mutations is confined to the dysplastic tissue and is mainly associated with the malformation phenotype, while the other is present also in the contralateral hemisphere and mainly contributes to epileptogenesis. The second mechanism is based on the possibility that the pathogenic mutation originates early during brain development, before the hemispheric cleavage, and asymmetrically distributes between the brain hemispheres. In this case, one hemisphere receives a number of mutated cells sufficient to develop a MRI-visible malformation, whereas mutant cells in the contralateral hemisphere, not sufficient in number to result in a visible malformation, would become epileptogenic only after removal of the overwhelmingly epileptogenic contralateral dysplasia. Finally, we discuss possible personalized pharmacological approaches with MTOR inhibitors to treat drug-resistant epilepsy in FCD.

EBN12 | Dissecting the role of HCN1 in Developmental and Epileptic Encephalopathy (DEE) by exploiting patient-specific models of cerebral cortex development

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Epilepsy is the most common neurological condition in childhood (1% prevalence), and constitutes a public health burden worldwide. Within the broad classification of infantile/pediatric epilepsies, particularly devastating are the developmental and epileptic encephalopathies (DEE), a group of heterogeneous conditions characterized by very early onset and recurrent, pharmaco-resistant seizures. Recent advancements in genome sequencing have expanded our knowledge on DEE, revealing a complex genetic architecture. Interestingly, the Hyperpolarization-activated Cyclic Nucleotide-gated (HCN1) gene has been recently linked to a particularly devastating subtype of DEE, namely DEE24. Indeed, growing evidence now supports a critical role for ion channels, which have been classically studied exclusively in mature neurons, in immature neuronal behavior and brain pathology.
In this work, we aim at understanding how developmental processes are altered by HCN1 pathogenic variants associated with DEE24. We selected 3 de novo HCN1 variants showing association with both epileptic, autistic/behavioral and morphological traits and evaluated their effect on the electrophysiological properties in both heterologous cell lines and neurons. By exploiting a highly reproducible human cortical organoids (hCOs) system, we generated hCOs from HCN1-DEE24 patient-specific iPSC lines to model HCN1 specific variants. Moreover, we generated chimeric brain harboring the patient-specific HCN1 variants via in utero electroporation and analyzed their cell intrinsic as well as not-cell autonomous effects on early cortical development. Our preliminary results suggest that the generation of personalized models allows to identify the specific endophenotypes of distinct genetic HCN1 variants and their effects on cortical assembly. These valuable models, bridging genetic and clinical data with cellular and molecular neurodevelopment, will potentially inspire tailored therapeutic avenues.

EBN13 | Nr2f1 haploinsufficiency affects immature granule neurons morphology and leads to an altered activation of neuronal ensembles within the adult mouse hippocampus

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The newly identified Bosch-Boonstra-Schaaf optic atrophy-intellectual syndrome (BBSOAS; OMIN#615722), is a rare neurodevelopmental disorder caused by mutations in the NR2F1 gene, also known as COUP-TFI, a transcriptional regulator playing pleiotropic functions in brain development. Although BBSOAS is characterised by a complex and wide array of clinical features, intellectual disability (ID) associated to global developmental delay, visual impairment, and autistic traits are the most common. Interestingly, alterations in postnatal neurogenesis and functional integration of adult-born granule neurons in the hippocampal circuit have been reported in animal models of ID and recent findings suggest that a deficit in hippocampal plasticity may contribute to BBSOAS. Here, to investigate the possible effects of Nr2f1 haploinsufficiency on the hippocampal circuit we took advantage of constitutive Nr2f1 heterozygous mice (i.e., Nr2f1-HET), a recently validated BBSOAS mouse model, and focussed on the dentate gyrus (DG). Our data indicate that Nr2f1 haploinsufficiency does not alter the total number of DCX+ neuroblasts/immature neurons in the adult DG. However, these cells in Nr2f1-HET mice show atypical and peculiar neuronal morphologies, which are usually associated with pathological conditions and aberrant hippocampal circuitry activation. We thus focussed on the expression of immediate early genes (e.g., Npas4, c-fos) and found that Nr2f1 heterozygous mice exhibit an increased activation of DG mature granule neurons both under basal conditions and in response to short-term exposure to a novel enriched environment. Accordingly, patch-clamp recordings of DG granule cells from hippocampal slices focussing on spontaneous GABA release, showed a reduced mIPSCs frequency with no changes in mIPSCs amplitude, suggesting reduced inhibition on granule cells. Experiments are in progress to further elucidate excitatory/inhibitory imbalance in the DG of Nr2f1-HET mice.

EBN14 | Oligophrenin-1 (OPHN1): a novel sumo target in synaptic function and dysfunction

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OPHN1 gene encodes Oligophrenin-1 (OPHN1), a Rho-GAP protein highly expressed in neurons. In humans, all OPHN1 mutations cause the loss of function of OPHN1 leading to syndromic intellectual disability (ID) characterized by cerebellum abnormalities. In neurons, OPHN1 regulates dendritic spine density and architecture, actin dynamics and AMPA receptor (AMPAR) trafficking. Interestingly, we identified for the first time Oligophrenin-1 (Ophn1) as a novel target of sumoylation. Sumoylation is a post-translational modification essential to the modulation of several neuronal functions, including neurotransmitter release and synaptic plasticity. Altered sumoylation has been associated with neurological disorders. Here, we combined molecular biology with live imaging and super resolution microscopy to address the role of sumoylation in controlling OPHN1 function in hippocampal neurons. Furthermore, since the sumoylation site is located close to the novel missense mutation (G412D) identified in ID patients, we explored thrilling hypothesis that compromised sumoylation may lead to synaptic dysfunction associated to the ID phenotype. Altogether, our results clearly demonstrate that sumoylation is a novel regulatory mechanism tuning OPHN1 activity. Furthermore, since the ID-linked G412D mutation impacts OPHN1 sumoylation and affects spine density and morphology as well as the AMPAR surface expression to a similar extent as the non-sumoylatable OPHN1 mutant, our data support the hypothesis that impaired OPHN1 sumoylation may participate to the etiology of ID in patients carrying the G412D mutation.

NI13 | Selective behavioral alterations after acute particulate matter exposure in a pre-symptomatic Multiple Sclerosis mouse model

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Exposure to air pollution, and particularly to particulate matter (PM), has been associated with higher rates of Multiple Sclerosis (MS) relapses and increased neuroinflammation in MS patients, suggesting that PM exposure may contribute to MS exacerbation. To address this issue, we have combined the induction of MOG35-55-induced experimental autoimmune encephalomyelitis (EAE) in mice and PM10 exposure. To study the effects of both short- and long-term PM exposures, mice were exposed to PM10 at dosages relevant for human exposure either acutely, before the immunization or during the pre-symptomatic phase or chronically for 7 days pre-immunization + 7 days post-immunization. Both chronic and acute PM10 exposures did not significantly modify the disease course or the neuropathology of EAE mice. Yet, few hours after exposure, EAE mice acutely exposed to PM10 during the pre-symptomatic phase (PM-EAE) showed behavioral alterations - that could not be detected neither in control EAE (Ctrl-EAE) nor in PM-exposed wild-type (PM-WT) mice. Namely, when tested in the Open Field, Elevated Plus Maze and Novel Object Recognition tests, PM-EAE mice showed reduced anxiety and a significant increase in novelty seeking. Stereotypic behaviors (i.e. grooming and rearing) instead did not appear selectively affected in PM-EAE mice. Since the observed behavioral phenotypes are frequently associated with alterations of the dopaminergic neurotransmission, along with neuroinflammation markers, we are now studying whether PM10 exposure in EAE mice is associated with changes in brain dopamine levels or in the expression of genes coding for dopamine receptors/transporters and their dynamics/recycling. Our data indicate that PM10 exposure did not alter the EAE course, probably due to the low PM10 dose used to mimic the human exposure. However, acute PM10 exposure selectively induces behavioral changes in EAE mice, possibly interacting with their altered neuroimmune/neurotransmission background.

NI14 | The spinal cord plasticity: regionalization and time-course of neurovascular events following peripheral nerve injury

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The central nervous system (CNS) plasticity is significantly perturbed following peripheral injuries. Structural or functional alterations of the neurovascular unit (NVU) can be pivotal and early changes leading to maladaptive rewiring of the CNS. The Spared Nerve Injury (SNI) model was used to trigger a perturbation of CNS homeostasis. Rats were sacrificed at 24 h, 48 h and 7 days after SNI or SHAM surgery. Immunohistochemistry, immunofluorescence, and western blot techniques were performed. Analysis of rat spinal cord sections revealed a time-dependent response was differently modulated for microglia and astrocytes. Astrocytic pedicles (AQP4) polarization and morphology were evaluated for their involvement in the constitution of NVU together with the endothelial and basal lamina. To confirm the pleiotropy of protein usually confined to the vascular compartment we found the overexpression of the thrombin receptor PAR-1 and the concomitant increase of the vascular endothelial growth factor (VEGF) influencing the CNS circuitry, without the direct lesion of vessels or parenchyma. Our results shed new light on the CNS maladaptive plasticity in the early phases following peripheral injury. These findings prompt further studies on NVU and novel multi-target and time-dependent therapies.

NI15 | Comparison of brain damages between male and female in a model of encephalopathy of prematurity: study of a sexual dimorphism

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Premature birth is defined as birth before 37 weeks of pregnancy. Children born prematurely are vulnerable to various injuries, including brain lesions, and have a high incidence of neurodevelopmental disorders. Brain lesions, described as encephalopathy of prematurity (EoP) affect both the developing gray and white matter and is characterized by mild to moderate diffuse brain damages. White matter lesions are caused by oligodendrocyte maturation arrest leading to hypomyelination while gray matter injuries consist of a reduction in the growth of cortical and sub-cortical regions. Our team has developed a model of EoP based on systemic injections of IL-1β, reproducing the deficits seen in premature infants. Indeed, mice injected with IL-1β present moderate diffuse damages in the white matter, a blockage of oligodendrocyte maturation at the progenitor stage without modification of proliferation or survival, a decrease in Myelin Basic Protein (MBP) expression at P15 and P30, and a decrease in myelination. However, those experiment have only been conducted in male mice as EoP presents a sexual dimorphism, with higher prevalence in male. We now sought to use our method in female mice in order to assess whether it reproduces some of the injuries and outcomes seen in male and study this sexual dimorphism.

NI16 | The promoter methylation status, mRNA expression and production of TNFα, IL6 and IL10 in Multiple Sclerosis patients

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Multiple sclerosis (MS) is an immune-mediated inflammatory demyelinating disease of the central nervous system and epigenetic alteration may modulate inflammatory mediators, playing a pivotal role in MS pathogenesis. To shed more light on the involvement of cytokines in MS pathogenesis, we have investigated the association between cytokine production, mRNA expression and promoter methylation status in naïve MS patients.

Promoter methylation status of tumor necrosis factor (TNF)α, interleukin (IL)6 and IL10 genes was performed on whole blood of 31 MS patients and 16 healthy controls (HC), using the pyrosequencing method. The gene expression was evaluated by Real-time PCR in peripheral blood mononuclear cells and cytokines levels were measured in serum by ELISA assay.

The average methylation index calculated by PyroMark CpG software was significantly declined for TNFα, IL6 and IL10 promoters in MS patients in comparison with HC. Our results indicate that TNFα and IL6 genes hypomethylation can predict their increased mRNA expression and production, helping to discriminate between HC and MS patients. Instead, the expression and production of anti-inflammatory IL10 were lower in MS patients than in HC and not related with its hypomethylated promoter.

Due to the complex regulation of cytokine signaling, epigenetics' role in MS needs to be clarified by the analysis of circular RNA and microRNA (miRNA) as epigenetic modulators. Preliminarily, we observed a significant downregulation of miRNA-124 in MS patients compared to HC, consistent with the support of inflammation. Thus, further research in larger groups of patients and on additional epigenetic regulators is ongoing to better elucidate the functions of epigenetic modifications in MS pathogenesis, progression and activity.

NI17 | Neuroinflammation in Fabry's disease: a new insight into a multisystemic disease

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Fabry's disease (FD) is a rare X-linked lysosomal storage disease characterized by defective lysosomal enzyme α-galactosidase A (α-Gal), leading to multisystemic manifestations due to the accumulation of the globotriaosylceramide (Gb3). A hallmark symptom of FD patients is neuropathic pain that appears in the early stage of the disease, as a result of peripheral small fibres damage and central nervous system disease. Evidence regarding the extend of neurocognitive impairment is still limited, even though different forms of cognitive dysfunctions have been recently reported in FD patients.

Cognitive impairment is commonly accompanied by neurodegenerative and mental disorders, in which the neuronal dysfunction is probably induced by an excessive inflammatory response. To clarify whether a neuroinflammatory process could be observed in FD animal model, we studied the level of neuroinflammatory markers in 3-4 months-old and 12 months-old α-Gal A KO mouse.

We observed an increase in the pro-inflammatory marker IL-1β in both cortex and hippocampus, together with an increasing trend in the expression of TNF-α in the cortex of 12 months-old α-Gal KO mice compared to WT ones. This observation could indicate the presence of an inflammatory process in 12-mounth FD since the expression of the abovementioned markers was not detected in young mice. Moreover, the reconstruction analysis of microglia morphology showed a decrease in the ramification of microglia in 12 months-old α-Gal A KO mice compared to WT animals, compatible with an activated phenotype in response to inflammatory stimuli.

Taken together, this evidence could indicate an inflammatory response driven by microglial cells toward a pro-inflammatory phenotype although the ameboid morphology has been preserved by confirming the heterogenicity of the cell population.

NI18 | Time-dependent modifications in glia cells, macrophages and extracellular matrix supporting glioblastoma progression

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Glioblastoma (GBM) is the most common malignant brain tumor. GBM progression is promoted by intricate and dynamic crosstalk with resident glia cells, which undergo several molecular and morphological changes also reflected as extracellular matrix (ECM) modifications. To better understand the GBM's mechanism of action and its effects on the tumor microenvironment, context- and disease stage-specific molecular targets should be determined.
GL261 glioma cells were injected into the right striatum of immuno-competent C57Bl6J mice and the brains extracted after 7, 14, and 21 days (7D, 14D, 21D). Immunohistochemistry and western blotting analysis were conducted. In the early stage, the GBM growth was barely boosted as showed by spotted ki67+ cells distribution and moderate reactive astrogliosis (GFAP+, glial fibrillary acidic protein), which was progressively enhanced at later phases. The tumor bulk was established at 14D and infiltration of phagocytic cells (CD68+: Iba1+) was detected in the peritumoral area, suggesting their functional role in the ECM remodelling. Among the ECM modifiers, metalloproteinase-9, tenascin-C, and fibulin-2 increased at this stage, indicative of a selective re-organization of the ECM. Surprisingly, microglia and macrophages were scarcely localized at the site of injection with a decreased expression of monocyte chemoattractant protein-1 (MCP-1/CCL2) and Iba1, which increased later. Despite the amount of Iba1-positive cells at 21D, the microglia (TMEM119+ cells) response appeared inhibited, suggesting a differential regulation for tumor-associated microglia and peripheral macrophages during GBM progression. Analyzing the glioblastoma time course and its interplay with resident glia cells may provide key information to reveal the cellular mechanisms that regulate the tumor development, encouraging a proper multi-targeted approach that could be translated to the human disease.

NI19 | N-acetyl L-cysteine counteracts cerebellar inflammation and autism-like behaviours in mice lacking the Cntnap2 gene

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Autism spectrum disorder (ASD) represents a heterogeneous group of neurodevelopmental syndromes classically characterized by social interaction deficits and repetitive behaviors as well as several associated neurological symptoms. These conditions affect children from the early childhood and produce clinically significant developmental impairments. Immune dysfunction has recently emerged as major contributor to the neurodevelopmental deficits observed in people affected by ASD. This condition is often linked with a strong inflammatory state, which contributes to neurodegeneration. Cntnap2 -/- mice have widely been considered a robust animal model of ASD, as they show typical autism-related behaviors such as hyperactivity, social impairments as well as sensory deficits. In the current study, we analyzed the expression of classical pro-inflammatory cytokines, chemokines and molecules related to damage in the cerebral cortex, hippocampus and cerebellum of adult (6-9 months old) mutant and control Cntnap2+/+ mice. The expression of all key pro-inflammatory molecules (among which IL-6, TNF and IFNγ), as well as chemokines CCL3, CCL5, CCL8, and metalloproteinases MMP3, MMP8, MMP12, MMP13, were increased in the cerebellum of Cntnap2-/-mice. Transcriptomic analysis has therefore been performed, with the aim of characterizing in detail pro-inflammatory impairments in the cerebellum of Cntnap2-/- mice. Interestingly, motor and social deficits in Cntnap2-/- mice, tested using open field, rotarod and 3-chamber sociability tests, were rescued after treating the animals with antioxidant/anti-inflammatory molecule N-acetyl L-cysteine (NAC). In parallel, the expression of pro-inflammatory cytokines, chemokines and metalloproteinases in the cerebellum of Cntnap2-/- mice was reduced at the level of Cntnap2+/+ mice after NAC treatment. Taken together, these results suggest that autism-related behaviors in Cntnap2 mutant mice may be reverted after targeting cerebellar inflammation.

NI20 | MiR-142-3p is a critical modulator of TNF-mediated neuronal toxicity in multiple sclerosis

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Multiple sclerosis (MS) is the main neurodegenerative autoimmune disease of the central nervous system (CNS) in young adults. In MS and its mouse model experimental autoimmune encephalomyelitis (EAE), proinflammatory cytokines trigger 'synaptopathy', an early and potentially reversible synaptic dysfunction that promotes excitotoxic damage. Therefore, the interest in identifying synaptotoxic biomarkers and inflammatory molecular axis is increasingly emerging to dampen excitotoxicity in MS. Notably, Tumor Necrosis Factor (TNF) stimulation is strictly responsible for synaptic alterations and neuronal damage in both MS and EAE, but the underlying molecular mechanism is still unclear.
Small noncoding microRNAs (miRNAs) are new modulators of gene expression circulating in the cerebrospinal fluids (CSF), recently proposed as diagnostic and prognostic biomarkers for MS.
Here, we investigated miR-142-3p, a synaptotoxic microRNA induced by inflammation in EAE/MS, as a potential downstream effector of TNF-signaling. High levels of both TNF and miR-142-3p were detected in the EAE striatum and in MS CSF, and patients with elevated CSF levels of both molecules present the most unfavorable progression index. Electrophysiological recordings in EAE mice, supported by histochemical and molecular analyses, show that low miR-142-3p levels in the inflamed striatum of miR-142 heterozygous mice impaired TNF-dependent synaptotoxicity. Accordingly, TNF treatment was ineffective in healthy striatal slices incubated with LNA-anti miR-142-3p. However, both preclinical and clinical data suggested that miR-142-3p levels are independent of TNF increase. To clarify these results, we investigated a new neuronal permissive role for miR-142-3p able to modulate TNF-mediated synaptotoxicity. In conclusion, we propose miR-142-3p as a critical modulator of TNF-mediated neuronal toxicity in neuroinflammatory conditions and highlight the potentiality of anti-miR-142-3p therapy.

NI21 | Stavudine “interferes” via alpha-7nAChR to inhibit NLRP3 in (LPS+Amyloid-beta) stimulated PBMC of AD Patients

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Alzheimer’s disease (AD) is marked by neuroinflammation, cholinergic hypofunction, and decreased nicotinic acetylcholine receptor (nAChR) density from the cortex and hippocampus. α7nAChR firstly identified in the autonomous nervous system, is a ligand-gated ion channel exerted as a regulator in cognitive processes through the modulation of specific neurotransmitters. Cholinergic neurons and more recently resident macrophages of different tissue proved to highly express α7nAChRs and the activation of these receptors inhibits inflammasome-NLRP3 assembly by arrestin-beta1 (ARRB1) and the production of cytokines thereby attenuating the local inflammatory response suggesting that α7nAChR activation represents a useful therapeutic strategy for AD. The current study was performed to investigate the effects of Stavudine (D4T), an antiviral drug normally use for HIV-treatment, to modulated inflammation through α7nAChR in in vitro assay of cultured PBMC of 15 AD patients compared to 12 sex and age matched healthy control (HC). PBMC of all subjects enrolled in the study were cultured in unstimulated (MED) or primed with LPS (1 μg/ml) for 2h and Aβ (10 μg/ml) for 24h in presence/absence of D4T (50 μM) for 22h to evaluate: 1)TNF-α, IL-1β, IL-6, IL-18 and caspase-1 protein production detected by ELISA in supernatants of cells; 2) detection of ACht and anti-Ab42 antibodies levels in plasma of AD patients and HC by ELISA; 3) α7nAChR, TNF-a, IL-1β, IL-6, IL-18, Nlrp3, ARRB1 and caspase-1 mRNA expression by qPCR. The efficacy of D4T to damper NLRP3 was previously demonstrated in in vitro cells line experiments, herein data confirmed that in PBMC Stavudine reduces: 1) down-stream inflammasome derived protein production (IL-18, caspase-1 and IL-1β) and inflammatory cytokines (IL-6 and TNF-a) both in AD and HC ($p < 0.001$); 2) α7nAChR and ARBB1 mRNA only in HC with the same production of plasmatic Acth both for AD and HC. Cholinergic neurons and more recently resident macrophages of different tissue proved to highly express α7nAChRs and the activation of these receptors inhibits the production of inflammatory cytokines, thereby attenuating the local inflammatory response suggesting that α7nAChR activation represents a useful therapeutic strategy for AD.

NI22 | Characterization of astrocyte reactivity in a model of encephalopathy of prematurity

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Premature birth caused by maternal infection represent an increased risk factor of brain lesions affecting both developing gray and white matter, known as encephalopathy of prematurity (EoP), and long-term neurodevelopmental disorders. It has been suggested that the set-up of a pro-inflammatory environment with the secretion of cytokines and chemokines might initiate an inappropriate inflammatory response driven by reactive microglia and astrocytes, which participate to neurodevelopmental disruption. Astrocytes, located at the interface between the brain parenchyma and the blood brain barrier, preserve homeostasis. They also participate in the inflammatory response and go through morphological and functional changes called astrogliosis. However, little is known about astrocytic reactivity during perinatal inflammation. Our team has developed a mouse model of EoP based on systemic injections of IL-1beta (a pro-inflammatory cytokine) reproducing the deficits seen in premature infants. Using this model, we aim to precisely characterize astrocyte reactivity in EoP. Astrocyte subpopulations are highlighted using flow cytometry, emphasizing their heterogeneity. Bulk RNA sequencing of purified astrocytes showed a transcriptomic signature of astrocytes during perinatal inflammation. Analysis of A1/A2 phenotypes by quantitative RT-PCR revealed a pro-inflammatory phenotype of the astrocytic response along time. Significant morphological changes of GFAP+ astrocytes in the subventricular zone have been shown by immunohistochemistry. Functional differences have been studied by quantitative RT-PCR and revealed a decrease of synaptogenesis factors secreted by astrocytes. This in-depth characterization of astrocytes will pave the way for designing new strategies to restore the homeostatic functions of astrocytes and protect the brain of preterm infants.

NI23 | Microglia-derived Extracellular Vesicles are involved in synaptic pruning in vitro

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During development, microglia is responsible for beneficial synaptic pruning by phagocytosis of aberrant synapses tagged by complement factors. Moreover, excessive complement-mediated synaptic pruning is activated during neurodegeneration, causing pathological loss of synapses. However, how complement factors are delivered to synapses is not yet completely clear.
Extracellular vesicles (EVs) released by microglia carry multiple signals implicated in synaptic pruning and scan the neuron surface before establishing a stable contact with dendrites, thus representing ideal vehicles to tag synapses with molecules guiding microglial removal.
To investigate the role of microglial EVs in synaptic pruning we cocultured mature hippocampal neurons with wild type (wt) microglia or C9orf72 knock out (ko) microglia, which produce a double amount of EVs and more complement factors (C1q/C3) associated to EVs compared to wt cells.
While wt microglia induced a decrease in the density of Shank-2-positive (postsynaptic) but not Bassoon (presynaptic) puncta, C9orf72 ko microglia reduced the density of both Bassoon and Shank-2-positive puncta in neurons. Parallel quantification of synaptic puncta in wt and C9orf72 ko microglia revealed increased uptake of pre-synaptic markers in C9orf72 ko microglia compared to wt cells.
Importantly, pretreatment of C9orf72 ko microglia with GW4869, an inhibitor of EV biogenesis that reduces EV production by 50%, restored normal pre-synaptic density in microglia-neuron cocultures, while addition of microglial EVs to neurons before co-culturing with wt microglia induced a selective decrease in Bassoon-positive puncta, leaving the density of post-synaptic puncta unchanged.
Our results indicate that microglial EVs promote pre-synapses engulfment, thus possibly influencing the synaptic density during developmental critical periods as well in brain pathologies.
Analysis of synaptic density in hippocampi of C9orf72 are ongoing to validate in vivo microglial EVs-mediated pre-synaptic pruning.

NI24 | Pro-resolving lipid mediator neuroprotectin D1 ameliorates chronic experimental autoimmune encephalomyelitis by modulating macrophage plasticity and polarization

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Multiple sclerosis (MS) is a chronic inflammatory neurodegenerative disease associated to uncontrolled inflammation and autoimmunity and for which there is still an unmet need for new diagnostic and therapeutic options. Recent studies suggest that chronic inflammation can be a consequence of failure to resolve inflammation, the resolution of which is mediated by a superfamily of bioactive lipids mainly derived from omega-3 essential fatty acids and termed specialized pro-resolving lipid mediators (SPMs). Since by means of targeted-metabololipidomics we previously found a significant impaired production of several SPMs, including neuroprotectin D1 (NPD1), in peripheral blood of MS patients, herein we assessed its in vivo role in modulating the plasticity and activation of monocyte-derived macrophages, key cells involved in the immunopathogenesis of MS. To do so, acute and chronic experimental autoimmune encephalomyelitis (EAE) was induced in mice and these were daily injected with NPD1 in both clinical phases. M1-like and M2-like macrophages were differentiated from monocytes obtained from spleen of acute and chronic vehicle- or NPD1-treated EAE mice and analyzed by high-dimensional flow cytometry for their signature activation markers. Although NPD1 could not impact on clinical symptoms at the peak of the disease (20-22 dpi), it significantly ameliorated disease course and severity at the chronic phase (35 dpi) and this was associated to significant changes in signature M1-like (CD86, MHC-II, CD62L, CD40 and CD68) and M2-like (Trem2, CD11c, CD44 and CD200R) markers, with an M1-to-M2 phenotypic shift in NPD1-treated mice. Altogether, these findings provide compelling evidence that boosting resolution of inflammation with a specific pro-resolving agent, could ameliorate the chronic course of MS by reprogramming macrophages plasticity and polarization and converting them into a protective and pro-resolving phenotype.

NP09 | Traumatic Life Experiences During Specific Critical Periods in Life Lead to Diverse Developmental Trajectories

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Life experiences wield particular influence over brain development during restricted time windows (i.e., critical periods), when the brain is more responsive to environmental stimuli. Positive experiences during critical periods lead to healthy brain wiring and development of proper behavioral skills. Conversely, diverse traumatic life experiences (e.g., physical/sexual abuse, prolonged hospitalization, warfare) can damage the brain structure and connectivity, leading to neuropsychiatric disorders (e.g. depression, anxiety, post-traumatic stress disorder, attention-deficit/hyperactivity disorders and personality disorders). In this context, although the types of traumatic life experiences can vary largely, the consequent neuropsychiatric conditions are in a restricted number. Moreover, no direct links exist between specific traumatic life experiences and the insurgence of certain neuropsychiatric traits, suggesting that a particular traumatic event can trigger different psychiatric consequences. In C57BL/6J mice, we here investigated whether the timing of the traumatic life experience (predator odor exposure) can play a role in the mismatch between the type of trauma and the specific neuropsychiatric outcome. Our results revealed the existence of critical periods for adult psychiatric disorders, which differentially modulate the adult behavioral outcome and impair brain connectivity in mice.

NP10 | From anatomy to functional connectivity in the mouse brain assessed through assembly detection methods

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It stretches back about 70 years the concept of cell assembly as a unit of neural processing. To date, many definitions have been provided, all of them converging on the concept of cell assembly as a functional network of neurons that fire with a defined temporal pattern and has information coding properties. We thus embarked on a series of studies using cell assemblies as a tool to investigate several aspects of mouse brain functioning, starting from the functional connectivity of a wide variety of brain areas with a magnified temporal and spatial resolution and ending, in future, in the analysis of assembly encoding of several task variables. In our studies, we used an algorithm for cell assembly detection that allows, for each assembly, the selection of the optimal bin and lag, enabling us to identify neural assemblies spanning more than 60 cortical and subcortical areas of the mouse brain in an available dataset of recordings. In the first study, we focused on the Zona Incerta (ZI) that establishes broad anatomical connectivity and through its outputs exerts mixed excitatory or inhibitory effects on many targets; this led us to expect a diverse profile of probability to detect assemblies that, being verified by our results, corroborate the biological realism of this method as a valid tool to qualitatively investigate interactions between areas. Beside confirming functional connections between anatomically connected areas and assessing their relative weight, our results led us to speculate about functional connections that may be underlined by structural connections yet unexplored. In a second study, following the validation offered by the first one, we focused on the hippocampal formation and the prefrontal cortex, objects of keen interest for their cognitive relevance, and explored their functional connections, providing a rich database of assembly distribution that can lay a solid foundation for future studies that will correlate assembly dynamics with behavior.

NP11 | Mir-34a selectively modulates GABAergic activation within Dorsal Raphe Nuclei in response to stressful but not rewarding stimuli

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The Dorsal Raphe Nuclei are the main source of serotonergic innervation in the brain. Within the DRN, GABAergic interneurons locally regulate 5-HT release in target structures, in response to external stimuli. Interestingly, similar physiological activation of DRN GABAergic neurons is observed in response to either stressful or rewarding stimuli, resulting however in very different behavioral responses, thus suggesting the presence of a diverse nature of DRN GABAergic neuronal control. However, the molecular signatures characterizing and regulating stimulus-specific GABAergic neurons in the DRN are unknown. We have reported that microRNA34a (miR-34a) is highly expressed in the DRN and plays a role in mediating behavioral and neurochemical alterations induced by stressful events.

Here we hypothesize that miR-34a could selectively modulate GABAergic acute response to stressful but not to rewarding stimuli in the DRN.

Using histological, molecular, and genetic approaches we first examined miR-34a cellular localization in the DRN. Then, by in vivo microdialysis and ex vivo patch clamp recording, we evaluated if miR-34a regulates acute stress and food-related GABAergic transmission in DRN.

Within the DRN, miR-34a seems to be specifically expressed in GABAergic neurons. Here, the exposure to either acute stress or palatable food similarly causes the inhibition of GABAergic activity in control mice. Notably, by two complementary pharmacological and genetic strategies, we show that the blockade of GABAergic miR-34 in the DRN enhanced local GABAergic activity-by means of local GABA release and mIPSCs frequency recorded in in 5HT-positive neurons – in response to acute stress but not to palatable food.

This study proposes miR-34a as a selective GABAergic regulator of the DRN activity, involved in the response to stressful but not rewarding stimuli. We thus propose miR-34a as a molecular signature characterizing stress-responsive GABAergic neurons in the DRN.

NP12 | Alterations of cholesterol metabolism in experimental models of Rett syndrome

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Rett syndrome (RTT) is an X-linked neurodevelopmental disorder, representing the most common genetic cause of severe intellectual disability in girls. It is caused by mutations in the epigenetic factor methyl-CpG-binding protein 2 (MeCP2), mainly affecting the central nervous system. Besides the well-known neuronal and synaptic alterations, perturbed lipid metabolism has been reported both in brain and peripheral tissues of RTT mouse models and patients. Of relevance, cholesterol is involved in brain primary functions such as synaptogenesis and neurotransmission, and its alterations are associated with several neurological disorders. Since peripheral cholesterol cannot cross the blood-brain barrier, a biosynthetic pathway within the brain is necessary to maintain its physiological levels and astrocytes are thought to produce most of brain cholesterol. To determine if cholesterol metabolism is defective in RTT, we started characterizing the brain cholesterol biosynthetic pathway both in cultured astrocytes derived from Mecp2 null mice and in brain areas collected from Mecp2 deficient animals. By exploiting our RNA-Seq data we uncovered a deregulation of cholesterol pathway in Mecp2 null models, further confirmed by qRT-PCR. In particular, we observed a downregulation of cholesterol associated genes in cortex and hippocampus of symptomatic Mecp2 null animals and cultured astrocytes. Specifically, Nsdhl, an essential enzyme in cholesterol synthesis, resulted strongly downregulated both in the mRNA and in the protein levels. To analyze if these alterations result in a functional defect, we will measure cholesterol levels in brain areas of Mecp2 null mice and in cultured primary astrocytes, and we will uncover the effects of cholesterol supplementation on the synaptic phenotype. The final aim of our research is to determine if targeting cholesterol metabolism pathway might be a possible therapeutic approach for treating RTT.

NP13 | Botulinum neurotoxin as a tool to study the plasticity of motor axon terminals

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A remarkable property of the vertebrate peripheral nervous system (PNS) is the ability to remodel and regenerate after damage. This is recapitulated at neuromuscular junction (NMJ), the specialized synapse formed between motor neurons and skeletal muscles, where a precise orchestration of signals between motor axon terminal (MAT), muscle fiber (MF) and perisynaptic Schwann's cells (PSCs) is responsible for NMJ maturation and maintenance throughout life. Here, we used Botulinum Neurotoxin type A (BoNT/A) - a bacterial exotoxin that blocks acetylcholine release causing prolonged neuromuscular paralysis - to stimulate PNS remodeling via MAT sprouting and formation of novel neuromuscular synapses. We found that slow motoneurons innervating the slow-type muscle soleus undergo intense remodeling, while fast motoneurons innervating the fast-type muscle extensor digitorum longus (EDL) undertook little, if any, changes. To identify the molecular determinants of MAT sprouting, we collected NMJs from the soleus and the EDL via laser capture microdissection (LCM) and performed RNA sequencing. We identified differentially expressed genes (DEGs) and performed gene ontologies (GO) and found that the soleus muscle exhibits major changes with respect to the EDL. GO analysis revealed increased expression of transcripts involved in protein translation and extracellular matrix, whilst mitochondria-related genes were down-regulated. To investigate the specific contribution of MAT, MF and PSCs to sprouting and NMJ remodeling, we deconvolved the DEGs by comparing our dataset with cell type-specific datasets present in literature through appropriate statistical methods. We found that DEGs belonging to the MF show greater differences in response to BoNT/A intoxication, suggesting a major contribution of muscle cells to the sprouting process. Our plan is now to examine the role of specific hits to identify signals and signaling axis involved in MAT plasticity and NMJ remodeling.

NP14 | Mothers and sons: how bisphenols target brain and behaviors

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Bisphenols (BPs), synthetic compounds used in the production of plastics, are an extremely abundant class of Endocrine Disrupting Chemicals, i.e., exogenous chemicals, or mixtures of chemicals, that can interfere with any aspect of hormone action. The first and still the most globally produced BP is the bisphenol A (BPA). Stricter regulations and increasing concerns about its impact on human health have led to an extensive search for safe alternatives. Bisphenol S (BPS) is one of the most used BPA substitutes, however, it seems to display the same, or even worse, endocrine disrupting properties as BPA. Interestingly, brain and behaviors appear to be targets of both BPA and BPS, particularly relevant when the exposure occurs during critical periods of development of adult life. Thus, this study aimed to evaluate the effects of oral exposure, either during pregnancy and lactation or during the perinatal period, to low dose (i.e., 4 µg/kg BW/day, EFSA TDI for BPA) BPA or BPS in mice. Dams were orally treated from mating to offspring weaning. Within the first postnatal week, we observed spontaneous maternal behavior. Finally, we analyzed the oxytocin immunoreactivity in the hypothalamic magnocellular nuclei, known to be involved in the control of maternal care. In the perinatally-exposed adult offspring, we evaluated the anxiety-related behaviors and the serotonin immunoreactivity in dorsal (DR) and median (MnR) raphe nuclei, which are highly involved in the control of these behaviors. Both BPs affected the sex ratio and the offspring mortality. Treated dams displayed impaired maternal behavior, but only the BPA-ones also showed alterations in the oxytocin immunoreactivity. In adult offspring, we observed different effects of the BPs exposure on anxiety-related behavior in males (anxiolytic) and females (anxiogenic), along with alterations of serotonin immunoreactivity in the analyzed nuclei. Both adult and perinatal periods are sensitive to BPs' exposure which can lead to impairment in fundamental behaviors and related neural circuits.

NP15 | Sexually dimorphic organizational role of estrogen receptors on different neuroendocrine systems controlling metabolism and reproduction

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Many hypothalamic systems, controlling metabolism and reproduction, are programmed and stabilized during critical periods of development by many factors, including gonadal steroids. In particular, estradiol (E2) appears to have an important role on organization of these circuits. E2 acts through three different receptors: ERα, ERβ and GPR30.
To understand the role of these receptors on organizational effect of E2, we treated male and female CD1 mice from post-natal day (PND) 5 to PND12 with subcutaneous injections of vehicle (corn oil), E2 and E2 associated with selective antagonist of estrogen receptors (MPP; PHTPP; G15) alone or together (mix). We analyzed, during the development, different physiological parameters related to food intake (body weight, food eaten, daily feed efficiency, gonadal and brown fat), reproduction (gonads, puberty onset, estrus cycle) and behavior (Y-maze, sexual behavior). Furthermore, in the adult, we have immunohistochemically highlighted the expression of some hypothalamic neuronal circuits closely associated with food-intake and metabolism, but also with the reproductive sphere: Pro-opiomelanocortin (POMC), Neuropetide Y (NPY), Orexin and Kisspeptin systems.
In general, E2 induced effects mostly in females both on sexual and feeding behaviors. The treatments with G15 alone or in combination (mix) altered all the considered parameters in both sexes. On the contrary MPP and PHTPP showed sexually dimorphic effects. MPP modified, in males, feeding parameters, but not those related to reproduction, whereas PHTPP modified parameters related to reproduction, but not those related to feeding. In females the situation was exactly the reverse. In conclusion, our data demonstrate that E2 has a strong organizational role on different neuroendocrine systems, acting primarily on GPR30 and, in a sexually different way, on ERα and ERβ.

NO06 | Preclinical testing of a novel therapeutic approach to counteract Glioblastoma Multiforme

BACK

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Glioblastoma Multiforme (GBM) is the most aggressive form of brain tumors. The therapeutic strategies used to counteract GBM are not effective and, as a result, the average survival for this pathology is of about 15 months after diagnosis. Hence, there is an urgent need to find innovative therapeutic approaches that produce a real impact on GBM patients' quality of life and survival.

In our lab has been recently designed and tested a new recombinant protein conjugating Chlorotoxin (CTX), a well-known peptide able of crossing the blood-brain-barrier and selectively targeting glioma cells, to CNF1, a protein that leads glioma cells to death through the activation of a senescence process. In vitro and in vivo studies have highlighted the potential of CTX-CNF1 in counteracting GBM. However, these experiments were carried out only in the GL261 mouse model, that is poorly infiltrative and lacks of glioma stem cells (GSCs). In order to confirm the potential of CTX-CNF1 in contrasting GBM growth, we decided to test the effects of this treatment on another syngeneic mouse model, enriched in GSCs: the CT-2A. CT-2A cells were injected into the primary motor cortex of C57BL6/J mice, which received a weekly intravenous injection of active/inactive CTX-CNF1 (i.e., treated and vehicle group respectively). Motor dysfunctions and GBM growth were longitudinally monitored using motor tests (i.e., Grip Strength and Grid Walk) and MRI.

The goal of preclinical studies is to use animals to model a specific disease in which testing the biological effect of potential drugs, with the final aim to predict a treatment outcome in patients. It is worth noticing that a single mouse model barely recapitulate all the features of the human condition; thus, testing potential drugs on more animal models is a crucial step before moving to clinic.

NO07 | Lactate Metabolism and YAP/TAZ tumorigenic effect in Glioblastoma

BACK

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Glioblastoma is considered the most aggressive type of tumor that affects the brain. It is rich in proliferative and invasive malignant cells with an aberrant neovascularization capacity. It is highly resistant to radio- and chemotherapy. The cancer cells display increased glucose metabolism which is essential to maintain high proliferation rate and cellular functions.
It is well documented that altered glucose fluxes and modulation of several glycolytic enzyme activities can affect signaling pathways associated with the cell cycle in cancer cells. Hippo signaling pathway is one of the pathways that is extremely significant in cancer research as it restrains cell proliferation and promotes apoptosis. Dysregulation in this pathway promotes translocation of the co-transcription factors Yes associate protein (YAP) and WW Domain Containing Transcription Regulator 1 (TAZ) complex into the nucleus and leads to cell proliferation and hyperplasia. Evidences demonstrate that the YAP/TAZ complex is active when cells have an enhanced glucose uptake and glycolysis. Interestingly, the glycolytic enzyme phosphofructokinase 1(PFK1) was found to translocate into the nucleus, where it stabilizes the complex YAP/TAZ. This process induces the expression of oncogenes as well as enzymes involved in glycolysis, such as 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3). The latter indirectly activates PFK1, and together with YAP/TAZ they form a proto-oncogene complex along with the transcription factor Transcriptional enhanced associate domains (TEADs).
Here, we aim to determine whether PFKFB3 and/or PFK1 can be therapeutic targets to treat glioblastoma by inducing cancer cells starvation. These approaches will help us to evaluate the impact of PFKFB3 on the activity of the YAP/TAZ/TEAD1/PFK1 complex, in addition to its subsequent effect on cell viability, proliferation of glioblastoma cells, and tumor migration.

NO08 | Braf activation and Pten deletion in peripheral neural stem cells give rise to Schwannoma and peripheral nerve tumors

BACK

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Schwannomas are Schwann cell-derived nerve sheath tumors that appear sporadically and in association with genetic tumor syndromes such as Neurofibromatosis type 2 (NF2). Recent studies suggest that Neurofibromatosis (NFs) arise as a result of gene mutation at earlier stages in the Schwannoma cell lineage possibly as early as neural crest stem cells. We recently developed a mouse model in which activation of BRAFV600E mutation and Pten deletion are driven by the Tamoxifen-inducible Sox2-CreERT2, a deleter specifically expressed in telencephalic neural stem cells (NSCs). Sixty days after Sox2-CreERT2 induction in adult mice developed tumors originating from the ventricular cavities, that were classified as oligodendroglioma. One hundred percent of Sox2CreErt BrafV600E Pten flox/flox tamoxifen-treated mice also showed a nodular lesion localized ventrally to the hindbrain, near the pons. At higher magnification, we found that the lesion contained ganglionic cells entrapped by whorls of hyperproliferating cells reminiscent of Scarpa ganglion schwannoma. Since Nestin is known to be expressed in Schwann precursor cells, we further crossed Nestin-Cre-ERT2 transgenic mice with BRafV600E Pten flox/flox mice. Thirty days after tamoxifen injection mice showed neurological signs of disease and were euthanized. Similar to Sox2-inducible mutant mice, histological analysis of brains revealed the presence of bilateral nodular lesions identified as Schwannoma that were immunoreactive for Egr2 and S100. In addition, the dissected spinal cord from tamoxifen injected mice also showed dorsal root ganglion (DGR) schwannomas as well as a plexiform neurofibroma that can transform into malignant peripheral nerve sheath tumor (MPNST). Our results suggest that Braf activation and Pten deletion in adult pluripotent Schwann cell precursors can be responsible for Schwannoma and peripheral nerve tumor formation.

NO09 | Cullin3/REN^{KCTD11} and SALL4/HDAC1 interplay promotes Hedgehog-dependent medulloblastoma through GLI1 deacetylation

BACK

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Medulloblastoma (MB) is the most common paediatric brain tumor that arises from alterations in cerebellum development. MB shows a high molecular heterogeneity and is associated with poor prognosis. Multi -omics approaches distinguished four main MB molecular subgroups. The Sonic Hedgehog variant (SHH-MB) is the best genetically understood, characterized by mutations in key component of the SHH signalling and cytogenetic alterations. We previously identified the tumor suppressor REN^{KCTD11} (REN) as a key negative regulator of the SHH pathway localized in chromosome 17p (a genomic region lost in ~30% of human SHH-MBs). REN encodes for an adaptor protein of Cullin3 E3-ligase complex that promotes the degradation of HDAC1, a well-known SHH pathway activator. The identification of new REN interactors is primary to unveil molecular circuitry whose deregulation could contribute to MB onset. Through mass spectrometry, we identified Spalt-like transcriptional factor 4 (SALL4) as REN interactor. SALL4 is mainly expressed in embryonic stem cells (ESCs) and its activity is stemness-related. In several human malignancies, SALL4 expression is reactivated in adult tissues and often correlates with worse prognosis and lower patient' survival. Here, we identified that SALL4 is a substrate of REN, which induces its poly-ubiquitylation and proteasome-mediated degradation. Interestingly, SALL4 binds GLI1 (the final effector of the SHH pathway) and works in complex with HDAC1 to promote GLI1 deacetylation and to induce its transcriptional activity. Of note, genetic depletion of SALL4 inhibits SHH-dependent tumor growth both in vitro and in vivo. Accordingly, high SALL4 expression levels correlate with worse overall survival in SHH-MB patients. Our findings highlight the relevance of ubiquitylation/acetylation interplay in the SHH pathway regulation and identify SALL4 as an interesting target in SHH-dependent cancer therapy.

NO10 | Glioblastoma Tunneling Nanotubes as potential targets for nanomedicines

BACK

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Tunneling nanotubes (TNTs) are thin, dynamic, long membrane protrusions that interconnect cells and act as a route for cell-to-cell communication, allowing the intercellular exchanges of signal clues, molecules, organelles and pathogens. The presence of TNTs has been observed in several types of cancer, glioblastoma (GBM) included, where they emerge to steer a more malignant phenotype. GBM is the most prevalent and aggressive brain tumor with high grade of recurrence. It is a challenging brain tumor characterized by a heterogeneous, complex, and multicellular microenvironment, which represents a strategic network for treatment escape. In this context, we are studying TNTs in GBM to deepen both structural and genesis features. Moreover, we are investigating if TNTs can be exploited to improve the intercellular distribution of nanomedicines. We identified structural differences of TNTs formed by two cell types: healthy astrocytes more frequently formed “thin” TNTs, while GBM cells tended to form more stable “thick” that are more efficient in the transport of nanomedicines compared to “thin” ones. TNTs formed by GBM cells have a length between 20-100 µm, with a thickness of 200-300 nm. The formation and disruption of GBM TNTs are dynamic processes that can be monitored over time for up to 24 h after cell seeding. GBM cells are able to form an average of 1-2 TNTs/cell, with a density of 15 TNTs/40 cells. Moreover, we showed that nanomedicines, at safe concentrations, could be efficiently transported via TNTs between GBM cells, but with less extent through the ones formed by healthy astrocytes. We also confirmed that nanomedicines transfer between GBM cells is predominantly mediated by cell-to-cell contact, likely through TNTs rather than cell release. In conclusion, the preliminary results showed that TNTs are potential channels in between cells that can be exploit for the delivery of drug-loaded nanoparticles in a highly specific manner.

NO11 | Addressing the significance of tumor-released microvesicles in glioblastoma aggressiveness and invasion

BACK

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Glioblastoma (GBM) is the most aggressive primary brain tumour associated with a very poor prognosis. Intra-tumour heterogeneity remains a substantial barrier as it prevents the efficacy of pharmacological therapies. The presence of GBM Stem-like Cells (GSCs) with varying degrees of stemness heavily impacts on treatment success rates. Spatially heterogeneity is defined from the core to the edge of the tumor, therefore paired GSC-core and -edge primary cell lines were investigated for invasiveness. To promote tumor invasion GBM makes use of different communication routes with the neighbour environment, which include extracellular vesicles (EVs). Indeed, evidence reported in the literature suggest the involvement of the EVs released by GSCs in inducing cellular migration. EVs are mainly divided into exosomes (EXOs) and microvesicles (MVs). The migration capacity of EXOs and MVs released by both GSCs-core and GSCs-edge were investigated. Both GSC-core and -edge displayed a significant higher migratory capacity when incubated in the presence of MVs, whereas no effects were observed in the presence of EXOs. Overall, a higher significant migratory potential of the EVs derived from GSCs-edge compared to the ones derived from GSCs-core was observed. To assess the contribution of the EVs released by non-tumoral cells present in the tumor microenvironment, paired GSC-core and -edge were treated with EXOs and MVs derived from the surgical aspirate. MVs from surgical aspirate demonstrated to be more powerful than EXOs, suggesting that not only EVs from tumoral cells but also EVs from the stroma (neurons, glial and immune cells) are actively implicated in GSC mobility in vivo. These findings show widespread heterogeneity between core and edge GSC cell lines, as well as reciprocal intercellular communication via tumor and microenvironment-derived MVs which increases invasiveness.

BACK

NIM05 | Novel BODIPY-based sensor for selective detection of misfolded Tau protein in retinal and cortical iPSC derived models for Frontotemporal Dementia

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Tauopathies, such as frontotemporal dementia (FTD) and Alzheimer's diseases, are characterized by the hyperphosphorylation and accumulation of the microtubule-associated protein Tau in the human brain, leading to a synaptic and neuronal loss. Numerous studies have shown a strong correlation between the number of neurofibrillary tangles of the Tau protein and Alzheimer's disease progression, making the quantitative detection of tau very promising from a clinical point of view. To investigate the complex Tau aggregation, it is beneficial to use fluorescence sensors that enable to detect and quantify pathological Tau aggregates. Here we describe the characterization of a fluorescent probe, consisting of a BODIPY core (BT1), with excellent photophysical properties and high selectivity. BT1 was tested onto human control and FTD iPSCs derived retinal and cortical neurons showing good affinity for hyperphosphorylated Tau protein filaments with minimal background noise.

NIM06 | Relationship between fatigue, disability, and reserve in patients with MS: a cross-sectional and longitudinal analysis

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Background and Aims: Fatigue is among most debilitating and common symptoms in multiple sclerosis (MS). Here, we hypothesized that individual resilience could affect motor and cognitive fatigue in MS patients, as already described for cognitive and motor disability, and explored the impact of clinico-demographic features and brain structural damage on fatigue.
Methods: Fifty-four MS patients were prospectively enrolled and underwent clinical examination (including Expanded Disability Status Scale-EDSS, Symbol Digit Modalities Test-SDMT and Beck Depression Inventory II-BDI) and MRI acquisition at baseline and after a mean follow-up of 14 months. Physical and cognitive MS-related fatigue was evaluated with the respective Modified Fatigue Impact (MFIS) subscales (MFIS-P and MFIS-C). Structural brain damage was estimated as white matter (WM) lesion load (JIM 6.0) and brain volume (SIENAX and SIENA). Percent change over time (%c) for clinical and MRI variables were also computed. A cognitive reserve index (CRI) was estimated by combining educational level, premorbid IQ and the participation in cognitive leisure activities. Brain reserve was expressed as sex adjusted intracranial volume (ICV). The association between putative risk factors (age, gender, phenotype, EDSS, SDMT-z, BDI, log transformed WM lesion load and normalized brain volume-NBV, brain reserve, cognitive reserve) and fatigue scores was assessed using bivariate correlations (preliminary screening) and hierarchical linear regressions. To explore the impact of risk factors on fatigue changes over time, partial correlations were tested between baseline features, their %c and fatigue scores %c, accounting for follow-up interval.
Results: At the cross-sectional analysis, MFIS-P was correlated with age, EDSS, BDI, NBV (r ranging from 0.01 to 0.001), but only marginally with brain reserve ($p=0.06$). The full regression model accounted for 32% of the variance in MFIS-P ($p=0.001$). The only variable accounting for significant variance was BDI ($p<0.001$). MFIS-C was correlated with BDI ($p<0.001$). As per the longitudinal analysis, none of the baseline features was associated to MFIS-P and MFIS-C %c. BDI %c was associated to MFIS-P and MFIS-C %c ($r=0.55$, $p<0.001$; $r=0.57$, $p<0.001$).
Conclusions: Among the explored features, only depression was strongly associated to both physical and cognitive fatigue. Brain and cognitive reserve did not affect fatigue symptoms in MS patients.

NIM07 | A Systematic Review of M-EEG evidence on value-based decisions in humans: experimental paradigms and spatiotemporal characteristics

BACK

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Decision-making is an integrative process that is crucial for humans and animals on a daily basis, and it has been at the centre of a long tradition of psychological and economic research. This systematic review focuses on the spatiotemporal dynamics of value-based decision-making. Since there is an abundance of fMRI, neuropsychological, and animal studies on the topic, we instead examined evidence collected from 100 magnetoencephalography and electroencephalography studies, which are known for their high temporal resolution. Additionally, we classified value-based decisions into 'external' (EDM) and 'internal' (IDM) and used this division as the theoretical framework of the present review. In 'external' decisions, the values of different options are objectively defined, whereas said values in 'internal' decisions are defined by the individual. The review aims to assess whether there is a convergent pattern of event-related potentials or fields (ERP/ERF) findings and how EDM and IDM processes have been studied so far with these methods. Based on precedents in the literature, we extracted statistically significant time intervals that result from contrasts between experimental conditions that are sensitive to differences in (external or internal) value. We also examined the topographical and source space distribution of these intervals. Finally, we classified the paradigms into specific clusters of experimental designs, an approach that will guide future research and inspire the development of novel tasks to study value-based decisions. Overall, our findings show that there are similarities as well as differences in the time course and the topography of EDM and IDM processes. To our knowledge, the current review is the first to directly contrast these two types of decision-making and to provide an in-depth description of the paradigms and ERP/ERF components that are most consistently reported in the field.

NIM08 | SANDIAMICO: an open-source toolbox for Soma And Neurite Density Imaging (SANDI) with AMICO

BACK

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The soma and neurite density imaging (SANDI) model has been recently proposed to estimate Magnetic Resonance Imaging (MRI) indices of apparent neurite and soma density noninvasively in the brain. The SANDI model assumes that soma (neuronal and glial cell bodies) and neurites (axons, dendrites, and glial processes) can be approximated as two non-exchanging compartments, modeled as spheres of certain size and cylinders of zero radius ("sticks"), respectively. However, the estimation of these parameters from Diffusion-Weighted Magnetic Resonance Imaging (dMRI) measurements is challenging and time consuming when using conventional fitting methods. To overcome this issue, we propose SANDI_AMICO: a new implementation of SANDI inside the Accelerated Microstructure Imaging via Convex Optimization (AMICO) framework.
SANDI_AMICO rewrites the SANDI model as a linear system Ax=y, where A is a matrix containing simulated signals (i.e., response functions) of each compartment, y is the vector of measured signals, and x is the vector of unknown contributions. In general, how we build matrix A impacts the results of the estimated model parameters. Here we propose a data-driven approach for the design of an optimal matrix A, given a dMRI acquisition and the SANDI model.
We used both analytical simulations and in vivo human data to compare the performance of AMICO with and without matrix A optimization under controlled and real conditions, respectively. On synthetic data, SANDI_AMICO showed higher robustness to noise, and it was over 30x faster than a conventional fitting method. On in vivo data, we observed that the variations of the apparent soma density over the midpoint cortical surface followed the expected cyto-architectonics of several Broadmann's areas. We showed that the SANDI_AMICO implementation provides a fast and robust estimation of SANDI parameters furthering their use in several neuroscience applications.

ND20 | Investigating the role of microglial TDP-43 in brain development

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TDP-43, encoded by the TARDBP gene, is a highly conserved DNA/RNA binding protein that shuttles between the nucleus and the cytoplasm, in both neurons and glial cells. Its aberrant cytoplasmic aggregation, commonly associated with depletion of nuclear TDP-43, is a hallmark of Amyotrophic Lateral Sclerosis and frontotemporal lobar degeneration. Microglia, the innate immune cells of the brain, are involved in a variety of physiological processes required for proper brain development and function. We previously showed that microglial TDP-43 regulates phagocytosis and that mice selectively lacking TDP-43 in microglia during adulthood display pathological synapse loss and defective motor behavior. Here, we hypothesize that microglial TDP-43 plays important roles for early brain maturation, thus providing susceptibility for developing neurological disorders. We investigated possible developmental defects in Cx3cr1CREert2;Tardbpflox/flox (cKO) and Cx3cr1CREert2;Tardbpwt/wt (control) mice at post-natal day 15. cKO mice display decreased microglial density and altered microglial morphology, characterized by increased cell volume and surface area. Unbiased diffusion MRI analysis of the whole brain revealed sex-dependent microstructure alterations specifically in the somatosensory and motor cortex (SMC) of cKO mice. Such structural changes were associated with myelin abnormalities in the SMC, reflected by increased size and number of MBP+ spheroids, and overall myelin disorganization. In parallel, we observed a decrease in the number of oligodendroglial progenitor cells (OPCs) identified as PDGFRa+ or Sox2+/Olig2+ cells, but not in mature oligodendrocytes (Sox2-/Olig2+). Interestingly, the decrease in OPCs was found in the SMC, but not in the hippocampus, suggesting specific structural alterations upon microglial TDP-43 KO in the brain. Overall, these findings indicate that microglial dysfunction induced by the loss of TDP-43 is important for postnatal SMC normal maturation.

ND21 | Tau aggregation affects glutamatergic genes expression

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The early stages of Alzheimer's Disease (AD) and tauopathies are characterized by neuronal hyperexcitability due to increased glutamate signalling, while the advanced stages undergo a progressive reduction in glutamate release. We recently demonstrated that at early stages, the accumulation of soluble non-aggregated tau protein occurs in the nucleus and modulates the expression of genes involved in glutamatergic transmission. Here, we focused on studying the nuclear aggregation of tau and its involvement in gene expression by reproducing late pathological stages in a neuronal cell line. We show that tau is able to aggregate into amyloidogenic inclusions directly in the nucleus without the contribution of cytoplasmic tau. Aggregates trap excess soluble tau and abolish the increased Tau-dependent expression of the glutamate transporter VGluT1. Furthermore, we observed an up- and down-regulation of VGLuT1 expression in the prefrontal cortex of AD patient's brain at early and late stages, respectively. The Gene Set Enrichment Analysis show that Tau modulation on gene expression along the disease progression can affect the protein pathways of the glutamatergic synapse. Altogether, these results provide a demonstration of how the alteration of the glutamatergic pathway is linked to a novel Tau function, giving an explanation to events, such as neuronal hyperexcipability, observed during AD progression.

ND22 | Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease by moving at the axon surface

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Synaptic dysfunction occurs early in Alzheimer's Disease (AD), involving progressively larger areas of the brain over time. However, how it starts and propagates is unknown. We show that amyloid-beta (Aβ) released by microglia in association with large extracellular vesicles (Aβ-EVs) alters dendritic spine morphology in vitro and impairs synaptic plasticity both in vitro and in vivo in the entorhinal cortex-dentate gyrus circuitry. 1h after Aβ-EV injection into the mouse entorhinal cortex (EC), long-term potentiation (LTP) is impaired in the EC, while 24 h later it is impaired also in the dentate gyrus (DG), revealing a spreading of LTP deficit between the two synaptically connected regions. Similar results are obtained by injecting EVs carrying Aβ naturally secreted by CHO7PA2 cells, while neither Aβ42 alone nor inflammatory EVs devoid of Aβ are able to propagate LTP impairment. Using optical tweezers coupled to time-lapse imaging to study Aβ-EV-neuron interaction, we show that Aβ-EVs move anterogradely at the axon surface and that their motion can be blocked by annexin-V coating. Importantly, when Aβ-EV motility is inhibited, no propagation of LTP deficit occurs along the EC-DG circuit, implicating large EV motion at the neuron surface in the spreading of LTP impairment. Accordingly, proteomic analysis displays differences in the composition of Aβ-EVs vs. EVs from microglia not exposed to Aβ. The influence of mesenchymal stem cell (MSC) indirect co-colture with microglia primed with Aβ on cell phenotype, EVs and functions is currently being explored. Our data indicate the involvement of large microglial EVs in the rise and propagation of early synaptic dysfunction in AD, and suggests a new mechanism controlling the diffusion of large EVs and their pathogenic signals in brain parenchyma, paving the way for novel therapeutic strategies to delay the disease. Fundings: Horizon2020#874721PREMSTEM, 2018-AARF-588984

ND23 | The synergistic role of SMN and eIF3e in ribosome heterogeneity and the impact of their loss in Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disorder caused by decreased expression of Survival Motor Neuron (SMN) protein. The mechanism through which the disease develops is unknown yet. Recently, it has been demonstrated that SMN protein is a Ribosome Associated Factor (RAF), and that SMN-primed ribosomes modulate the translation efficiency of a specific subset of transcripts related to the disorder. To understand the extend of SMN-primed ribosome heterogeneity, we obtained the entire catalogue of protein interactors of SMN-primed ribosomes. By proteomics analysis of SMN-primed ribosomes obtained from control mouse tissues, we identified hundreds of RNA-independent interactors. This finding supports the hypothesis that ribosomes are much more complex molecular machines than previously thought. To identify putative direct SMN interactors on ribosomes, we intersected our proteomics analysis with the SMN proximal interactome and found several proteins belonging to the eIF3 complex. In addition to its classical role in translation initiation, eIF3 has been recently described as a RAF involved in muscle physiology. By integrating SMN- and eIF3e-specific multi-omics data, we observed a robust and significant overlap between down-regulated transcripts upon SMN loss and eIF3 silencing, and RNAs associated with SMN-specific and eIF3-specific ribosomes. Intriguingly, both SMN and eIF3e-specific ribosomes display a preference for binding mRNAs with IRES-like sequences. Next, we confirmed the association of SMN and eIF3e to ribosomes and ribosomal subunits in multiple control tissues and the loss of their association in SMA or in eIF3e KO-cell lines, suggesting that decreased levels of SMN lead to a corresponding alteration in the co-sedimentation profile of eIF3e with ribosomes. These results highlight the existence of a functional interplay between SMN and eIF3e on ribosomes, strengthening our hypothesis that SMN plays a crucial role in translation regulation.

ND24 | A preliminary in vitro study to assess the stressor effect on Amyotrophic Lateral Sclerosis onset and progression

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Nowadays, worldwide people are continuously exposed to many stressors, due to exhausting lifestyle, increased population density, pollutants and environmental/global changes. These conditions can trigger several cellular alterations, in turn predisposing to neurodegenerative diseases, as Amyotrophic Lateral Sclerosis (ALS). ALS is a motor neuron (MN) disease, determining weakness, muscle atrophy and premature death. It is characterized by excitotoxicity, oxidative stress and neuroinflammation, cellular processes activated also by stressor exposure. This study aims to clarify the contribution of different stressors in causing/anticipating ALS, since many mechanisms are still unclear. Preliminary experiments have been set-up in vitro, using NSC-34 cells expressing hSOD1(G93A) gene under the control of a doxycycline-inducible promoter. To differentiate the cells in MN-like cells, different retinoic acid (RA) concentrations have been tested: RA (1, 5, 10, 15 or 20µM) was added to the culture medium for 2, 4, 6 and 8 days. Based on the MTT assay results, 20µM RA for 4 days represents the most proper condition to induce cell maturation. Concerning the overexpression of hSOD1(G93A), the cells were grown in complete medium and 5µg/ml of doxycycline for 24h, confirming hSOD1 expression by WB, both in undifferentiated and differentiated cells. Finally, to mimic a stress condition, cells underwent oxygen glucose deprivation: CoCl2 was used as hypoxic agent and its toxicity was measured by MTT assay: different concentrations of CoCl2 were evaluated in both high and low glucose medium, suggesting 100µM CoCl2 in low glucose as optimal stress condition. With these preliminary experiments, we have set-up the conditions for the next analyses, to evaluate genetic/epigenetic mutations and cellular/molecular alterations, and to clarify the stressor impact on the neurons and on the predisposition to neurological pathologies.

ND25 | Multifunctional liposomes increase synaptic transmission strength in mouse cortical neurons

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Alzheimer's disease (AD) is characterized by the accumulation of plaques of β-amyloid (Aβ) peptide in the brain. Given its pivotal role, new nanotechnological therapeutic tools targeting Aβ are in demand. For this purpose, we synthesized bifunctional liposomes (mApoE-PA-LIP) with a peptide derived from the apolipoprotein-E receptor-binding domain (mApoE) for blood–brain barrier (BBB) targeting and with phosphatidic acid (PA) for Aβ binding. Our previous results indicate that mApoE-PA-LIP can destabilize brain Aβ aggregates and promote peptide removal across the BBB and its peripheral clearance both in vitro and in vivo models with memory improvement. We firstly evaluated the impact of mApoE-PA-LIP on intracellular Ca2+ dynamics in hCMEC/D3, as an in vitro human BBB model, and in cultured astrocytes, that are both among the main components of the neurovascular unit. Our results show that the mApoE-PA-LIP pre-treatment increase the ATP-evoked intracellular Ca2+ waves, both in presence and in absence of extracellular Ca2+, indicating that this effect is mainly due to endogenous Ca2+ release from endoplasmic reticulum (ER). Indeed, blocking Sarco-ER Ca2+ ATPase (SERCA) activity with cyclopiazonic acid, ATP failed to trigger any intracellular Ca2+ waves, indicating that metabotropic purinergic receptors (P2Y) are mainly involved. So, we assessed whether mApoE-PA-LIP can modulate the neuronal synaptic transmission in the cortical area. For this purpose, we performed electrophysiological recordings, using the whole-cell patch-clamp technique, on mouse brain slices. We evaluated inward and outward total currents, firing pattern, spontaneous Excitatory Post-Synaptic Currents (sEPSCs) frequency and amplitude before and after the perfusion with mApoE-PA-LIP. Our preliminary data suggest that mApoE-PA-LIP trigger an increase of sEPSCs frequency. The here outlined results could give additional support to promote mApoE-PA-LIP as putative therapeutic tool for AD treatment.

ND26 | Co-ultramicronized Palmitoylethanolamide/Luteolin prevents alteration in astrocyte-oligodendrocyte crosstalk relevant for myelination in an in vitro model of β-amyloid toxicity

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Astrocytes are cells pivotal for the correct functioning of the brain. One of their less explored functions concerns their ability to affect myelination by supporting oligodendrocytes precursor cells (OPCs). Brain imaging studies link myelin impairments with Alzheimer's disease (AD), appointing beta-amyloid (Aβ) deposition as a possible etiological factor. However, recent evidence reported that Aβ stimulates OPCs maturation. To clarify this counterintuitive evidence, we studied the interaction between astrocytes and oligodendrocytes, with particular emphasis on astrocyte reactivity and the release of trophic factors required for OPCs maturation, by setting up an in vitro model of Aβ1-42 toxicity. In a transwell system we posed in co-culture rat primary astrocytes and OPCs. We treated astrocytes with human Aβ1-42 and analyzed what astrocytic alterations relevant to myelination occurred , as well as how that challenge affected the maturation of co-cultured OPCs. As a possible treatment to dampen Aβ1-42 effects, we tested a formulation of palmitoylethanolamide combined with the flavonoid luteolin (co-ultra PEALut), that we previously demonstrated to be able to counteract Aβ-induced alteration.
Astrocytes exposed to human Aβ1-42 increased the transcription of pro-inflammatory cytokines and reduced that of specific factors implicated in OPCs maturations. We also observed severe morphological changes in co-cultured OPCs, that lose the complexity of arborization, indicating aberrant maturation. Co-ultra PEALut prevented such pathological changes and some of these effects were mediated by the peroxisome proliferator-activated receptor alfa.
Considering that co-ultra PEALut is already approved for human use as a dietary supplement, altogether our findings open new opportunities for the treatment of diseases characterized by myelination impairments such as AD.

ND27 | Zebrafish as a model for Alexander disease

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Alexander disease (AxD) is a rare pathology affecting astrocytes, occuring as Tipe I and Tipe II, characterized by different clinical manifestations ranging from seizures to developmental delay. AxD Tipe I is the most common form of the disease and is characterized by an early onset and a faster progression with respect to AxD Tipe II. AxD is caused by heterozygous mutations in the glial fibrillary acidic protein (GFAP) gene, encoding the GFAP protein, an astrocyte's intermediate filament. The mutated GFAP is prone to form aggregates together with Heat Shock Protein 27 (HSP27), αB-crystallin, ubiquitin and proteasome subunits. These aggregates, also known as Rosenthal Fibers, are cytotoxic and are considered a hallmark of the disease. The aim of this study has been the creation of a transgenic zebrafish model with R239C mutation causing AxD, based on Tol2 transposon approach. Our results demonstrate that the transient mutant well reproduces the main features of AxD, such as glial localization of aggregates. By high-density microelectrode array platform (HD-MEA), we studied the electrophysiological response observing a significant difference in the mean burst duration and rate in mutants compared with control. Stated the reliability of the transient zebrafish line for AxD, we proceeded creating a stable transgenic line on which we conducted a transcriptomic analysis to further investigate and better characterize the molecular pathways involved this disease.

ND28 | Mitochondrial SMN1-anticorrelated genes as potential targets for Spinal Muscular Atrophy therapy

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Spinal Muscular Atrophy (SMA) is a pediatric disease caused by the mutation of survival motor neuron 1 (SMN1) gene and, consequently, low levels of SMN protein. It determines not only motor neuron (MN) loss in brainstem and spinal cord, but also the impairment of peripheral tissues (i.e. skeletal muscles and heart). Nowadays, the identification of new targets and therapeutic strategies is necessary to overcome some limitations of the SMN-dependent available therapies. Recently it has been demonstrated that stress conditions can induce a morphofunctional "switch" in mitochondria that are considered the "powerhouse of the cells". Since all the affected tissues in SMA require a lot of energy, mitochondria can be studied as promising targets for the investigation of new treatments.
In this context, through a bioinformatic approach, we searched for SMN1-anticorrelated mitochondrial genes whose expression could be normalized to regulate mitochondrial functionality. In particular, we identified some genes expressed in the most affected tissues in SMA (spinal cord, brain, skeletal muscles and heart): GCSH, COX7A1, BAG1, GOLPH3, DNAJC5, SLC25A36, GLRX2 and UQCRC2. To assess their levels in SMA, we analyzed tissues obtained from SMNdelta7 mice (an intermediate model of SMA) after a period of behavioral testing, from postnatal day 2 (P2) to P5, sacrificing WT and SMA animals in an early symptomatic stage of the pathology (P5). Molecular analysis on collected samples revealed a marked upregulation of GCSH in SMA spinal cord, brain and gastrocnemius, compared to controls.
Starting from this evidence, we can hypothesize an existing relationship between SMN1 and GCSH expression. Therefore, normalizing GCSH levels could lead to the restoration of mitochondrial integrity and to a recovery from cellular disease impairments.

ND29 | The chrOMICles of ALS spinal cord organoids - OMIC characterization of patient-derived spinal cord organoids to unravel new therapeutic targets in C9ORF72 form of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a rare neurodegenerative disorder involving motor neurons (MNs) in brain and spinal cord, resulting in progressive muscle atrophy and weakness that eventually compromise diaphragm functionality. No efficacious treatment is currently able to halt or reverse disease progression, making it mandatory to develop novel therapeutic approaches that would improve the lives of the patients affected by this devastating disease. In the study of pathophysiology, 3D models are a promising powerful tool that can recapitulate the complex architecture of tissues in a more accurate manner than 2D cultures. The objectives of this work included the characterization of spinal cord organoids to refine the reliability and reproducibility of the differentiation protocol, as well as to delineate the ALS phenotype with omic techniques; over and above, the selection of ALS-related candidates from the outlined transcriptomic and proteomic profiles. Our spinal cord-like organoids displayed neural progenitors that progressively decreased, post-mitotic neurons, MNs, and glia. Organoids were collected at 30, 55, and 80 days in vitro (DIV) and evaluated for their morphology and neurodevelopmental features by IHC and qPCR. Specifically, DIV80-organoids expressed SMI32, TUBB3, MAP2, DCX, OLIG2, PAX6, HOXB4, GFAP, and S100β. Besides astrogliosis, the C9 condition interestingly showed PRPH aggregation, as described in literature. Mass spectrometry and gene ontology depicted an enrichment in pathways related with cytoskeletal coordination, synaptic functionality, astrocyte reactivity, and stress response in C9-ALS condition. Single-cell RNA sequencing and gene annotation disclosed the predominance of neuroectoderm and neural cell populations in the samples, remarking the potential of this disease model. Overall, this project might allow the assessment of novel candidate genes linked with C9ORF72-ALS pathogenesis and their potential as therapeutic targets.

ND30 | Towards understanding the role of translational heterogeneity in SMA disease

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A significant body of work utilizing a range of different animal and cellular models has expanded our understanding of the diverse role(s) of the Survival Motor Neuron (SMN) protein, depletion of which is the root cause of SMA. First-generation antisense oligonucleotide treatment and gene therapy to enhance the levels of SMN protein represent a landmark achievement in the treatment of SMA patients, however none of the single treatment/therapies can be considered as a 'cure' for the disease. The limitations of these treatments suggest a need to develop a next-generation of therapies. The association of SMN protein with ribosomes has been recently discovered. These ribosomes, termed "SMN-primed ribosomes", are known to control the translation of specific mRNA transcripts, which showed translational defects at pre and early stages of disease in brain of 'severe' Taiwanese SMA mice. In this study, we have extended these investigations in order to ask whether translational defects can be detected in different tissues and at stages of disease in the milder Smn2B/- mouse model. Using two complementary techniques, namely ribosome and polysome profiling, we aim to establish whether translational defects are common across multiple mouse models of the disease. These findings will be crucial to identify whether translation defects are dependent on the SMN levels present within individual SMA mouse models. Preliminary results revealed a decrease in the fraction of ribosomes in polysomes in Smn2B/- mice, consistent with previous observations in a more severe model of SMA, suggesting that these defects are associated with defective association of SMN to the translation machinery in vivo. Interestingly, initial results also indicate that the splicing derangements during SMA may be resulted due to the defective translation of particular transcripts at the pre and early stage of the disease.

ND31 | Cognitive frailty and oxygen-ozone therapy: differential expressed genes as predictive biological markers of response/improvement to treatment

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Frailty is a multidimensional geriatric syndrome characterized by increased vulnerability to stressors as a result of the reduced functional capacity of different physiological systems. This heterogeneous clinical syndrome is known to show not only a physical or biological dimension but also a multidimensional concept, that includes cognitive frailty (CF). Biological mechanisms and specific treatments for CF are still understudied. Oxygen-Ozone (O2-O3) therapy is a no-invasive/no-pharmacological low-cost procedure with no side effects based on therapeutic effects of low O3 concentrations that can induce a mild oxidative stress stimulating antioxidant defenses, and preventing the inflammatory response and cell damage. We hypothesized that O2-O3 therapy might induce a significant effect on those oxidative and inflammation processes, strongly altered in CF. We thus conducted the first pilot double blind randomized controlled trial where the group of elderly frail subjects aged between 65 and 80 were stratified as untreated, treated with O2 and O2-O3 mixture. The methodology consisted in rectal insufflations for 5 weeks (3 sessions for week), and a total amount of 150cc of O2-O3 mixture at the concentration of 30 µg of O3 per cc of O2 over a 5-10 min period was administered. The mRNA profiling was analysed in whole blood from 72 CF subjects by Agilent microarray, and measured before (T0) and 3 months post-treatment (T1). Preliminary results highlighted a specific mRNA response to treatment, which involves differentially expressed mRNAs of several pathways. These biomarkers will be integrated with clinical and neuropsychological data, allowing to predict and optimize the therapeutic response to O2-O3 therapy.

ND32 | A new intrabody based optogenetic tool to degrade the aggregation prone proteins

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Neurodegenerative diseases, such as ALS, are characterized by the presence of misfolded protein that produce toxic aggregates. Since the mechanism of the aggregation remains unclear, one strategy could be to find a way to induce the degradation of the aggregates. The ability of intracellular antibodies to bind a pathological protein within the cell environment is not enough to reduce the toxic phenotype. The degradation of the complex, composed by intrabody and toxic target, can only be achieved by introducing a degron domain which is able to activate the ubiquitin proteasome system. The selection of an appropriate degron is a key aspect to develop a tool for the modulation of the proteasome activity, but it may not be sufficient to control the timing of the degradation pathway. In this work, we create a target specific photosensitive degron domain fused to a camelid intrabody directed against TDP43 protein to modulate the degradation of TDP43 protein by using a blue light stimulation protocol on mammalian cell cultures. Our data show that the photosensitive degron, made by photoreceptor light oxygen voltage (LOV) domain and ornithine decarboxylase enzyme fused with a nanobody directed against TDP43 protein, is a powerful tool to detect and reduce the protein level. We provide the proof of concept for the optimization of the illumination protocol in mammalian cells using the aggregation prone protein TDP43 as target for degradation and we also show the application of our tool against different targets involved in neurodegeneration.

ND33 | The neuropathology of the SARS-CoV-2: an autoptic COVID-19 "biobanking" of brain specimens for future translational biomedical research

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Neurological symptoms are detected in Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infected individuals and biomarkers of viral involvement of the nervous system in individuals who died of coronavirus disease 2019 (COVID-19) related respiratory failure are documented (*NEUROCOVID*). Autopsy is the "*gold standard*" for understanding the etiopathogenetic mechanisms associated with the onset of morbid processes, including the COVID-19 pandemic. Since 2019 up to now we performed 500 autopsies , including 200 on deceased patients with diagnosis of SARS-CoV-2 related pneumonia to provide clinicians with appropriate feedback. About 27% of cases (range 74-93 years) were hospitalized patients affected by neurodegenerative processes such as Alzheimer's Disease, Senile Dementia and Lewy Body Dementia. The diagnosis of SARS-CoV-2 infection was initially carried out by RT-PCR on oropharyngeal swabs and, then, confirmed on post-mortem lung tissues. We performed a trasnethmoidal approach to the skull using Jamshidi needle, in order to obtain a "window" to the brain and to reduce the risk of exposition to the virus for the autopsy staff. A minimally invasive autopsy technique was also executed for body sampling. We collected specimens of all tissues that were formalin-fixed and paraffin embedded while brain and olphactory bulbs and CSF were stored at -80°C. Histological (H/E) and molecular (RT-PCR) investigations revealed the presence of SARS-CoV-2 in all organs, tissues, cells and biological fluids. The SARS-CoV-2 presence was documented especially in lung parenchyma and upper respiratory tract secretions with concordance with clinical data greater than 95%. We propose that the large number of clinical data and biological samples can represent a valuable collection for future BBMRI.it COVID-19 *"biobanking"* activities and for translational researches aimed at understanding the pathogenetic mechanism underlying the SARS-CoV2 infection and developing effective therapies.

ND34 | Transcriptome-phenotype relationship in unmutated sporadic ALS patients highlights phenotype-specific gene expression patterns

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Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disorder affecting human motor system, characterized by heterogeneity and phenotypes variability. This background highlights a critical need for identification of an adequate strategy to stratify ALS patients and to identify reliable biomarkers for early diagnosis, prognosis, and disease progression. The discovery of the involvement of RNA-mediated toxicity, which could be controlled by alteration of gene expression, is considered a key event in ALS. Regulation of gene expression represents a novel opportunity to identify specific traits in ALS subgroups. For these considerations, the classification based on specific clinical phenotypes could be associated with different gene expression patterns that are shaped during lifespan, representing a novel opportunity to identify specific ALS subtypes with homogeneous clinical and biological features.
Our objective is to identify the transcriptomic signatures of distinct ALS phenotypes, and to use this information for biomarker assessment and personal therapy development.
We characterized 36 unmutated sALS patients by clinical and paraclinical phenotype, and subdivided them in "Classic" (n=12), "Bulbar" (n=7), "Flail Arm" (n=6), "Flail Leg" (n=6) and "Pyramidal" (n=5). Then, RNAs extracted from PBMCs isolated from patients (n=15) and healthy controls were sequenced. By performing a Principal Component Analysis (PCA) of DE genes in each group of patients compared to controls, we observed a gene expression clusterization of patients and controls, except for "Flail Arm" group. Interestingly, we found only one gene commonly deregulated in all groups (Y RNA, a component of the Ro60 ribonucleoprotein), while the rest of DE genes were phenotype-specific.
The identification of phenotype-specific pathogenic mechanisms will be crucial for the prognosis and the identification of new therapeutic targets to delay onset or attenuate disease progression rate.

ND35 | The possible role of cholesterol metabolism in the onset and progression of Huntington's disease

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Huntington's disease (HD) is an autosomal dominant neurodegenerative disorder caused by the abnormal expansion of the CAG repeats in the first exon of the IT15 gene, which encodes for an expanded PolyQ tract in the huntingtin protein (HTT). One of the affected pathways is cholesterol (Chol) metabolism. Many data in vivo and in vitro have shown that the presence of mHTT causes a reduction in cholesterol synthesis and an alteration of its turnover. 24OH-cholesterol (24OHC) is the main metabolite of Chol in the brain; unlike Chol, it can cross the blood-brain barrier and enter peripheral blood circulation, and 24OHC can be considered an indirect indicator of Chol metabolism in the brain. A biomarker is needed to monitor disease progression. This project aimed to characterize the cholesterol metabolism in an HD mouse model using an LC-MS method and evaluate 24OHC as a biomarker of disease progression in a clinical trial. The HD mouse model efficiently represents the disease progression at a behavioral level. In the striatum, the results show a significant difference between WT and HD animals at 12 weeks in desmosterol and 24-OHC levels when the worst behavioral defects were found in the animals. There is no difference between 24OHC levels between HD and WT mice in plasma. In parallel, a clinical trial is ongoing to study plasmatic 24OHC in HD patients to evaluate the metabolite as a biomarker of HD progression.
These preliminary data suggest that a deficit of cholesterol metabolism correlates with the worst HD phenotype in animals and suggests that a decrease of desmosterol and 24OHC production occurs only at 12 weeks of age when HD symptoms are very severe in HD animals. Still, it will be necessary to use a mouse model with slower disease progression to deepen our understanding of the deficit in cholesterol synthesis.

ND36 | Mitochondrial alterations in subjects with idiopathic REM sleep disorders as a predictive biomarker for conversion to Parkinson's disease

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One of the most important prodromal marker of Parkinson's disease (PD) is the presence of idiopathic rapid eye movement (REM) sleep disorders (iRBD). Nonetheless, only a few studies have looked into the mechanisms that may be involved in the development of PD in iRBD patients. Since mitochondrial dysfunction has been linked to sleep disturbances in PD (Smith et al., 2018; Milanese et al., 2019), we investigated potential mitochondrial alterations in fibroblasts of subjects with iRBD in order to identify a biochemical profile that can characterize this condition and predict the future onset of PD.
The project included 23 subjects so far, divided into three experimental groups: healthy subjects (HC), iRBD subjects, and iRBD subjects subsequently converted to PD (iRBD-PD).
The evaluation of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) using a XFe24 Seahorse Analyzer, indices of the mitochondrial respiration efficiency, revealed a reduction in maximal and spare respiration levels in the fibroblasts of iRBD patients if compared to HC, though the difference was not statistically significant. Instead, a significant worsening of the bioenergetic profile was observed in the iRBD-PD patients, as evidenced by lower levels of adenosine triphosphate (ATP) production and reduced basal, maximal, and spare respiration. Moreover, the presence of mitochondrial fragmentation and a significant decrease in the expression levels of electron transport chain complexes III and V are associated with mitochondrial dysfunction in iRBD-PD patients. Similar, but less severe changes were observed in iRBD subjects.
These findings imply that mitochondrial alterations (e.g., a decreased ability to respond to increased energy demand) observed in iRBD subjects' fibroblasts may predispose to worsening of the bioenergetic profile observed in iRBD subjects already converted to PD, indicating a potential mechanism underlying the progression of PD in iRBD patients.

ND37 | RNA Expression Profiling in Lymphoblastoid Cell Lines from Mutated and Non-Mutated Amyotrophic Lateral Sclerosis Patients

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Amyotrophic Lateral Sclerosis (ALS) is a progressive and fatal neurodegenerative disease of adulthood characterized by the loss of upper and lower motor neurons responsible for the control of voluntary muscles. ALS manifests predominantly in a sporadic form (SALS, 90-95%), with no family history, but a small percentage of patients (5%-10%) may present a familial form, which is mainly transmitted in an autosomal dominant manner. Mounting evidence suggests that altered RNA metabolism plays a significant role in ALS pathogenesis. Given the difficulty to recover biological material from patients, lymphoblastoid cell lines (LCLs) were used as a convenient and practical model for ALS, since several pathways normally found in neurons, were also found to be active in these cells. A transcriptome profiling of unmutated SALS and mutant patients (FUS, TARDBP, C9ORF72, and SOD1), as well as matched controls, was performed to assess the expression of coding and long noncoding RNAs in LCLs. Hence, Differentially Expressed Genes (DEGs) were investigated. For both controls and ALS patients, peripheral blood was processed, and Peripheral Blood Mononuclear Cells (PBMCs) were isolated, immortalized into LCLs via Epstein-Barr Virus (EBV) infection and cultured. Total RNA was extracted, and RNA-sequencing analysis was performed. LCL gene expression profiles were genetic background specific, with only 12 genes being commonly deregulated in all groups compared to controls. Nevertheless, DEGs-enriched pathways in each group were also compared and a total of 89 KEGG terms were found to be shared among all patients. Finally, to evaluate how immortalization affects basal transcriptomic profile and to assess whether LCLs maintain a disease-specific gene expression patter, our obtained data were matched with a transcriptome profile realized in PBMCs of the same patients. As a result, we concluded that LCLs may be a useful model for studying RNA deregulation in ALS.

ND38 | Contingent intramuscular boosting of P2X7 axis improves motor function in transgenic ALS mice

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Muscle weakness plays an important role in neuromuscular disorders comprising Amyotrophic Lateral Sclerosis (ALS), a fatal neurodegenerative disorder that leads to progressive degeneration of motor neurons and severe muscle atrophy without effective treatment. Most research on the disease has been focused on studying motor neurons and supporting cells of the central nervous system. Strikingly, recent observations have shown that the expression of the SOD-1G93A mutation in skeletal muscles causes denervation of the neuromuscular junctions, inability to regenerate and consequent atrophy, all clear symptoms of ALS, suggesting that these morpho-functional alterations in skeletal muscle precede motor neuron degeneration, bolstering the interest in studying muscle tissue as a potential target for the delivery of therapies.
We previously showed that the systemic administration of the P2XR7 agonist, 2′ (3′) - O - (4 - benzoylbenzoyl) adenosine 5 - triphosphate (BzATP), enhanced the metabolism, improved the innervation and promoted the myogenesis of new fibres in the skeletal muscles of SOD1G93A mice. Here we further corroborated this evidence showing that intramuscular administration of BzATP improved the motor performance of ALS mice by enhancing satellite cells and the muscle pro-regenerative activity of infiltrating macrophages. The preservation of the skeletal muscle retrogradely propagated along with the motor unit, suggesting that backward signalling from the muscle could impinge on motor neuron death. In addition to providing the basis for a suitable adjunct multisystem therapeutic approach in ALS, these data point out that the muscle should be at the centre of ALS research as a target tissue to address novel therapies in combination with those oriented to the CNS.

CN05 | Investigation on the neuroprotective role that astrocytes exert on neurons in the context of Riboflavin Transporter Deficiency

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Riboflavin (Rf) is the precursor of Flavin Adenine Dinucleotide (FAD) and Flavin Mononucleotide (FMN), two biological cofactors involved in different metabolic redox reactions, especially in mitochondrial processes. Mutations in the genes which encode for human Riboflavin Transporters hRFT2 and hRFT3 (called SLC52A3 and SLC52A2 respectively), cause a rare autosomal recessive disease that arises in childhood: Riboflavin Transporter Deficiency (RTD). To better understand the patho-mechanisms which characterize this neurodegenerative disease, induced pluripotent stem cells (iPSCs) have been used as in vitro cellular model. RTD is considered a motoneuronal progressive disease, as motoneurons (MNs) represent the most affected cell type. Since astrocytes have a neuroprotective role, we decided to investigate if iPSC-derived astrocytes (ASTROs) in co-culture with RTD MNs can improve their morphology and neuronal activity. To this aim, we differentiated RTD and Ctrl iPSCs into ASTROs and examined their effect on RTD MNs. The results obtained show that both RTD and Ctrl ASTROs are able to increase the neurites length and intracellular calcium (Ca+2) levels of RTD MNs. These results suggest that astrocytes may have a strong neuroprotection role on RTD neurons.

CN06 | Disease Modifying Therapy specifically impacts on microRNAs expression profiling in Relapsing-Remitting Multiple Sclerosis

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Multiple Sclerosis (MS) is an autoimmune demyelinating and degenerative disease of the Central Nervous System characterized by heterogeneous clinical phenotypes, disease progression and response to disease-modifying therapies (DMTs). Diagnosis, monitoring and therapeutic choices are guided by neuroimaging and clinical neurological features that are considered to predict long-term disability. DMTs can change the disease course, especially for the Relapsing Remitting, RR-MS. Among DMTs, drugs depleting immune cells emerge for their efficacy: they can deplete specifically B or both B and T cells. However, the current clinical approach involves clinical and neuroimaging follow-up for at least one year before being able to define whether the drugs adequately control the disease. The unsatisfying availability of non-invasive and easily detectable molecular biomarkers represents an unmet need in clinical practice, for a more accurate diagnosis and better prognostic or response to treatment predictions. In this study, we focused on Cladribine and Ocrelizumab, that are widely used immune cells-depleting DMTs in Italian clinical MS Centres. Although the DMT mechanisms of action is relatively defined, their impact on gene expression is still unknown. Thus, we investigated the microRNAs profiling, proposed as diagnostic and prognostic tool for neuroinflammatory diseases, and their response to the Cladribine or Ocrelizumab treatment. The microRNA profiling was analysed in peripheral blood mononuclear cells from 20 patients with RR-MS (Ethical approval Rif. 6361, Prot. 0635/2021), by Agilent microarray, and measured before (T0) and 6 months post-treatment (T1). Results highlight a specific microRNAs response to DMT, which involves differentially expressed microRNAs of the neuroinflammatory, immune-regulatory and neurodegenerative pathways. These microRNA candidates, integrated with clinical and imaging data, might allow to predict and optimize the therapeutic response to DMT.

CN07 | Stathmin-2 in Spinal Muscular Atrophy (SMA): assessing molecular and therapeutic role in SMA human and murine models

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Spinal muscular atrophy (SMA) is a severe genetic neuromuscular disease with early onset, and represents the most common genetic cause of infant mortality. SMA is caused by mutations in the survival motor neuron 1 gene (SMN1) that impair the function and survival of lower motor neurons (MNs) in the spinal cord. The majority of current SMA therapeutic approaches are focused on increasing the levels of full length SMN protein. However, finding SMN-independent approaches to target downstream pathological events can be valuable, particularly in the symptomatic phase of the disease.

To develop a complementary approach, one possibility is to identify downstream genes responsible for selective MN dysfunction. Stathmin-2 (STMN2), a gene involved in neurite outgrowth, cytoskeleton metabolism and axonal regeneration, was already observed to be a target in other neurodegenerative diseases as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Therefore, we investigated the role of STMN2 in the context of SMA pathogenesis by studying its expression in SMA in vitro iPSC-derived MNs and in vivo murine models. Furthermore, we demonstrated that STMN2 overexpression, obtained using a lentiviral vector or a pharmacological drug, was able to increase survival, axon length and neurite complexity in patients iPSC-derived MNs.

Overall, the investigation of the molecular and therapeutic role of STMN2 in SMA could offer new insights into increased MN vulnerability, and may also support the finding of downstream modifier genes or SMN-independent therapeutic targets for a complementary SMA therapy that combines SMN-independent and SMN-dependent strategies.

CN08 | Oxygen-Ozone Therapy and Cognitive Frailty: a non-pharmacological approach to potentially resolve immune and inflammatory dysfunctions

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Introduction. As the world's population ages, Cognitive Frailty (CF) is becoming one of the most serious health problems and elucidating its biological mechanisms along with prevention and treatments becomes increasingly important also considering the associated health costs. We thus performed a clinical randomized trial where CF subjects received a non-pharmacological therapy based on the regenerative properties of ozone (O3) known to act on immune/inflammation processes, strongly altered in CF.
Methods. A cohort of 75 patients was stratified in non-, mildly- or severely frail rate and treated with placebo, oxygen (O2) or O2-O3. The serum levels of 27 peculiar pro- and anti-inflammatory cytokines and chemokine cell signalling molecules were measured by using the Bio-Plex Pro Human Cytokine 27-plex immunoassay. The student's t-test and analysis of variance (ANOVA) followed by Tukey's post hoc test were used for comparison of means between the groups.
Results. Preliminary analyses evidenced the implication, at different levels, of some molecules in relation to the frailty rate. Noteworthy, we observed modulations of immune (i.e inteleukin, IL-9) and inflammation (i.e IL-1β) biomarkers at baseline (Time, T0) and after treatment (T1=3 months). Correlations between clinical CF profiles and peripheral levels of the considered biomarkers are ongoing to predict the response to O2-O3 therapy.
Conclusion. Although preliminary, these results confirm that the immune-inflammation systems are involved in the aetiopathogenetic mechanisms of CF, and that the related molecules could be potential therapeutic targets/biomarkers for the O2-O3 therapy. These data will further permit to validate a new non-pharmacological treatment approach for this condition.

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EBN15 | Generation and characterization of iPSC-derived neurons to model Radio-Tartaglia syndrome

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Radio-Tartaglia syndrome (RATARS) is a neurodevelopmental disorder characterized by global developmental delay, behavioral abnormalities and craniofacial dysmorphism. The disorder is caused by de novo inactivating variants in SPEN. No clear genotype/phenotype correlation has been identified. SPEN encodes a hormone inducible transcriptional repressor expressed in many tissues, and its role during prenatal cortical development has been suggested. As many rare diseases, RATARS lacks reliable models that can be manipulated in vitro. In this context, iPSC technology allows to study human disease-relevant cells. Thus, we reprogrammed fibroblasts derived from two RATARS patients into iPSCs, and obtained clones expressing pluripotency markers, i.e. SOX2, TRA1-60, OCT4, and SSEA4. Consistently, the alkaline phosphatase test confirmed the pluripotency of RATARS cells. Interestingly, the mitotic cycle was examined by calculating the percentage of abnormal mitosis out of the total number of dividing cells, showed an increase of altered mitosis in RATARS iPSCs vs. control cells. Patient-specific iPSCs were then differentiated into motor and cortical neurons and RATARS cells displayed reduced differentiation efficiency compared to controls, as showed by phase contrast and fluorescence images of SMI-32 and ßIII tubulin immunostaining. Morphometric analyses were also performed using neurotrack analysis which showed a significant reduction in RATARS neurites' length vs. controls. Such difference was more evident following cortical neuron differentiation rather than following motor neuron differentiation. Finally, Tunel assay was performed to assess possible activation of cell death processes, demonstrating a significant increase of apoptotic cells in RATARS cells. Overall, our data extend the knowledge on RATARS pathology, demonstrating altered mitotic cycle and increased cell death in patient's cells associated with impaired neuronal differentiation.

EBN16 | Remodulation of Rac1 GTPase pathway in cytoskeletal related Intellectual Disabilities

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Intellectual disability (ID) is a neurodevelopmental disorder characterized by limited intellectual functioning and adaptive behavior. Alterations in neurites and spine morphology, as well as in neuronal migration properties, have been consistently associated with ID and other neurodevelopmental disorders and rely on cytoskeleton dynamics and functions, whose upstream regulation is exerted by small GTPases (i.e., Rac1, RhoA, and Cdc42). The Rac1 pathway is hypoactive as the result of several gene mutations associated with ID, including RAC1 itself, its regulators (e.g., ARHGEF6 and TRIO), and effectors (e.g., PAK3), pointing out the urgency of a positive modulation of Rac1 pathway. To achieve a GTPase-specific, modest, and controlled remodulation of Rac1 activity, the full characterization of the protein::protein interaction between Rac1 and its negative regulator ArhGAP15 allowed the design of a peptide able to interfere with this interaction. Moreover, to envision a translation to the human setting for pharmacological screens and studies, we started the generation of human iPSCs isogenic clones harboring in-del inactivating mutations in ARHGEF6 and TRIO and differentiated them in cortical-like progenitors and neurons.

EBN17 | A new role of NBS1 in the regulation of primary cilium

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Microcephaly is caused by the depletion of neuronal progenitors and is usually associated with defects in proteins that localize at or regulate the centrosome. Notably, also defects in proteins of the DNA damage Response (DDR) can lead to microcephaly. Curiously, many DDR proteins localize and regulate centrosomal proteins, suggesting a functional link between centrosomes and DDR proteins that seems to converge in the control of the neuronal progenitor's expansion. In interphase the centrosome becomes the Basal Body (BB) and enucleates the Primary Cilium (PC), an organelle that is essential for the transduction of many mitogenic pathways also in neuronal progenitors. Indeed, ciliopathies, caused by defects in PC proteins, can be characterized by defects in brain development. We recently demonstrated that a DDR protein, NBS1, stably localizes at the centrosome/BB. Moreover, its depletion lengthens PC, affects the expression of PC-dependent pathways and perturbs the proliferation of neuronal progenitors, in vitro and in vivo. Because centrosome is known as regulator of PC assembly/disassembly and trafficking, we speculate that NBS1 could regulate PC working as an adapter at the BB. In order to address this issue, we propose to investigate the physical interactors of NBS1 at the BB. To this end, we will use an innovative method called "proximity-dependent biotin identification". We will stably transfect RPE-1 cells with a plasmid expressing the sequence of NBS1 fused with a mutant of E.coli BirA biotin ligase. The cells will be incubated with an excess of biotin to allow the covalent biotinylation of protein near to NBS1. Then, we will purify the centrosome fraction performing mass spectrometry analysis on putative NBS1 interactors isolated by streptavidin beads. Our studies can better explain the mechanisms that regulate PC and they could give a possible justification for the neuronal phenotypes associated with defects in centrosome and DDR proteins.

EBN18 | 3D Human Cortical Organoids to investigate developmental and epileptic encephalopathy

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The human cerebral cortex is characterized by an extraordinary complexity of neuronal and non-neuronal cell types wired together for the execution of high-order cognitive functions. Alterations, during fetal development as well as after birth, in the assembly of cortical neuronal circuits can lead to aberrant neuronal activity and abnormal firing patterns, shared signs of neurodevelopmental disorders. Developmental and Epileptic Encephalopathy (DEE), a heterogeneous group of devastating childhood epilepsy disorders with a strong genetic component, constitute the most precocious syndromes that can affect infants as early as in the womb. DEE mutations are associated with a variety of proteins implicated in a wide range of developmental processes, from neuronal migration and cell adhesion to transcriptional regulation and synaptic transmission (i.e. ARX, PCDH19, HCN1, FOXG1, SCN1A, GABAA, SLC2A1). Despite the number of genes linked to DEE is growing the etiology remains unknown for most cases, and it is challenging to decouple the patient-specific genetic make-up from the effect of the aberrant activity per se on brain development. To answer this fundamental question and address unique features of human development, we are exploiting a highly reproducible human cortical organoids (hCOs) system, on which we are inducing acute seizure-like currents, to model infantile/pediatric epilepsy in vitro. To address the implications of activity on circuit assembly, we are mapping at the single-cell level the epigenetic and transcriptional landscape of treated and untreated hCOs, with the final goal of deciphering the epigenetic fingerprints produced by exacerbate activity in distinct classes of cortical neurons along their specification trajectory. Identification the of aberrant pathways associated with epileptic seizures will constitute an invaluable resource to discover novel drug targets for infantile/pediatric epilepsy.

EBN19 | In vivo functional validation of new disease-genes and variants impairing trafficking and cytoskeleton dynamics as underlying cause of undiagnosed neurodevelopmental diseases

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Rare diseases represent a serious societal burden, with at least 70% of the cases manifested already during childhood in chronic forms often affecting the nervous system and resulting in extremely debilitating conditions, which include early onset neurodegeneration. The lack of a fundamental understanding of the underlying pathophysiological mechanisms, which might involve a variety of cell populations and developmental processes, make them difficult to diagnose and treat. New and potentially pathogenic gene variants are continuously identified in undiagnosed patients thanks to advanced genomic technologies, resulting in an increased need for effective in vivo disease-models to obtain functional validation. In the framework of the "Undiagnosed Patients Program" at Ospedale Pediatrico Bambino Gesù (OPBG, Rome) more than 20 new rare diseases have been clinically and genetically classified since the launch in 2015, in collaboration with healthcare and research premises worldwide. Here, we show the most recent examples which benefited from functional validation through ad hoc modeling and analysis in zebrafish obtained at the newly established OPBG zebrafish laboratory. In this "in-house" in vivo workflow we utilize transient and stable protein knockdown and overexpression approaches for loss and gain of function conditions, coupled to whole embryo imaging-based phenotype characterization at cellular and subcellular levels. We present zebrafish data which recently contributed to: 1.validate the pathogenicity of new disease genes and variants involved in endosomal trafficking and Golgi homeostasis affecting neurodevelopment; 2.provide in-depth insights into the role and function of small GTPases and microtubules chaperons for central nervous system and motoneurons development; 3.characterize the impact of previously unidentified gene variants and the efficacy of newly synthetized molecules inhibiting the altered RAS/MAPK signaling involved in RASopathies.

EBN20 | Towards an in vitro model for therapeutic opportunities in Lafora disease

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Lafora disease (LD) is a rare, autosomal recessive, severe, and progressive myoclonus epilepsy. The onset occurs during adolescence with an average life expectancy of about ten years. Main symptoms include myoclonus and/or generalized seizures, visual hallucinations, and progressive neurological decline.
LD is mainly caused by loss-of-function mutations of the EPM2A gene (encoding laforin) or NHLRC1 gene (expressing malin). These proteins are involved in the metabolism of glycogen, ubiquitinating protein targeting to glycogen (PTG) which positively regulates the glycogen synthesis. In pathological conditions, PTG escapes from degradation due to dysfunctional laforin-malin complex, leading to the accumulation of structurally abnormal, insoluble glycogen into Lafora bodies and driving the neurodegeneration.
Nowadays, neither a cure nor a relevant cellular model is available to allow a complete depiction of the pathophysiological mechanisms behind LD as well as the development and test of effective therapeutic strategies.
Therefore, we employed LD patient-derived induced pluripotent stem cells, reprogrammed from peripheral blood mononuclear cells. These have been characterized for the expression of pluripotency markers and their ability in generating the three germ layers. We managed to induce the neuralization into neural progenitor cells to further differentiate into pan-neurons, as an in vitro patient-derived neuronal model of LD. In parallel, we genetically modified a commercially available and stable human neural stem cell line (AF22) for the overexpression of PTG to boost the accumulation of glycogen.
On the other hand, we are performing structural studies on PTG crystals to test hit compounds able to bind the protein with the aim of inhibiting its activity.
Taken together, these models should allow the screening of drug hits emerged from the structure-based drug discovery approach and drug repurposing to select the best candidates for further pre-clinical trials.

EBN21 | A new role of Nijmegen Breakage Syndrome gene in Neuronal development

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The DDR-defective syndromes are characterized by immune deficiency, cancer predisposition and neuronal defects (microcephaly and ataxia). An example of them is the Nijmegen Breakage Syndrome (NBS) caused by mutations in the NBS1 gene. Why DDR-defective syndromes patients show neuro-developmental defects has never been explained. We recently demonstrated that NBS1 stably localized at the centrosome/Basal Body and regulates Primary Cilia (PC) length and functionality. The PC is a non – motile organelle that works as an antenna for external stimuli and is essential for many mitogenic pathways. Defects in PC lead to diseases termed 'ciliopathies', some of which are characterized by defects in brain development and mental retardation (i.e. Joubert and Meckel Syndromes). Therefore, we propose a model in which PC deregulation, due to NBS1 defects, is responsible for the neuronal defects observed in NBS patients. In order to address this issue, we need to verify whether hypomorphic mutations in the NBS1 gene affect PC structure and functionality. Coherently with this hypotesis, we found a significant increase in the PC length in human fibroblasts (HF) from NBS patients compared to the healthy ones. However, this isn't a syngenic model. Therefore, in order to better evaluate our hypothesis in a syngenic context we propose to generate RPE-1 cells in which we will both introduce the 675D5 mutation, the most frequently observed in NBS patients, in the NBS1 gene by Crispr/Cas9 technology and stably transfect a plasmid expressing a WT form of NBS1 under the control of an inducible promoter. In this cell model we will analyze the localization of NBS1 and its interactors at the centrosome and we will study morphology, dynamic and functionality of the PC. Our study can represent an important step in understanding the molecular mechanism that causes neuronal defects in NBS and potentially in other DDR-defective Syndromes and could reclassify NBS as a Ciliopathy.

EBN22 | Expression of a secretable, cell-penetrating CDKL5 protein enhances the efficacy of AAV vector-mediated gene therapy for CDKL5 deficiency disorder

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CDKL5 deficiency disorder (CDD) is a severe neurodevelopmental disease caused by mutations in the CDKL5 gene. CDD is characterized by early-onset epileptic seizures, hypotonia, intellectual disability, motor and visual impairment and respiratory dysregulation. There is currently no cure or effective treatment to ameliorate cognitive and behavioral symptoms for CDD. Although delivery of a wild-type copy of the mutated gene to cells represents the most curative approach for a monogenic disease, proof-of-concept studies highlight significant efficacy caveats for brain gene therapy. Herein, we develop a cross-correction-based strategy to enhance the efficiency of a gene therapy for CDD. We created a vector for gene therapy that produces an Igk-TATk-CDKL5 fusion protein that can be secreted via constitutive secretory pathways and, due to the transduction property of the TATk peptide, be internalized by neighboring cells. We carried out a comparative evaluation of CDKL5 and TATk-CDKL5 protein biodistribution in vivo and the effect of intravascular treatment with the AAVPHP.B_CDKL5 vector or AAVPHP.B_Ikg-TATk-CDKL5 vector on brain structure and behavior in adult symptomatic Cdkl5 knockout (KO) mice. We found that, although AAVPHP.B_Igk-TATk-CDKL5 and AAVPHP.B_CDKL5 vectors had similar brain infection efficiency, the first one led to a higher CDKL5 protein replacement due to secretion and transduction of the TATk-CDKL5 protein into the neighboring cells. Importantly, Cdkl5 KO mice treated with the AAVPHP.B_Igk-TATk-CDKL5 vector showed a behavioral and neuroanatomical improvement in comparison with vehicle-treated Cdkl5 KO mice or Cdkl5 KO mice treated with the AAVPHP.B_CDKL5 vector. These results indicate that a gene therapy based on a secretable recombinant TATk-CDKL5 protein is more effective at compensating Cdkl5-null brain defects than gene therapy based on the expression of the naive CDKL5.

NI25 | Effects of interleukin-9 on striatal synaptic dysfunction in a mouse model of multiple sclerosis

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Multiple Sclerosis (MS) is an autoimmune neuroinflammatory disease of the central nervous system (CNS) characterized by the infiltration of lymphocytes into the CNS resulting in a diffuse demyelination, neuroinflammation, neuroaxonal loss and dysfunction. Clinical and preclinical studies revealed that CNS inflammation drives a synaptic damage, named synaptopathy, independently of demyelination. Interleukin (IL)-9 is a cytokine that plays an important immunoregulatory role in MS and experimental autoimmune encephalomyelitis (EAE), however, the exact role of IL-9 in MS disease is still unclear.

Here, we studied the effect of IL-9 on clinical disability and striatal synaptic alteration in EAE mice. Our data clearly indicate beneficial effects of systemic IL-9 treatment in both presymptomatic and therapeutic stages in EAE mice. In particular, we observed that IL-9 treatment is able to induce a less severe disease course accompanied by a recovery of the spontaneous glutamatergic current kinetics in the striatum of EAE mice. Intracerebroventricular infusion (ICV) of IL-9 with osmotic minipumps implanted in the brain of EAE mice also ameliorates clinical disability and glutamatergic alteration suggesting a direct role of IL-9 into the CNS. The study of the receptor localization of IL-9 in the brain reveals a detectable expression of IL-9 receptor on microglia cells membrane, whereas no signal was observed from neurons or infiltrating lymphocytes, suggesting its indirect role on neuronal cells. Overall, these data suggest that IL-9 significantly amieliorates EAE pathogenesis by a direct effect into the CNS, likely due to modulation of resident immune cells activity.

NI26 | The role of SK channels and the vagus nerve in turning on AgRP neurons in experimental autoimmune encephalomyelitis

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Hypothalamic AGRP (agouti-related neuropeptide)-expressing neurons sense caloric needs to coordinate homeostatic feeding. Activation of AgRP neurons by fasting is mediated by N-methyl-D-aspartate receptors for glutamate and by the down-regulation of the inhibitory small-conductance calcium-activated potassium (SK) channels. Activation of AgRP neurons impairs hematopoiesis and increases the generation of regulatory T lymphocytes. Sensory fibers of the vagus nerve are involved in conveying glutamatergic signals from the periphery to the hypothalamus. AgRP neurons are activated upon induction of experimental autoimmune encephalomyelitis (EAE), and AgRP neuropeptide, produced by activated AgRP neurons, is increased in patients with multiple sclerosis (MS). We have analyzed, at different times upon immunization, the mRNA expression of AgRP, which increases when AgRP neurons are activated, in the hypothalamus of EAE-induced mice that underwent or not unilateral cervical vagotomy. We found that the increase in Agrp expression upon EAE induction is significantly less in mice that have undergone unilateral cervical vagotomy than in non-operated mice, indicating that the vagus nerve is implicated in AgRP neuron activation. Moreover, we observed that expression of AgRP is inversely correlated with the expression of Knnc1, the gene coding for SK1 channel, suggesting that modulation of SK1 channels is involved in activation of AgRP neurons in EAE. Our results support the possibility that glutaminergic signals transmitted by the vagus nerve and SK1 channels are involved in the modulation of AgRP neuron activity in EAE. Further experiments are necessary to establish whether electrical stimulation of the vagus nerve and/or pharmacological modulation of SK1 channels may be used to modulate the functionality of AgRP neurons and, thereby, hematopoiesis and lymphopoiesis in EAE.

NI27 | Nerve Growth Factor influences microglial activity in vivo via TrkA receptors

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The neurotrophin Nerve Growth Factor (NGF) supports neuronal survival, differentiation, and plasticity. However, it also has pleiotropic actions on non-neuronal cells, especially regarding the immune system. For instance, peripheral immune cells (mast cells and macrophages) do secrete and respond to NGF, by expressing TrkA receptors. Early reports demonstrated that NGF promotes in vitro migration and proliferation of the resident brain immune cells, microglia. More recently, our lab discovered potent immunomodulatory properties of NGF via TrkA on microglia in vitro: NGF steers them toward a neuroprotective and anti-inflammatory phenotype, by modulating their motility and phagocytosis. In addition, we showed that TrkA is expressed by microglia in mouse brain acute slices and is upregulated in microglia of Alzheimer's disease model 5xFAD. Here, we provide in vivo evidence of a role for NGF signaling in brain microglia. Indeed, to better and directly assess the functional role of microglial NGF-TrkA signaling, we generated a novel transgenic mouse line (CX3CR1CreERT/+::TrkAfl/fl), in which TrkA can be specifically deleted in microglia by administering tamoxifen. We report that dampening microglial NGF-TrkA signaling leads to a reduction in cortical and hippocampal microglial density, while overall morphology is unaffected with respect to controls. Moreover, TrkA-deprived microglia show higher phagocytosis of spines, leading to a striking reduction of cortical spine density. Lastly, behaviorally, microglial NGF-TrkA signaling deletion affects motor learning. Altogether, these data suggest that CNS resident microglia express functional TrkA receptors in vivo and that TrkA signaling influences pivotal microglia activities.

NI28 | Dendritic cells educated through exposure to specialized pro-resolving mediators acquire a tolerogenic phenotype

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In experimental autoimmune encephalomyelitis (EAE) the loss of immunological tolerance is one of the main autoimmune pathological mechanisms. In this context, dendritic cells (DCs) play a role in inducing both immunity and tolerance by acting as modulators of thymic and peripheral immune tolerance. We propose to generate tolerogenic DCs using a novel approach whereby DCs are exposed to specialized pro-resolving mediators (SPMs), a novel class of lipid autacoids that reduce tissue infiltration and activation of pro-inflammatory macrophages and T lymphocytes. Accordingly, we hypothesize that DCs conditioned by exposure to SPMs (DCsSPMs) could acquire a tolerogenic phenotype and could reduce the activation of T cells, thus ameliorating EAE. qPCR analysis showed that differentiation of bone-marrow-derived DCs induced to mature with LPS-INF in the presence of SPMs imparts a tolerogenic phenotype to these cells, with downregulation of pro-inflammatory markers (Cd40 and Il1b) and concomitant upregulation of tolerogenic markers Lilrb4, Cd274 and Pdcd1lg2. Moreover, mature DCsSPMs maintained the upregulation of those tolerogenic markers after overnight, 24h and 48h, migrated less upon SDF-1 and CCL19 engagement. Flow cytometry experiments confirmed that DCsSPMs upregulate the surface markers of tolerance, ILT3 and PD-L1, as well as other anti-inflammatory markers, such as MerTK, and CTLA4. Activated T cells co-cultured with DCsSPMs or in the presence of supernatant of DCsSPMs, or their derived extracellular vesicles, produced low levels of pro-inflammatory cytokines INFγ and IL-17 and displayed a reduced mRNA expression of the transcription factors Tbx21 and Rorc, related to the inflammatory T-cell phenotypes. Furthermore, metabolic assessment of DCsSPMs revealed a complete coupling between ATP synthesis and oxygen consumption, suggesting that these DCs are anti-inflammatory. Our preliminary data suggest a novel role of SPMs in the induction of a tolerogenic phenotype of DCs.

NI29 | Altered expression of specific HSPs in the spinal cord in an animal model of rheumatoid arthritis

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Rheumatoid Arthritis (RA) is a chronic inflammatory and autoimmune disease characterized by an attack of the immune system on the joint lining. This disease results in an increased release of inflammatory mediators peripherally and centrally. Some of the major peptides involved are glutamate, Substance P (SP) and Calcitonin gene-related peptide (CGRP) which sensitize sensory neurons and cause further hyperexcitability, the severe symptoms of RA and the persistent neurogenic inflammation. Several studies have highlighted the protective role of HSPs (27, 60, 70 and 90) in different animal models of pain and inflammation. The aim of the present study is to examine the time course of HSPs expression in the different regions of the spinal cord specifically sensory neurons and glial cells in the dorsal horn, and their role following induction of inflammation by intra-articular injection of Complete Freund's adjuvant (CFA) into the joint and to examine if increased cellular proliferation in the spinal cord of arthritic rats correlate with nociceptive behaviour and the altered HSPs expression. Male Sprague-Dawley rats were assessed for sensory and motor behavioural changes prior to and at 7, 14 and 21 days post induction of inflammation. In addition, spinal cord tissues, serum, and synovial membranes were collected at the same time points. The level of different HSPs expression in the spinal cord (centrally) and in the synovial membrane (peripherally) was assessed using Western blot analysis. Immunofluorescence experiments were also done to reveal the expression site of these proteins in the spinal cord. All inflamed rats developed sensory hypersensitivity and motor incoordination. Alteration in the expression level of different HSPs was found in spinal cord tissues at different time points: a significant increase in the heat shock protein 90 was detected at day 14 while heat shock protein 70 was significantly increased at day 21, whereas heat shock protein 60 showed major decrease at day 21, heat shock protein 27 showed a trend of increase at day 14 but it was not substantial. Increased cellular proliferation was detected in different regions of the spinal cord of inflamed rats and peaked at day 14. HSPs (27, 60, 70 and 90) can be found in both neurons and glial cells in the dorsal and ventral horns of the spinal cord. Our preliminary data provide evidence for dual role either HSPs are playing an anti-inflammatory role contributing to the alleviation of progression of this disease or an inflammatory role which involves the development of pain and inflammation associated with RA. However, the role is to be confirmed reliant on whether it is upregulated or downregulated relatively to the time points of variation, cellular events and behavioural changes occurring.

NI30 | Counteract the outer Blood-Retinal Barrier breakdown targeting ocular inflammation to delay vision loss in Retinitis Pigmentosa

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Vascular barrier breakdown is a hallmark of neuroinflammation. The retinal pigment epithelium (RPE) is a monolayer of cells joined by tight junctions (TJs) constituting the outer blood-retinal barrier (oBRB), which separates choroidal vessels from the neural retina contributing to its immune privilege. In 3 mouse models of retinitis pigmentosa (RP), we showed that photoreceptor degeneration is accompanied by decreased integrity of the oBRB, where immuno-labelling for zonula occludens-1 (ZO-1), an essential regulatory component of TJs, shows structural discontinuities, corresponding to leakage areas of in vivo administered fluorescent dextran from choroidal vessels to the RPE-retinal interface. We also showed that subcutaneous Dexamethasone administration to rd10 mice (mimicking autosomal recessive RP) during the period of maximum photoreceptor death, prolongs cone survival and preserves photopic vision lowering retinal inflammation. Since this drug also rescues RPE structure, we started to test efficacy of long-lasting intravitreal Dexamethasone, to increase ocular bioavailability and avoiding the side effects of general administration. Hence, we adapted a surgical procedure of human ophthalmology performing intravitreal implants in mice. Analysis of whole RPEs upon ZO-1 immunostaining was combined to image analysis to assess barrier integrity. Paired observations were obtained by studying glucocorticoid receptor (GR) distribution and response to oxidative agents in ARPE19 cells, a human cell line with barrier organization at confluence. We show that mouse RPE and ARPE19 cells express GR and that corticosteroids might rescue simultaneously retinal cones and the RPE. Similar to the RPE recognized role in the pathogenesis of Macular Degeneration, our studies point out the relevance of the oBRB in RP progression and treatment and reinforce the notion that is possible to limit photoreceptor degeneration and vision loss targeting oBRB breakdown and damage.

NI31 | Influence of the sympathetic nervous system on the thymus: β3-adrenergic receptor-expressing stromal cells as sentinels of the thymic function

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The thymus is organized into discrete areas, in which the interaction of thymocyte precursors with stromal cells drive the maturation and the specification of T cells. The thymus is innervated by fibers of the sympathetic nervous system (SNS), that release norepinephrine (NE) in thymic environment. NE interacts with α and β2 adrenergic receptors (AR) expressed by thymocytes and with β3AR expressed by stromal cells. Hence, we have speculated that SNS signals acting on thymic stromal cells through β3AR, may promote T cell maturation and egress from the thymus. As NE increases in the thymus upon induction of experimental autoimmune encephalomyelitis (EAE), we have assessed the effect of the activation of β3AR on thymus homeostasis in EAE-induced mice treated with an antagonist of β3AR. To monitor T-cell maturation we have performed a FACS analysis of T lymphocytes within the thymus, and to evaluate the egress of mature lymphocytes from the thymus we have quantified TREC (T-cell Receptor Excision Circles) in the blood by Real-time PCR. We found that activation of β3AR increases the frequency of regulatory T (Treg) cells in thymus and increases the expression in stromal cells of IL15, a cytokine involved in the maturation process of Treg cells. The quantification of TREC in blood revealed an increase in newly generated T cells that move from the thymus into the blood upon activation of β3AR. As the decreased expression of sphingosine 1 phosphate receptor (S1P1) by T lymphocytes is the main mechanism involved in the retaining of mature T lymphocytes and in the expansion of Treg cells, we evaluated the expression of S1P1 and of Klf2, a transcription factor mediating the expression of S1P1; activation of b3ar induce a decrease expression of both molecules. Overall, our results indicate that the SNS controls the generation of Treg cells and the egress of newly-generated T lymphocytes from the thymus through a mechanism that involves β3AR-expressing stromal cells.

NI32 | Nutritional overload promotes inflammatory synaptic damage and disease course worsening in clinical and experimental multiple sclerosis

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Multiple sclerosis (MS) is a chronic autoimmune demyelinating disease with an unpredictable course also influenced by lifestyle factors. Increasing evidence, obtained in both MS and its mouse model, the experimental autoimmune encephalomyelitis (EAE), has revealed that proinflammatory cytokines and miRNAs trigger reversible synaptic dysfunctions as early hallmarks of the disease. The persistence of this inflammatory synaptopathy can cause excitotoxic damage and neuronal death, contributing to a silent disease progression independent of demyelination. Considering that nutritional overload has recently been shown to enhance chronic inflammation in several autoimmune diseases, we aimed at clarifying its effects on neuroinflammation and synaptic damage in EAE and MS.

We explored the impact of high-fat diet (HFD) compared to standard diet (SD) in EAE and control mice by evaluating clinical, behavioral, electrophysiological and molecular parameters. As expected, HFD-obesity worsened EAE clinical score and EAE-dependent weight loss. Importantly, our results also indicated that HFD significantly increases striatal inflammation and excitatory transmission (sEPSC) in both control and EAE mice. In particular, control mice fed on HFD showed an enhancement of both sEPSC frequency and duration, reproducing the striatal synaptopathy observed in EAE SD mice.

Moreover, fecal 16S rDNA sequencing revealed that HFD induces gut microbiota dysbiosis in control mice and exacerbates it in EAE mice. Mechanistically gut dysbiosis likely alters immune homeostasis and increases inflammatory synaptopathy.

Clinical studies have confirmed the high synaptotoxic potential of HFD by showing serum triglyceride levels to directly correlate with both CSF glutamate levels and MS severity (EDSS) at diagnosis.

Overall, we demonstrated that HFD negatively affects EAE and MS course by altering glutamate signaling and inflammatory synaptopathy with an exacerbation of the consequent neurodegenerative processes.

NI33 | Evaluation of early aging following perinatal inflammation-driven encephalopathy of prematurity in a mouse model

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Background: 15 million infants are born prematurely every year, 40% of the time following maternal infections. The resulting neuroinflammatory processes affecting these newborns are driven by glial cells' reactivity (microglia and astrocytes) and increase the onset of brain lesions collectively termed Encephalopathy of Prematurity (EoP). EoP is associated with neurodevelopmental disorders in children and, in recent studies, with mental deficits such as mood disorders in young adults. Evidence demonstrated that long after initial reactivity, glial cells are keener to react exaggeratedly to later inflammatory stimuli, most probably through cellular priming. Recent literature focusing on the normal aging brain highlighted a low-grade chronic inflammatory state playing a potential role in the brain's susceptibility to neurodegeneration. Glial cells primed by perinatal inflammation could therefore increase the age-related inflammation state leading to early aging.
Aims: We sought to determine whether a perinatal inflammatory challenge could accelerate the brain aging trajectory.
Methods: This study is based on a mouse model of perinatal inflammation responsible for EoP-like lesions. Months later the onset of EoP, in middle-aged mice, we used transcriptomic (RNA sequencing), functional (Ultra-fast Doppler imaging, flow cytometry, histology), and behavioral analyses (open-field, 3-chamber test…) to evaluate the impact of perinatal inflammation on age-related inflammation and neuronal impairments, including functional brain connectivity and its behavioral consequences.
Results: Our results showed signs of ongoing inflammation and glial reactivity while brain connectivity defects were recorded in middle-aged mice exposed to perinatal inflammation.
Conclusion: Preliminary data tended to confirm long-term consequences of perinatal inflammation suggesting early brain aging.

NI34 | Targeting the brain 5-HT7 receptor to prevent hypomyelination in a rodent model of perinatal white matter injuries

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Children born prematurely (1 birth out of 10) are at higher risk of developing perinatal brain lesions, especially white matter injuries (WMI). Evidence demonstrates that systemic inflammation-induced microglial and astrocyte reactivity, and are the prominent processes of WMI. Thus, a new challenge is to develop new neuroprotective strategies to target neuroinflammation to prevent WMI. Serotonin (5-HT) and its receptors play an important role in inflammation and emerging evidence indicates that 5-HT may regulate brain inflammation by the modulation of microglial reactivity and astrocyte functions. The present study is based on a mouse model of WMI induced by intraperitoneal (i.p.) injections of IL-1β during the first five days of life. In this model, certain key lesions of preterm brain injuries can be summarized by (i) systemic inflammation, (ii) pro-inflammatory microglial and astrocyte activation, and (iii) inhibition of oligodendrocyte maturation, leading to hypomyelination. We demonstrate that Htr7 mRNA (coding for the 5-HT7 receptor) is significantly overexpressed in the anterior cortex of IL-1β-exposed animals, suggesting it as a potential therapeutic target. LP-211 is a specific high-affinity HTR7 agonist that crosses the blood-brain barrier (BBB). When co-injected with IL-1β, LP-211 treatment prevented microglial and astrocyte reactivity and the down-regulation of myelin proteins (MBP and PLP) linked to hypomyelination. Thus, HTR7 may represent an innovative therapeutic target to protect the developing brain from preterm brain injuries.

NI35 | Specialized pro-resolving lipid mediator neuroprotectin D1 attenuates motor disability by reducing synaptotoxic a

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BACKGROUND • Specialized proresolving mediators (SPMs) from ω-3 polyunsaturated fatty acid control inflammation of several physiological and pathological processes. Since SPMs, including neuroprotectin D1 (PD1) is reduced in patients with Multiple Sclerosis, we evaluated its potential therapeutic role on experimental autoimmune encephalomyelitis (EAE) mice.
MATERIALS AND METHODS • C57BL/6 female mice at 8-10 wk of age were active MOG-immunized as a model of multiple sclerosis. Then, 100 ng/mice of PD1 were daily intraperitoneally administered starting from the peak of disease (15-day post immunization). The antinflammatory activity mediated by PD1 treatment was evaluated measuring disability and synaptic transmission of EAE mice. Motor activity of EAE mice was measured after 10-12 days of treatment using specifical behavioral task made to measure endurance and muscle strength on mice. In order to study neuronal network, both excitatory and inhibitory striatal transmission were evaluated respectively during the acute and the chronic phase of disease by patch clamp recording.
RESULTS • PD1 attenuated motor impairment on EAE mice starting from 10-12 days of treatment, improving score disability from 25 dpi until the end of experimental study (35 dpi), muscular strength and endurance. By electrophysiological recording, although PD1 did not influence glutamatergic transmission measured in the acute phase (18-24 dpi), probably due to a duration of treatment, the GABAergic transmission measured at 26-35 dpi showed an increase of GABA release from presynaptic neuron on PD1-EAE mice compared to Vehicle-EAE mice. Furthermore, we found that cannabinoid activity on GABAergic transmission was restored on PD1-EAE mice, highlighting the antinflammatory ability of PD1.
CONCLUSIONS • Overall, restoring resolution of Inflammation with PD1 ameliorates the motor disability of EAE mice by impacting on synaptopathy in a cannabinoid-dependent manner.

NI36 | Inflammatory pathways signal transducers analysis in iPSC-derived neurons and 3D cerebral organoids

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Aicardi-Goutières syndrome (AGS) is a rare severe genetic disorder characterized by constitutive type I interferon (IFN) upregulation due to mutations in genes involved in nucleic acid metabolism and sensing. Clinically AGS is characterized by microcephaly, brain atrophy and leukodystrophy. To date some treatments with immuno-modulatory drugs that block interferon alpha (IFNa) signaling, seem to improve the immunological conditions of patients, but the lack of therapies for their neurological degeneration is particularly pressing. The inaccessibility of autologous neurons to test new pharmacological compounds is hindering improvements in the field. The possibility to derive neurons from inducible pluripotent stem cells (iPSCs) obtained from patients offers the opportunity for in vitro modeling. We have deepened the in vitro 2D neuronal differentiation, generating and characterizing neural stem cells (NSCs) and neurons from iPSCs of three AGS patients mutated in different genes. Given the central role of IFNa in AGS, we investigated the IFNa signaling in NSCs, analyzing STAT1 as its principal signal transducer. Despite what we observed in lymphocytes and monocytes of AGS patients that showed a statistically significant increase of STAT1 activation and expression, NSCs seem to be anergic to IFNa stimulation. No increment of STAT1 activation was in fact detected in NSCs, while STAT1 expression was still present in iPSC-derived NSCs. Our results suggest that neuronal degeneration observed in AGS could not be caused directly by IFNa, but can be a consequence of an unhealthy state of glia, that, stimulated by IFNa, assumes the antiviral phenotype that impairs the typical glia functions such trophic support and myelination. Moreover, we generated 3D cerebral organoid in dynamic suspension from control and AGS iPSCs as a better in vitro model to explore the pathogenetic contributions and interaction between neurons and glia.

NP16 | Enhancing dendritic spine plasticity by coupling physical activity with non-invasive brain stimulation

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There is a huge interest in coupling transcranial direct current stimulation (tDCS) and physical activity as an effective strategy to further enhance cortical excitability in physiological and pathological conditions. Nevertheless, the mechanisms underlying this phenomenon are not well understood yet. Animal studies revealed that tDCS affects the motor cortical plasticity by modulating dendritic spines, similarly to voluntary physical exercise. Thus, in this study we investigated the effects of combining tDCS and physical activity in healthy mice. For this purpose, we studied the effects of coupling anodal tDCS and physical activity on the morphological plasticity in primary motor cortex (M1) layer II/II and layer V in both young (2-3 months) and middle-aged (14-16 months) mice. At both ages, the combination of stimulation and physical activity results in an increased number of activated cells and in a higher density of spines in basal and apical dendrites of both hemispheres, compared to single interventions only. In young mice spine morphology analysis highlighted increased mushrooms spines, while middle-aged mice showed higher number of thin spines after the association of tDCS and physical activity. Altogether, the coupling between tDCS and physical activity results in a significant inter-hemispheric plasticity enhancement in physiological conditions, maintained with aging. However, the spine morphology is differently displayed in young and middle-aged mice, probably indicating a different effect of the combination in the aging.

NP17 | Exploring the Dynamics of Cell Excitability by Optogenetics in ex vivo Neuronal Cultures

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It is currently unclear whether or how neuronal excitability dynamics differ between types of neurons over extended (little-studied, yet highly relevant) timescales.
We approach the subject via a medium-throughput methodology, coupling extracellular recording with optogenetic stimulation of subpopulation specific, ShChR/CaMKIIα-expressing i.e., putative glutamatergic cells. We analyze spike probability and spike latency as proxies of intrinsic excitability.
In accordance with previous findings (Gal et al., 2010), across a wide range of frequencies of repeated photoactivation, we report a transient and an intermittent phase. Based on the latter, we classify the considerable heterogeneity of responses. These observations question the validity of the established glutamatergic type, which is essential for understanding the computations it performs and its role within a circuit. Furthermore, we find the macroscopic properties of the evoked spike trains to be identical under different stimulation regimes above a critical stimulation rate. We show that the fluctuations in the spike probability which occur over extended observation windows follow the statistics of a fractional random process, indicating that intrinsic neuronal activity is significantly affected by past stimuli.
Our work is crucial to the development of more accurate generative models which are capable of accurate and versatile description of neuronal excitability a) across spatiotemporally diverse electrophysiological types and b) over behaviorally relevant, extended timescales.

NP18 | Olfactory stimulation reverses anxiety and depression-like behaviours induced by acute and chronic stress

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Major Depressive Disorder (MDD) is a debilitating illness which is estimated to be affecting 3.8 % of the adult population worldwide. MDD is characterized by low mood, irritability, reduced self-care and anhedonia, all symptoms which can lead to social isolation and, in worst cases, to suicide. Among the different pharmacological options available for MDD, many patients show treatment-resistance and heavy side-effects, underlying the necessity to investigate other therapeutic strategies, such as olfactory stimulation. In fact, the olfactory bulb and related structures are in tight connection with the limbic system and it has been reported that, also in humans, odorants can influence mood, memory and autonomic responses. Here, we show that in a paradigm of acute restraint stress, olfactory stimulation using a mixture of vanillin, limonene and green odor (trans-2-hexenal and cis-3-hexenol) decreases anxiety behaviour in the elevated plus maze. Indeed, animals subjected to acute restraint stress treated with olfactory stimulation show reduced time in the closed arms and a higher number of entrances in the open arms of the apparatus compared to stress-exposed only animals. This effect is independent from locomotor activity, as assessed by the open field test. Furthermore, in a mouse model of depression, the chronic unpredictable mild stress paradigm, both male and female wild-type mice treated with the olfactory stimulation protocol showed increased grooming time in the sucrose splash test and reduced immobility in the forced swim test. Again, this effect was independent from alterations of total locomotor activity therefore suggesting reduced depressive-like symptoms. Collectively, our data indicate that olfactory stimulation counteracts the detrimental effects of acute and chronic stress on anxiety and depressive-like behaviours.

NP19 | Inhibitor of the excitation-contraction coupling machinery act as enhancer of Botulinum Neurotoxin type A pharmacological activity

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Botulinum neurotoxin type A (BoNT/A) is a blockbuster drug preparation for the treatment of cholinergic nerve hyperactivity and very effective in aesthetic medicine. Exceptional specificity and persistence of action are key features of its pharmacological activity. Yet, a major challenge in BoNT/A therapy remains to quicken its onset of action, which can take up to one week after injection, without increasing the dose. Here, we report an innovative treatment combining BoNT/A with blockers of the excitation-contraction coupling (ECC) machinery, including an inhibitor of Nav1.4 voltage-gated sodium channels in the sarcolemma, dubbed FTP-101. Commercial preparations of BoNT/A were mixed with FTP-101 or saline and injected locally in the hind limb in mice. Paralysis was monitored via the digit abduction score (DAS) assay and electrophysiology techniques. FTP-101 quickened BoNT/A effects with myorelaxation starting already at 1 hour, compared to the 6 hours of control. Interestingly, the drug combinations also prolonged BoNT/A effects causing a substantial enhancement in the overall duration of action, as assessed by measuring nerve-muscle transmission via the Evoked End-Plate Potentials 21 and 45 days after injection. Importantly, we examined the cleavage of SNAP-25 by immunofluorescence on the NMJs of the soleus throughout the first 18 hours after the injection of either BoNT/A or the combination. We found a significantly faster accumulation of cleaved SNAP-25 at the motor axon terminal (MAT) when BoNT/A was injected in combination, which roughly corresponded to a threefold dose of BoNT/A. This suggests that the faster onset was not only due to the direct muscle relaxant effect of FTP-101 but also to an anticipated BoNT/A entry within MATs, indicating a striking synergistic biological action between BoNT/A and ECC blockers.

NP20 | The effects of anesthetics on glycogen concentration in microwave-fixed brain samples

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Astrocytes are the most abundant type of glial cells in the brain. They are required for neuronal functioning, neurogenesis, maintenance of brain vascular tone and are crucial in supporting neuronal metabolism. Glycogen, the largest cerebral energy reservoir, is specifically localized in astrocytes under physiological conditions. Astrocytes possess the enzymatic machinery necessary for glycogen breakdown and its further conversion to lactate, which could be shuttled to neurons via monocarboxylate transporters to fuel their tricarboxylic acid cycle (a mechanism known as Astrocyte-Neuron Lactate shuttle, or ANLS). Therefore, one of the primary roles of glycogen is to provide a metabolic buffer during neurotransmission. Recent studies report the crucial role of glycogen metabolism in long-term memory formation, maintenance of long-term potentiation, and learning-dependent synaptic stabilization. In the present work, we aim to compare the glycogen concentration in the brain using different anesthetics prior to euthanasia by microwave fixation. We also compare the glycogen levels in paraformaldehyde-perfused and microwaved-fixated tissue. Our results confirm that microwave-fixation better preserves glycogen stores by halting enzymatic activities and extend the analysis to optimally combine fixation of brain metabolites in vivo while maintaining anatomic integrity. Finally, we compared different working protocols in the microwave-fixation system to determine the adequate condition for glycogen quantification. Our results support the idea that proper biochemical quantification of metabolites related to brain energy requires a special experimental setup that needs to be finely tuned.

NP21 | Tetanus Toxin Injections into the Rat Motor Cortex and Striatum Impair the Narrow Beam Walking Performance

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Abnormalities in the motor cortex and basal ganglia excitability play a central role in the onset of movement disorders such as dystonias and parkinsonism. Basal ganglia and motor cortex integrate the sensory proprioceptive input arriving from the periphery for the planning and execution of movement. Tetanus neurotoxin (TeNT) prevents the inhibitory neurotransmission in central synapses. The aim of present the study was to examine the behavioral effect of neuronal disinhibition in mentioned brain regions induced by low, non-convulsive doses of TeNT. The rats were unilaterally injected into the caudate putamen or motor cortex with 0.5 ng TeNT. The injections were repeated into the contralateral motor regions after 2 weeks, and the effect of TeNT was assessed for another 2 weeks. Different behavioral tests were performed repeatedly to assess the effect of TeNT induced disinhibition on rats motor performance, as well as to exclude possible epileptogenic action of tetanus neurotoxin. After unilateral toxin applications, the animals appear to be able to compensate for the proprioceptive motor deficit, which is then aggravated and becomes longer lasting after contralateral regional disinhibition. The plantar misplacement of the hind-limb during the narrow beam traversing were more evident on the hind-paw contralateral to the injected brain region. Open field test, pre-pulse inhibition and attempted audiogenic seizure tests did not indicate the possible epileptogenic actions of TeNT. The motor cortex or striatal disinhibition with TeNT induces subtle motor impairment during the relatively complex motor task of crossing the elevated narrow beam, requiring correct prediction of paw placement. Since only 50% of treated animals have developed the described impairments, it is necessary to make additional experiments. Experiments with higher doses of TeNT or application into the different regions such as the internal globus pallidus are necessary.

NP22 | Role of group I metabotropic receptors in the synaptic alterations in the dorsal striatum of theR451C-Nlgn3 mouse model of autism

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Autism Spectrum Disorders (ASD), comprise heterogeneous neurodevelopmental disorder-scharacterized by early onset of communication and social interaction difficulties, restricted interests,repetitive and stereotyped behaviors. Gene sequencing studies have identified hundreds of genespotentially implicated in ASD, which converge on two main biological pathways: gene expressionregulation and neuronal communication. The projection neurons of the striatum, the input nucleusof the basal ganglia, are characterized by a particularly high expression of ASD-associated genes. Indeed, structural and functional alterations of the striatum were described in ASDpatients, and a correlation of the dorso-ventral anatomo-functional subdivisions of the striatumwith specific domains of ASD symptoms was proposed. In our previous work, we described the lossof corticostriatal long-term depression (LTD) in the dorsal striatum of the R451C-Nlgn3 knock-in(KI) mouse model of ASD. LTD was partially rescued by enhancing the endocannabinoid tone oractivating CB1 receptors.Here, we aimed at identifying more effective strategies to rescue corticostriatal LTD. Activation ofgroup I metabotropic glutamate receptors (mGlu1 and mGlu5 receptors) activates downstreamsignaling pathways, involving production of endocannabinoids. We therefore attempted apharmacological rescue of LTD in KI mice, by targeting group I mGlu receptors. The mixed mGlu1and mGlu5 receptor agonist 3,5-DHPG was able to rescue LTD expression. By means ofimmunoblotting experiments, we found that the amount of mGlu5 receptor protein was significantlyreduced in the synaptosomes from the dorsal striatum of KI mice, suggesting a molecular basis ofcorticostriatal LTD impairment.

NO12 | Breast cancer susceptibility gene 1 (BRCA1) is a critical component of the DNA damage response after CITK inhibition in Medulloblastoma

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Standard therapy consists in surgical resection, followed by radiotherapy and high-dose multiagent chemotherapy. Despite the improvement in patient survival, many patients still die and those who survive suffer from neurological and endocrine disorders. Hence, there is a pressing need develop more efficient therapies. An attractive target for MB-directed drug development is represented by Citron Kinase (CITK). CITK knockdown in MB leads to cytokinesis failure, DNA damage accumulation and apoptosis in vitro and in vivo. Here we found that CITK knockdown in MB reduces Breast cancer susceptibility gene 1 (BRCA1) levels. BRCA1 is a tumor suppressor fundamental for Homologous Recombination (HR)-mediated DNA repair. We found that CITK knockdown reduces BRCA1 recruitment to the DNA double strand breaks lesions without altering phospho-RPA recruitment in MB cell lines. Consequently, we observed reduction in BRCA2 and RAD51, other players of the HR pathway. These data indicate that CITK plays a crucial role for BRCA1-BRCA2 complex activity. Our future effort will be to elucidate how CITK regulates BRCA1 recruitment at the DNA damage sites in MB. Moreover, we will assess if CITK knockdown could sensitize MB cells to PARP inhibitors, treatments used in clinical practice in BRCA1 mutated cancers.

NO13 | Cancer-neuronal crosstalk in glioblastoma

BACK

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Glioblastoma (GBM) is a highly aggressive and invasive brain tumor with rather unique features. Interestingly, it is of recent acquisition that GBM also exploits neuronal activity as fuel to boost proliferation and aggressiveness and it induces hyperexcitability of surrounding network creating a vicious cycle. However, the mechanisms and the specific contribution of brain cells to the interplay between neurons and cancer cells are largely unknown. We set up an in vitro co-culture model to study the molecular mechanisms underlying the GBM-neuronal crosstalk. Primary human GBM Stem-like Cell Lines (GSCs) were established from patient post-surgical specimens. To model the neuro-tumoral unit, GSCs were cocultured with primary neurons at either immature (4DIV) or mature (11 DIV) stages. After 7 days of coculture, both immature and mature neuronal cultures boosted GSCs proliferation. In addition, neuronal conditioned medium or astrocytes alone were not able to sustain cancer cell proliferation likewise, suggesting a putative mechanism based on cell-to-cell interaction.

To explore the possible causes, neuronal network activity was measured in presence/absence of tumor cells using High Density-Multi Electro Array. After 24 hours of neuron-cancer co-culture, the mean firing rate of neurons was increased indicating that GSCs promote network excitability. By using the genetically encoded intracellular Glutamate-Sensitive Fluorescent sensor (iGluSnFR), we found that GSCs were able to sense electrically-evoked glutamate released from neurons, with kinetic properties resembling those observed in neuron-to-neuron synapses. In summary, here we described a GBM-neurons vicious cycle in which neurons boost GSCs proliferation and cancer cells trigger neuron hyperexcitability, probably involving glutamate released by neuronal activity. These results represent an entry point to investigate the molecular mechanisms underpinning cancer-neuronal crosstalk.

NO14 | Reduction of lipoprotein receptors levels synergistically potentiates the anti-tumour activity of Givinostat on human glioblastoma cancer cells

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Dysregulation of histone modifying enzymes (HDACs) is commonly identified in many tumors and has been linked to cancer proliferation, changes in metabolism and drug resistance. These events also sustain the onset and progression of glioblastoma (GBM), the most common and aggressive brain tumor. Accordingly, HDAC inhibitors (HDACis) represent a promising class of anti-tumor agents. In this context, we analysed the activity of Givinostat, a pan-HDACi, in a GBM cell model. The treatment of GBM cells with Givinostat inhibited HDACs activity, affected cell viability in a dose- and time-dependent manner and induced caspase-mediated cell death. Givinostat also display a natural selectivity for cancer cells versus healthy cells that was maintained up to the dose of 2.5 µM. In addition, the expression levels of several receptors involved in cholesterol uptake (low-density lipoprotein receptor, very low-density lipoprotein receptor and low-density lipoprotein receptor-related protein 1) were significantly decreased, unravelling an unprecedented mechanism of action of Givinostat on GBM cells. This effect was confirmed using ApoE-lipoprotein-like particles in 2D and 3D cellular models, providing evidence of the key role played by HDACs in tumor metabolism. We also provide the proof of concept for a delivery system that can improve the pharmacokinetic of Givinostat. Liposomes composed of cholesterol and sphingomyelin embedding Givinostat showed a 2.5-fold increase in the drug plasma half-life and a 6-fold increase of the drug brain exposure in healthy mice. These results strongly suggest that Givinostat may have a clinical potential for HDACi-based therapeutic strategies against GBM and liposome valuable candidates for its brain delivery. IMMUNHUB “Sviluppo di nuove molecole di seconda generazione per immunoterapia oncologica”, CUP E51B19000550007–Call HUB Ricerca e Innovazione, cofunded by POR FESR 20142020 (Regional Operational Programme, European Regional Development Fund).

NO15 | CTX-CNF1 treatment boosts the immune system

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Glioblastoma Multiforme (GBM) is the most destructive type of gliomas, with an average survival rate of 15 months after diagnosis. The currently used standard of care is not effective, thus there is a compelling need to find innovative approaches to counteract GBM and preserve the surrounding healthy tissue. In our lab has been recently designed and tested a new recombinant protein CTX-CNF1 conjugating Chlorotoxin (CTX), a well-known peptide able of crossing the blood-brain-barrier and selectively targeting glioma cells, to CNF1, a protein that leads glioma cells to death through the activation of a senescence process. In vitro and acute in vivo studies have highlighted the potential of our chimeric protein in counteracting GBM growth. To recapitulate what happens in clinic, we performed weekly systemic administrations of CTX-CNF1 (80 nM) for 3 weeks starting from MRI diagnosis. This repetitive treatment ameliorated glioma-bearing mice motor deficits (seen with Grip Strength and Grid Walk tests), progressively reduced their tumoral mass and significantly increased their survival. Indeed, 50% of glioma-bearing mice were still alive 6 months after tumor induction and no glioma mass was detected at this time point. These surviving mice were rechallenged with glioma cells (injected into the contralateral hemisphere) and, comparing with same-age mice, they were less susceptible to develop another GBM. This suggest an involvement of immune system, that was also confirmed by immunofluorescence, where we found that CD8 marker was upregulated in peritumoral tissue 48h after treatment. Our preclinical data strongly point out that CTX-CNF1 represents a very promising approach for GBM treatment. However, further studies need to be done to better understand which are the underlying molecular pathways.

NO16 | The role of the cytoskeleton regulator inverted formin INF2 in medulloblastoma tumorigenesis

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Medulloblastoma (MB) is the most common and aggressive pediatric brain malignancy. The high heterogeneity of MB makes extremely difficult determining a successful therapy. Among MB's molecular subgroups, Sonic Hedgehog (SHH) is the most abundant and genetically understood. SHH-MB is characterized by genetic alterations in key components of SHH signaling, a developmental pathway emerged as an attractive therapeutic target for MB treatment. However, the molecular circuitries governing SHH-MB remain unclear. Recently, we identified INF2, a formin involved in the regulation of actin and cytoskeletal dynamics, as a negative regulator of SHH signaling involved in SHH-MB tumorigenesis. We found that INF2 counteracts the transcriptional activity of GLI1, the final and most powerful effector of SHH signaling. INF2 protein levels, but not mRNA, were strongly reduced in murine and human SHH-MBs, suggesting a regulation of INF2 at post- translational level. Indeed, we observed that FBXW7, an E3 ligase and tumor suppressor highly mutated in MB, promotes the ubiquitylation of INF2 and its protein stability, suggesting that the loss of INF2 expression, due to mutations of FBXW7, might play a key role in SHH-MB tumorigenesis. Importantly, we showed that the overexpression of INF2 in SHH-MB primary cells repressed tumor cell proliferation and this correlated with the decrease of GLI1 expression levels. Overall, these findings support a negative role of INF2 in the control of SHH signaling and SHH-dependent tumor growth and could further illuminate on the role of cytoskeleton in SHH-dependent cancers.

NO17 | Molecular changes underlying decay of sensory responses and enhanced seizure propensity in peritumoral neurons

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In recent years, the interaction between glioma and brain cells has emerged as one important regulator of tumor progression. In particular, it has been proved that glioma growth impacts the structure and physiology of peritumoral neuronal networks, altering the activity of pyramidal neurons which drives further tumor progression. Using the GL261 syngeneic murine model, we performed in vivo electrophysiological recordings of visual evoked potentials (VEPs) to longitudinal assess modifications of peritumoral neurons along with glioma progression. With respect to controls, glioma-bearing mice showed a dampening of visual responses that started from day 14 after tumor induction (TI). At this stage, we microdissected layer II-III pyramidal neurons and evaluated the expression of a panel of genes involved in synaptic transmission and neuronal excitability. Among all genes, only gabra1 and SNAP25 were significantly reduced in peritumoral neurons from glioma-bearing mice. No significant changes were detected in glutamatergic markers. We also performed LFPs recordings in freely moving glioma-bearing and control mice. We found interictal spikes in 50% of glioma-bearing mice 18 days after TI. An intraperitoneally treatment with a subconvulsive dose of DMCM triggered epileptiform activity in glioma-bearing mice but not in controls, suggesting an involvement of the GABA-A receptor in seizures' susceptibility. Elucidating the mechanisms underlying the decay of the sensory response and the propensity to seizures in glioma-bearing animals could add useful information to develop more effective therapeutic approaches aimed at ameliorating patients' quality of life and survival.

NIM09 | An unexpected culprit: intracerebellar hemorrhage in at-term newborn

BACK

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Cerebellar hemorrhage in at term neonates is very rare. In the past, it was usually diagnosed postmortem, but nowadays, its detection is increasing thanks to the new radio-imaging techniques. It is associated to traumatic birth (breech presentation), prolonged labor, vacuum application and maternal factors such as infections. They could be due to severe distortion of the venous structures with laceration of the falx or to direct cerebellar damage with vermis laceration. Clinically, the patients could present severe asphyxia requiring intubation and seizures. The outcomes are relatively unknown but they can vary from normal to severe disabilities including motor alterations, cognitive and psychiatric disorders and autism.

In our case, the neonate had a gestational age of 39+5 weeks (spontaneous delivery with a labor of 18 h and double-wrapped cord around the neck) and mild hypoxic ischemic encephalopathy at birth. He was hypotonic, lethargic, with reduced motility and reflexes. He was treated with whole body hypothermia and the cerebral function monitoring was started. The electroencephalogram was moderately altered. After some hours the patient improved and was extubated.

In the 2nd day of life, the patient suffered from severe apnea requiring intubation and drug-resistant seizures (phenobarbital), thus levetiracetam was added. The ultrasound detected an intraventricular hemorrhage. While the magnetic resonance imaging highlighted a bilateral intraparenchymal cerebellar hemorrhage (18.8x10 mm in the right hemisphere and 9.8x5 mm in the left hemisphere), a subdural hemorrhage in the same region and an intraventricular hemorrhage was also detected. Then the patient recovered his respiratory function, with amelioration of his general conditions and was discharged after 2 weeks.

At 2 months of life, during the follow up examination, the patient displayed a normal psycho-behavioral and motor profile.

NIM10 | In vivo evaluation of dentato-thalamo-cortical tract integrity in friedreich ataxia using diffusion MRI

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Background and Objective • Brain involvement in Friedreich Ataxia (FRDA) is characterized by widespread microstructural alterations, extending beyond brainstem and cerebellum. Nevertheless, no information about the degree of involvement of the dentato-thalamo-cortical tract (DTT), the cerebellar motor system main efference, is available. Aim of this study was to explore the microstructural integrity of this tract in FRDA using diffusion MRI (dMRI).
Methods • Scans of 57 FRDA and 52 healthy-controls (HCs) from three different sites were evaluated. In all subjects a volumetric T1-weighted sequences, for brain parcellation purposes, and a high resolution dMRI sequence, for the quantification of DTT bundles, were obtained. Tracts computation was obtained as follows: fibers connecting each dentate nucleus (DN) to the contralateral thalamus, encompassing ipsilateral red nucleus and ending in the primary motor cortex were calculated for each HC. A study specific template was calculated as the average of all tracts, and then applied to each patient's space to extract microstructural indices of bundle integrity (fractional anisotropy -FA-, radial -RD- and mean diffusivity -MD-).
Results • After excluding subjects with poor image quality, data of 50 FRDA patients (mean age 34.8±13.9;M/F=29/21) and 38 HCs (mean age 36.1±12.7;M/F=18/20) were compared. A significant decrease in FA in FRDA, compared to HCs, emerged on both sides (0.38±0.03vs0.42±0.02, on the left; 0,39±0.03vs0.43±0.02, on the right, p-values<0.001), coupled to a significant increase in MD and RD (all p-values<0.001).
Discussion and Conclusion • Our analysis further expands the current knowledge about brain involvement in FRDA, by showing the presence of significant microstructural abnormalities at the level of the main cerebellar efference in these patients. This finding is in line with the hypothesis of an anterograde secondary degeneration arising from the DN to the primary motor cortex and corroborate the possibility of employing dMRI to longitudinally evaluate damage spread and possibly treatment response in FRDA.

NIM11 | Examination of whole-brain structural and functional connectivity in Fabry Disease

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In Fabry Disease (FD), a rare X-linked lysosomal storage disorder, the central nervous system is heterogeneously involved but the macro-scale connectivity is not yet been investigated. In this study, we processed diffusion and resting-state functional MRI data of 46 patients (FDs, 28F, 42.2±13.2y) and 49 healthy controls (HCs, 21F, 42.3±16.3y). To build structural connectomes (SC) we employed probabilistic tractography and Convex Optimization Modeling for Microstructure Informed Tractography, weighting each connection by the total intra-axonal signal fraction of the corresponding bundle. Functional connectomes (FC) were built by computing the correlation between BOLD timeseries and using a modified AAL parcellation with 100 regions (used also for SC). By exploring the between-group differences in terms of 5 global network metrics extracted for each brain network, we found that FDs have a significantly reduced global efficiency ($p=0.005$) and mean strength ($p<0.001$) in SC and an increased modularity ($p=0.005$) in FC. Moreover, we employed network-based statistics to explore for the presence of connected subnetworks associated with a significant between-group difference. As result, we identified a subnetwork, involving mainly frontal areas, with decreased structural connectivity in FDs w.r.t. HCs. Finally, we tested the relationship between the altered properties of SC and FC and the cognitive performance of a FDs subset (n=11). Significant correlations arose between SC mean strength and RAVLT-immediate score ($r=0.72$, $p=0.03$) and between FC modularity and DGS score ($r=-0.77$, $p=0.02$). Instead, the subnetwork showed a mean structural disruption of -0.62 in FDs compared to HCs, which resulted significantly correlated to 3 different neuropsychological tests (WCFST, CBTT and RAVLT-delayed). These findings show widespread structural disconnection and functional reorganization in FDs, supported by loss in axonal integrity and with some associations with cognitive performance.

ND12 | The β amyloid-derived peptide Aβ1-6A2V protects from tau toxicity in vivo

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Altered amyloid β (Aβ) and tau species are the hallmarks of Alzheimer's disease (AD) and the primary targets for therapeutic intervention. Although several attempts to find a treatment for AD have been made over the years, there are still no effective therapies, and the drugs available provide only modest symptomatic benefit. To design an innovative pharmacological strategy, we took inspiration from a clinical observation on a subject naturally protected from the onset of a genetic AD form. In this subject, the 673 Ala-to-Val substitutions in heterozygous form in the APP gene resulted in the production of an Aβ carrying A2V mutation able to interact with Aβ wild-type, thus interfering with its polymerization. Based on this observation, our group developed the Aβ1-6A2V synthetic peptide able to interfere in vitro and in vivo with Aβ polymerization and protect from its proteotoxicity.

To test whether this peptide can also interfere with tau aggregation and toxicity, we applied an integrated approach involving recombinant human tau, the nematode C. elegans, and two murine models of tauopathy: the transgenic P301L mice and 3xTg-AD mice subjected to traumatic brain injury (TBI). Aβ1-6A2V inhibited tau aggregation in vitro and counteracted the toxicity induced in C. elegans by brain homogenates from P301L mice. The intranasal administration of Aβ1-6A2V to injured 3xTg-AD mice caused a reduction in the TBI-induced cognitive impairment. Noteworthy, brain homogenates from Aβ1-6A2V treated animals were not toxic when administered to C. elegans, indicating that the peptide reduced the cerebral toxic forms of tau in mice.

These findings indicate for the first time that Aβ1-6A2V can interact with tau in vitro and in vivo, protecting from its toxic effects, and suggest that this peptide can be an ideal therapeutic strategy for treating not only AD but also other tauopathies.

ND39 | Emerging roles of SLITRK family members in αSyn- p.A53T mediated synaptic dysfunction

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Alpha-synuclein (αSyn) is a highly expressed and conserved pre-synaptic protein, which when dysregulated leads mainly to sporadic Parkinson's disease (PD). Familial forms of the disease also exist and are due to mutations in the SNCA gene that encodes αSyn. The best-characterized mutation of αSyn is the p.A53T (G209 SNCA), which leads to early onset progressive parkinsonism and is studied in numerous mouse and human-based experimental systems. Our team employing a human induced pluripotent stem cell (iPSC)-based model that harbors the p.A53T mutation and displays PD-associated phenotypes, showed early distortions in a variety of pathways, including those that are related to synaptogenesis. Of relevance, transcriptomic analysis suggested defects in synapse formation and function and showed dysregulated expression of genes involved in synaptic signaling. Here we try to investigate the potential role of three members of the post-synaptic adhesion molecules family, so called-SLITRKs in p.A53T-αSyn mediated synaptic dysfunction. A transgenic mouse model that expresses the human p.A53T-αSyn and hiPSC-derived neurons are used to examine the ultrastructural and molecular defects of the p.A53T synapse and establish the link between pathological αSyn expression and SLITRKs expression and subcellular localization. E.M. analysis of p.A53T synapses showed distorted organization and less synaptic vesicles compared to control synapses. Additionally, artificial synapse formation assays revealed defects in early synaptogenesis as p.A53T neurons formed more inhibitory synapses than control neurons, while excitatory synapse formation remained unaffected. This imbalance was also obvious when naturally-forming p.A53T synapses were analyzed. Furthermore, dysregulation in SLITRK1/2/4 RNA and protein expression was observed from early stages of p.A53T pathology while subneuronal localization was also greatly affected. Altogether, our work aims to identify the link between SLITRKs dysregulation and p.A53T-αSyn induce synaptopathy and characterize the molecular and cellular mechanisms.

ND40 | The functional coupling between the NaV1.6 voltage-gated channel and the Na+/Ca2+ exchanger 3 promotes an endoplasmic reticulum Ca2+ refilling in a transgenic model of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disorder with a clinical symptomathology ranging from cognitive disabilities to severe dementia. Although the AD etiology is complex, the Amyloid-β (Aβ) peptide is recognized as the main culprit of AD neurodegeneration. The remodeling of ionic homeostasis is crucially involved in neuronal and glial responses to Aβ injury and determines detrimental modifications in cellular homeostasis, neuronal excitability and synaptic activity. Previously, we demonstrated that the upregulation of the NaV1.6 voltage-gated channel is involved in the hippocampal hyperexcitability of the Tg2576 mouse, a transgenic model of AD displaying Aβ accumulation in the brain parenchyma. Since disturbances in Ca2+ homeostasis are a crucial cellular event contributing to AD development, in the present study we have explored the impact of the NaV1.6-mediated aberrant inward Na+ currents on Ca2+ homeostasis of primary hippocampal neurons from the Tg2576 mouse. By means of functional studies, we assessed the activity of the Na+/Ca2+ exchanger (NCX), a master regulator of Ca2+ and Na+ concentrations in neuronal cells. We found that the Ca2+ influx through the NCX isoform 3 was increased in Tg2576 neurons at 12 days in vitro, when the maximum upregulation of NaV1.6 was observed. Intriguingly, this increased Ca2+ influx resulted in the enhancement of the endoplasmic reticulum (ER) Ca2+ content, without affecting cytosolic Ca2+ levels. While NCX3 protein expression was unaltered, immunocytochemical analyses revealed that NaV1.6/NCX3 co-localization was increased in Tg2576 neurons, hence supporting their functional coupling. Indeed, in the presence of a NaV1.6 internalization-inducer or NaV1.6 silencing the increase of Ca2+ influx through NCX3 was prevented. Collectively, our results reveal that the upregulation of NaV1.6 affects the filling state of the ER of Tg2576 neurons through NCX3 and provide new insight into ionic dysregulation in AD.

ND41 | Sleep fragmentation accelerate dementia in transgenic 5xFAD AD mice model inducing astrogliosis and affecting glymphatic system

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Alzheimer's disease (AD), the most common form of dementia, is characterized by genetic and multifactorial risk factors. Many studies correlated AD to sleep disorders. The acute effect of sleep disorders results in an increase in amyloid-b (Ab) concentration, due to a decrease in its clearance, besides the sleep quality in AD patients is impaired, leading to a possible further accumulation of Ab. In this study, we performed and validated a mouse model of AD and sleep fragmentation in 5xFAD mice of 2 months of age. All the animals underwent to behavioral studies to analyze anxiety and spatial and working memory. We had validated sleep fragmentation and its effect through EEG and biomolecular analysis, by observing all the macro-areas implied in sleep regulation. As regard behavioral activities, we observed a significant memory impairment and an increase in anxiety in fragmented mice compared to control. These results were confirmed in biomolecular analysis. In particular, Ab accumulation increased in all the interested areas, while tau phosphorylation appeared only in the dentate gyrus, and these data correlate to a significant increase in neuroinflammation, evaluating both microglia and astrocyte markers. Aquaporin-4 (AQP4), the astrocyte transporter for Ab clearance, was significantly increased in fragmented mice compared to control. This is not evident analyzing older 5xFAD mice (6 months old) which underwent sleep fragmentation, where we observed a decrease of AQP4 levels without differences in the density of GFAP. In conclusion, we can assert sleep fragmentation worsen AD pathology by accelerating Ab accumulation, which in turn triggers neuroinflammation and tau phosphorylation, but there is still an attempt from the brain to rescue the faster progression of the pathology by increasing AQP4 levels. While, in an advanced pathological system in 6 months old mice, the AQP4 clearance mechanism fails, perhaps due to the decreased expression of the channel.

ND42 | Histone Deacetylase inhibition in Retinitis Pigmentosa rescues cone cells

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Retinitis Pigmentosa (RP) is a family of heterogeneous genetic retinopathies leading to progressive photoreceptor loss and blindness. Hallmarks of RP are the primary death of rods (usually expressing the primary mutation) and a secondary degeneration of cones. RP is a complex and still not completely understood disease, yet without a cure. A promising approach to treat RP is delaying the secondary death of cones, which suffer from a so-called bystander effect; rescuing cones is important to maintain visual acuity and ensure to RP patients some independence in daily activities. It has been shown that an alteration of Histone Deacetylases (HDACs) activity occurs in various neurodegenerative pathologies, including RP and modulation of these enzymes is a major goal of epigenetic approaches. We tested a newly synthesized molecule (NF2902) inhibiting specifically HDAC6/8 expressed in cone cells as potential therapy for RP using the rd10 mutant mouse, mimicking autosomal recessive RP due to a rod-phosphodiesterase mutation. Different doses of the drug were employed to determine the most effective to promote cone maintenance. Preliminary data obtained by immunohistochemistry (IHC) and Electroretinogram (ERG) recordings demonstrated that in vivo administration by a single intravitreal injection of NF2902 results into a visible preservation of cone morphology and a measurable higher survival of cone cells in treated mice. This was confirmed by maintenance of cone-mediated responses recorded by ERG in photopic conditions. Western blot analysis of α-tubulin and acetylated α-tubulin (a known downstream product of HDAC6 inhibition) confirmed that the drug reached the retina and effectively regulated the activity of the targeted HDAC. In conclusion, this study underlines the capability of a novel and cone-specific HDAC inhibitor to promote a morphological and functional preservation of cone cells, extending the time window of useful vision in RP.

ND43 | The role of astrocytes in β-Amyloid- and magnetite nanoparticles-induced neurotoxicity

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Air pollution is thought to be one of the causes of the increased occurrence of several neurodegenerative diseases such as Alzheimer's. Very recent studies have shown that magnetite (iron oxide) nanoparticles (MNP), produced by urban traffic, can be inhaled and reach the brain. This ultrafine particulate matter is toxic to the brain as it can promote the formation of reactive oxygen species and induce oxidative stress, a condition often associated with Alzheimer's disease and other neurodegenerative disorders. Iron has been shown to facilitate Amyloid-β (Aβ) deposition, a hallmark of Alzheimer's disease, leading to neuronal damage. During oxidative stress, astrocytes can activate the transcription factor Nrf2 a regulator of several phase II detoxifying and antioxidant genes, such as the System Xc- subunit xCT. Here, we studied (i) the effect of the Aβ fragment 25-35 (Aβ25-35) and MNP on Nrf2-dependent System Xc- expression in U373 human astroglial cells and (ii) the effect of Aβ25-35- and MNP-induced astrocytic response on neuronal cell viability using an in vitro co-culture system. We found that Aβ25-35 as well as MNP were able to activate an antioxidant response in astrocytes, by inducing both Nrf2 activation and System Xc- up-regulation. However, this astrocytic response caused an enhanced cell mortality of co-cultured SH-SY5Y cells, taken as a neuronal model. Consistently, the specific System Xc- inhibitor sulfasalazine prevented the increase of both neuronal mortality and extracellular glutamate levels, thus indicating that the neurotoxic effect was due to an augmented release of glutamate through the transporter. The present study sheds light on the Nrf2/system Xc- pathway in the toxicity induced by Aβ25-35 and may help to better understand the involvement of astrocytes in neuronal death during Alzheimer's disease.

ND44 | Compensatory myogenesis and acetylcholine receptor clustering delay symptoms onset and progression in SOD1 mutant mice

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Amyotrophic lateral sclerosis (ALS) is a heterogeneous disease with high variability in the speed of progression even in cases with a defined genetic cause such as superoxide dismutase 1 (SOD1) mutations. SOD1G93A mutation on mice with distinct genetic backgrounds (C57 and 129Sv) show consistent differences in speed of disease progression resembling what is observed in ALS patients. We recently hypothesized that the difference in the peripheral neuromuscular system rather than the extent of spinal motor neuron loss reflects the phenotypic difference between these two mouse models. Therefore, we redirect our attention to the skeletal muscle as an early component of ALS pathogenesis, aiming to discover the molecular mechanisms contributing to the distinct phenotypes and to identify factors underlying fast and slow disease progression. In this work, we compare the functional, morphological and molecular profiles of the gastrocnemius muscle (GCM) from these two SOD1G93A mouse strains at the pre-symptomatic and onset stage of the disease. Data collected clearly defined the extent of NMJ stability and muscle regeneration as a discriminator between rapidly and slowing progressing ALS mice. Notably, the slow-progressing mice, despite the premature denervation and muscle atrophy, activate different compensatory mechanisms including the expression and clustering of the AChR, myogenesis and inflammatory response, which are able to delay the onset and progression of their symptoms. On the contrary, the fast-progressing mice that are unable to activate these responses exhibit a rapid decline of muscle force. This study highlights a set of key genes and molecular pathways indices of fast or slow disease progression, which may prove useful in identifying potential disease modifiers responsible for the heterogeneity of human Amyotrophic Lateral Sclerosis, which may provide new opportunities to hamper the disease progression.

ND45 | Therapeutic potential of nanoformulations in a zebrafish model of retinal degeneration

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Retinal degenerative diseases are the leading cause of blindness worldwide and currently effective treatments to counteract the loss of photoreceptors are not available, mostly due to the peculiar physiology and anatomy of the eye. Using zebrafish model, we developed a drug delivery system creating acrylic nanoparticles (ANPs) to ameliorate the effects of oxidative stress, which is actually one of the main factors involved in these disorders, because of the continuous exposition of the retina to the visible light. Indeed, retinal structure in zebrafish closely resembles that of humans for physiology, tissue anatomy, and molecular pathways involved in retinal development and function. We established a condition of retinal damage by intravitreal microinjection of increasing doses of hydrogen peroxide (H2O2) in the eye of zebrafish larvae at 5 days post-fertilization. First, we identify the optimal dose able to induce apoptosis of retinal cells, evidenced by the activation of caspase 3 using confocal fluorescence microscopy. Next, we produced ANPs conjugated with nerve growth factor (NGF), which plays a main role in the development and regeneration of neural circuits in the visual system of vertebrates, to possibly extend its half-life through nanoformulation. In addition, the ANPs were functionalized with peanut agglutinin (PNA) that would have improved their localization in the retina. By intravitreal microinjection of the nanoformulated NGF and the free one, we analysed the capability of ANPs to prevent the apoptosis induction by H2O2, compared to the free NGF. Analyzing the active caspase3-positive cells, we confirmed that nanoformulated NGF is effective in counteracting H2O2-induced apoptosis and ongoing investigation are devoted to evaluate bioavailability and localization of ANPs in the retina as well as the possible amelioration of visual function in this zebrafish model of retina degeneration.

ND46 | Single-cell transcriptomic comparison of human microglia in Alzheimer's disease and Multiple Sclerosis

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Microglia activation has been reported to play a significant role in the progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Multiple sclerosis (MS). This has raised some interest in identifying potential microglia commonalities and molecular targets to investigate further. Previous studies highlighted a partial overlap between the transcriptional profile of microglia from the whole brain of AD animal models (5xFAD mouse) and MS plaques. However, a direct comparison of human microglia from AD and MS patients is missing. Thus, we compared microglial transcriptomic profiles using human MS and AD single nucleus RNA-seq (snRNA-seq) datasets. In particular, we compared the entorhinal and frontal cortex from AD patients (Braak stage 2 and 6) vs chronic active lesions from MS patients vs non-neurological control brains. We filtered the microglia population from the selected datasets and performed differential gene expression (DE) comparing control and disease states. We defined consensus gene signatures for each disease state for both upregulated and downregulated sets from the list of DE genes. These signatures were used to score the microglia enrichment of AD vs MS and MS vs AD by GSEA. Interestingly, we have identified a strong enrichment score for both upregulated and downregulated signatures from AD on MS and vice versa. To further confirm the similarity between the two disease states, we generated pseudobulks and built a correlation matrix between samples. The clustering analysis also supports the hypothesis that microglia from AD Braak 2 and chronic active MS lesions have some transcriptional similarities. In conclusion, we provided initial evidence supporting the hypothesis of similarity concerning the transcriptomic profile of activated microglia between AD and MS in specific disease stages. The signatures and the leading edges defined in the specific gene scoring can be used as reference for experimental validation.

ND47 | n-3 PUFA improves psychological well-being during menopausal transition

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Females show an increased risk of cognitive impairment when approaching menopause because of the loss of ovarian function and estrogen deficiency occurring during the climacteric. In addition, menopause is closely associated with emotional disorders, such as anxiety and depression. Data on risk and protection factors have yielded robust evidence on the effects of lifestyle factors, such as diet, in preserving emotional and cognitive functioning. The impact of specific lifestyle factors on psychological health indicates that there may be potential to improve (or at least stabilise) declining trajectories of emotional and cognitive functions in menopause.
This work focused on the effects of omega-3 polyunsaturated fatty acids (n-3 PUFA) supplementation on cognitive functions, depression and anxiety during the menopausal transition.
This systematic review, performed according to PRISMA guidelines, considered all articles published until December 31st 2021 and the search was performed on two databases, PUBMED and SCOPUS. The fields of interest were “menopausal transition”, “n-3 PUFA” and “cognitive and affective aspects”.
Out of the 361 articles found on PUBMED and 283 on Scopus, 17 met the inclusion criteria. They encompassed 11 human and 6 experimental studies.
Most clinical and preclinical studies report relieved depressive symptoms in relation to n-3 PUFA intake in menopause. Controversial results have been found in menopausal women on anxiety and cognitive functions, while in the few studies carried out in animal models n-3 PUFA reduced anxiety symptoms and improved cognitive functions.
Taken together, the current results show beneficial effects of n-3 PUFA on emotional and cognitive behaviours during menopause transition. However, further investigations should be performed to increase knowledge about the real effectiveness of n-3 PUFA on psychological well-being in this delicate period of feminine life.

ND48 | Microglia-released extracellular vesicles to slow down the aging process in relation to the gender

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Aging is a progressive deterioration of physiological functions characterized by accumulation of cellular damage, oxidative stress and cellular senescence. Although aging is not a disease, it represents a significant risk factor for cognitive impairment and neurodegenerative disorders.
Aged brain presents a chronic, low-grade inflammatory state defined "inflammaging", characterized by high oxidative stress, chronic inflammation and high production of inflammatory compounds. During aging, modifications occur in microglia cells (the immune "sentinel" cells of the brain) which become hyper-responsive and nonfunctional. Consistently, these cells undergo the most prominent aging-related changes in both the morphological and functional phenotypes, affecting differently males and females. These events lead to dramatic consequences for brain homeostasis and central nervous system (CNS) cellular interactions.
Extracellular vesicles (EVs) are key players of the inter-cellular communication and are exploited by the cells to exchange information consisting of lipids, proteins and nucleic acids. In the brain, EVs participate to neuron-glial cross-talk, synaptic modulation and can contribute to spreading disease in many CNS pathologies. Given their properties, EVs are emerging as a promising tool to develop revolutionary non-invasive therapies for a wide range of diseases.
Considering the above stated background, we investigated the effect of EVs released by not-inflamed microglia and intranasally administered to both male and female mice during the old age (16-18 months). We evaluated in vivo memory and motor coordination by behavioral tests and ex vivo inflammatory state of glia by RT-qPCR for inflammatory markers (IL-6, TNFα, IL1β, CD86). In EVs treated mice we observed an increased memory and motor coordination and a reduction of all pro-inflammatory genes analyzed. The findings indicate EVs as an innovative strategy to slow down the effects of aging on brain functioning.

ND49 | Biophotonics platforms for the characterization of multifunctional nanoliposomes for Alzheimer's disease and Glioblastoma

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In recent years, the steadily growing and ageing world population has led to an increased incidence of neurological disorders. Despite the development of innovative therapies, the main problem is the delivery of drugs to the brain. The blood brain barrier (BBB) has unique anatomical features that reflect its protective role, but prevent the access of therapeutics against brain disorders. To overcome this limitation, the design of nanocarriers capable of transporting drugs across the BBB can be an effective strategy to treat neuro-inflammation. In this study, we propose Raman Spectroscopy (RS) and Surface Plasmon Resonance imaging (SPRi) for the characterization of multifunctional nanoliposomes (LPs) intended for the control of neuroinflammation and associated microglial dysfunctions in Glioblastoma and Alzheimer's Disease. LPs, encapsulating specific drugs, were functionalized with a modified peptide derived from the receptor binding domain of apolipoprotein E (mApoE) for BBB crossing, and with a lipo-peptide containing a sequence sensitive to metalloproteases. Through RS, the spectra of each LP component and of different LP formulations were acquired using CaF2 as substrate. Principal Component Analysis and Linear Discriminant Analysis allowed to differentiate spectra collected from LPs functionalized or not functionalized with mApoE, drug-loaded LPs and empty LPs, demonstrating that RS can identify the LP components and compare the composition of multiple formulations and batches. Using SPRi, we evaluated the binding affinity and kinetics of the interaction between LPs and specific receptors. SPRi results confirmed the presence of mApoE, and the preservation of its binding affinity, thanks to specific interactions with selected receptors mainly expressed in the brain. In conclusion, our results demonstrate the potential of biophotonics-based techniques for the biochemical characterization of LPs, to evaluate their quality, reproducibility and binding kinetics.

ND50 | Counteracting alpha-synuclein aggregation: a novel role for GM1 oligosaccharide

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Parkinson's Disease (PD) is the second most common neurodegenerative disorder, characterized by the progressive loss of dopamine (DA) releasing neurons in the substantia nigra (SN). Fibrillary aggregated α-synuclein (αS) is a PD neurologic hallmark, considered to play a causative role in the disease. Although the causes leading to αS aggregation are not clear so far, the interaction with GM1 ganglioside is recognized to prevent this process. Recent evidence is showing that the GM1 deficiency can lead to a failure of trophic plasma membrane signaling and to the αS accumulation, increasing the susceptibility to neuronal death. How GM1 exerts these functions is not clear, though a primary role of its soluble oligosaccharide portion (OligoGM1) is emerging. Indeed, we recently demonstrated that OligoGM1 is the bioactive portion of the ganglioside, able to modify the PD phenotype.

By Real-Time Quaking-Induced Conversion (RT–QuIC) assay, we demonstrated that OligoGM1 is able to prevent both the spontaneous and the prion-like (+PFF) αS aggregation. By circular dichroism spectroscopy of recombinant monomeric αS, we found that the administration of OligoGM1 do not induce any change in αS secondary structure (~5% helical, 95% random coil). Following, we proved the OligoGM1 efficacy in an in vitro model of PD: its administration significantly increases neuronal survival and preserves neurite networks of rat DA neurons affected by αS oligomers. Finally, using a PD mouse model, based on partial deletion of GM1 ganglioside, we found that OligoGM1 systemically administered is able to reduce the αS aggregates, completely rescuing the DA neurons and the motor impairments.

Our data demonstrate that GM1 ganglioside prevents the αS pathogenic aggregation through its oligosaccharide head, suggesting a possible role of age-dependent GM1 deficiency as possible initiator for sporadic PD and the use of OligoGM1 as a possible therapeutic strategy.

ND51 | Trafficking of the glutamate transporter is impaired in LRRK2-related Parkinson's disease

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The Excitatory Amino Acid Transporter 2 (EAAT2) accounts for 80% of brain glutamate clearance and is mainly expressed in astrocytic perisynaptic processes. EAAT2 function is finely regulated by endocytic events, recycling to the plasma membrane and degradation. Noteworthy, deficits in EAAT2 have been associated with neuronal excitotoxicity and neurodegeneration. In this study, we show that EAAT2 trafficking is impaired by the leucine-rich repeat kinase 2 (LRRK2) pathogenic variant G2019S, a common cause of late-onset familial Parkinson's disease (PD). In LRRK2 G2019S human brains and experimental animal models, EAAT2 protein levels are significantly decreased, which is associated with elevated gliosis. The decreased expression of the transporter correlates with its reduced functionality in mouse LRRK2 G2019S purified astrocytic terminals and in Xenopus laevis oocytes expressing human LRRK2 G2019S. In LRRK2 G2019S knock-in mouse brain, the correct surface localization of the endogenous transporter is impaired, resulting in its interaction with a plethora of endo-vesicular proteins. Mechanistically, we report that pathogenic LRRK2 kinase activity delays the recycling of the transporter to the plasma membrane via Rabs inactivation, causing its intracellular re-localization and degradation. Taken together, our results demonstrate that pathogenic LRRK2 interferes with the physiology of EAAT2, pointing to extracellular glutamate overload as a possible contributor to neurodegeneration in PD.

ND52 | The role of Nrf2-mediated System xc- activation in HIV-1 Tat-induced neurotoxicity

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HIV-associated neurocognitive disorders (HANDs) affect a large part of HIV-infected patients, despite highly active antiretroviral therapy. HANDs occur in the absence of a direct infection of neurons. Nevertheless, viral proteins (e.g., Tat) are capable to cause neuronal dysfunction via oxidative stress, but the cellular pathways leading to HANDs are not yet fully defined. Here, we investigated the effects of Tat on Nrf2-mediated antioxidant response and system xc- expression in U373 human astroglial cells. The role of Tat-producing astrocytes on neuronal cell viability was assessed using SH-SY5Y cells as a culture model. We demonstrated that Tat produced by astrocytes was able to induce Nrf2 activation and system xc- expression in astrocytes, thus reducing cell viability of co-cultured neuronal cells. Sulfasalazine, a specific system xc- inhibitor, was able to reduce extracellular glutamate and to prevent the reduction of neuronal viability, thus demonstrating that the neurotoxic effect was dependent on an increased glutamate release through the transporter. Moreover, we investigated on the efficacy of bovine lactoferrin (bLf), in both its native and iron-saturated (holo-bLf) forms, in counteracting oxidative stress in astroglial cells constitutively expressing HIV-Tat protein. Our findings provide evidence of the involvement of astroglial Nrf2/system xc- pathway in the neurotoxicity induced by HIV-1 Tat protein, thereby suggesting how astrocytes may exacerbate neurodegeneration through the conversion of an antioxidant response to excitotoxicity.

ND53 | Mitochondrial dysfunctions in Spinal Muscular Atrophy: mitochondrial aconitase as a potential biomarker of the disease

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Spinal Muscular Atrophy (SMA) is a paediatric and juvenile onset neurodegenerative disease due to a mutation/deletion of the Survival Motor Neuron 1 (SMN1) gene, which causes the selective and early death of spinal cord (SC) motor neurons following the decrease of functional SMN protein levels. Despite the genetic cause of SMA is known, many aspects of its pathogenesis are still elusive. In the last years, mitochondrial alterations have been found already during the pre-symptomatic stages of the disease and now are considered a risk factor for SMA. Therefore, we decided to deepen the study of such dysfunctions in SMA both at central (SC of postnatal day 7 SMNΔ7 mice, a severe SMA model) and peripheral (SMNΔ7 and human fibroblasts) level. From a screening with 2-DE-MALDI-TOF-MS on pure mitochondria isolated from SC, we noticed altered expression and post-translational ubiquitination of the mitochondrial Aconitase (mAcn) enzyme, together with a strong reduction (<40%) of its functionality. Moreover, by western blotting analysis we identified alterations in mitochondrial dynamism (increased fission and decreased fusion) and respiration without any change in mitochondrial content. Interestingly, mAcn alterations were present also in fibroblasts derived from SMNΔ7 embryos and SMA patients. Murine fibroblasts showed a decreased mAcn functionality (<60%) and there was completely no mAcn activity in 2 over 3 SMA patients. MitoTracker staining of SMA murine and human fibroblasts followed by Mitochondrial Network Analysis revealed an increase of mitochondrial footprint and individuals confirming the tendency to network fragmentation. Moreover, SMA human fibroblasts displayed also a remarkable reduction (-3 folds) of mitophagic processes. Overall, such data show alterations of mAcn activity also in peripheral cells and suggest this enzyme as a potential biomarker of the disease, eventually to be tested even in blood cells that can be collected by minimally invasive procedures.

ND54 | Niclosamide ameliorates disease progression in a model of amyotrophic lateral sclerosis

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Niclosamide ameliorates disease progression in a model of amyotrophic lateral sclerosis
Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease caused by interactions between genetic, epigenetic, and environmental factors, with consequent dysfunction of multiple cellular and molecular pathways. The multifactorial nature of the disorder could explain the modest results obtained by the treatments proposed so far and highlights the need for multi-target therapies acting synergistically on different aspects of the disease. Niclosamide is on the WHO list of essential medicines, already used for decades as an anthelminthic. It has recently been repurposed in clinical trials for its potent anti-inflammatory and anti-fibrotic properties. It is well documented that niclosamide can inhibit different molecular pathways (e.g., STAT3, Wnt/b-catenin, SQSTM1/p62, NF-kB), which, importantly, are dysregulated in ALS, suggesting its potential use to interfere with these mechanisms in the pathology. We found that niclosamide inhibits microglia reactivity, reduces inflammation and fibrosis, and promotes autophagy in familial and sporadic ALS fibroblasts. Further, in a proof-of-concept study conducted in a model of ALS, i.e. hFUS mice, niclosamide strongly inhibited inflammation and fibrosis and promoted autophagy and regeneration in the nervous system and skeletal muscles. This work aims to perform a preclinical validation of the drug in the hFUS-ALS model to analyze disease progression and investigate pathogenic mechanisms targeted by niclosamide. We demonstrated that niclosamide injected intraperitoneally starting at symptom onset ameliorates grip strength and behavioral scores, increasing mice disease duration and survival. Moreover, niclosamide increases BBB integrity and decreases motoneuron loss, axonal damage and microgliosis in the spinal cord of hFUS-treated mice. These data suggest that a cheap and well-explored drug, like niclosamide, can slow down the progression of the disease in ALS mice, making it a promising candidate to be repositioned for ALS.

ND55 | Defective protein O-GlcNAcylation in Parkinson's disease patients brain and blood cells

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The loss of dopaminergic neurons in the substantia nigra (SN) pars compacta and the accumulation of misfolded α-synuclein (α-syn) are considered the pathological hallmarks of Parkinson's disease (PD). We previously reported that NF-κB/c-Rel deficient mice develop a late-onset parkinsonism, encompassing nigrostriatal degeneration, L-DOPA-reversible hypomotility and caudal-rostral α-syn deposition. To assess whether c-Rel dysregulation can be implied in PD pathophysiology, we investigate c-Rel DNA-binding activity in both SN and peripheral blood mononuclear cells (PBMCs) of healthy controls (HC) and PD patients. DNA-based ELISA revealed a significant reduction in c-Rel activity in both post-mortem SN and PBMCs from PD patients when compared with age-matched HC ones, although no differences in c-Rel protein level were observed. c-Rel DNA-binding activity has been shown to depend on post-translational modifications (PTMs) such as O-linked-β-N-acetylglucosamine (O-GlcNAc). Interestingly, altered O-GlcNAc glycosylation (O-GlcNAcylation) have been found in multiple neurodegeneration-related pathway. To assess the impact of O-GlcNAcylation on c-Rel DNA-binding capability, cultured PBMCs from HC and PD patients were exposed to 11 mM glucose (not stimulated) or 30 mM glucose and 0,1 mM PUGNAc (stimulated) in order to increase the extent of total O-GlcNAcylation. Stimulated HC PBMCs displayed a significant increase in c-Rel DNA-binding activity when compared with not stimulated ones, while we didn't see any marked differences in PD patients PBMCs. Finally, an increase in total O-GlcNAc levels was only recorded in HC PBMCs when exposed to high level of glucose and PUGNAc. These results are functional to our ongoing studies on c-Rel O-GlcNAcylation state in PD. Taken together these data suggest that c-Rel dysregulation is implied in the pathophysiology of PD, whereas an altered O-GlcNAcylation state could explain the defect in c-Rel DNA-binding activity.

ND56 | Eye as a mirror of brain neurodegeneration: retinal characterization of neuroinflammatory and neurodegenerative aspects in a mouse model of NGF deprivation

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Neurodegenerative diseases are multifactorial disorders characterized by molecular signalling, neuronal and glial dysfunctions, finally leading to cognitive impairment. The eye represents a unique mirror of the brain that can be easily assessed via non- invasive ocular imaging. Moreover, some visual pathological symptoms can precede the onset of neurodegeneration. Therefore, ocular measurements can be potential sources of biomarkers for the early detection and management of neurodegenerative progression. The AD11 transgenic mice is an NGF-deprivation model, in which the postnatal expression of an anti-NGF antibody leads to a progressive neurodegeneration with a well distinct phase of neuroinflammation at P30-P90. In our study, we exploited this mouse model to investigate cellular and molecular changes in the retina as potential biomarkers for the early detection of neurodegeneration. Using immunohistochemical and biochemical analysis, we examined and compared the main neurodegenerative and neuro-inflammatory markers in the retinas of 4, 6, 12 and 18 months-old AD11 mice (both VH control and VHVK mice), as well as into their aged-matched brains. Our results indicate that degenerative and inflammatory changes detected in AD11 retina mice, in term of neuronal degeneration, alterations in glial morphology and NGF receptors expression, mirrors the events observed in the brain parenchyma, confirming the eye as a useful tool for the study of neurodegenerative processes. However, the time onset of retina neurodegeneration in AD11 mouse model does not fully support this ocular structure as predictive platform of neurodegenerative hallmarks. Further investigations will be done to deeper understand the crosstalk among neurodegeneration, NGF deprivation and senescence.

ND57 | Characterization of the early cognitive, emotional, motor, and behavioral features of a mouse model of Parkinson's disease

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Parkinson's disease (PD) is a chronic and progressive neurodegenerative disorder mainly characterized by resting tremor, rigidity, bradykinesia and postural instability. PD is a genetically heterogeneous disorder. Among the monogenic forms, the PTEN-induced putative kinase 1 (PINK1) mutation is the second most frequent cause of early-onset PD, being associated to a pathological mechanism involving the slow progressive loss of physiological functions. Although PD is considered primarily a motor disorder, growing evidence suggests the presence of a wide spectrum of non-motor symptoms appearing from early stages of the disease.
Cognitive impairment is one of the most common and important non-motor features of PD. Indeed, several animal and human studies demonstrated the presence of cognitive and behavioral disorders in patients with PD, which may affect different domains such as attention, visuospatial and executive functions, learning, memory, and neuropsychiatric symptoms such as anxiety and depression.
The present study, funded by the Italian Ministry of Health (RF-2019-12370182), was aimed at investigating a PINK1 mouse model of PD to evaluate the presence of motor, cognitive, emotional, and behavioral symptoms at an early stage of the disease and characterize them. We compared 2-month-old PINK1 knock-out mice with PINK1 wild-type controls. All mice were submitted to a behavioral assessment battery consisting of: Novel Object Recognition Test and Y-Maze Spontaneous Alternation Test (cognitive functions); Elevated Plus Maze Test, Forced Swim Test, and Splash Test (emotional behaviors); Rotarod Test (locomotor capabilities). Preliminary data suggested the presence of alterations in specific aspects of cognitive and emotional components, which appeared not accompanied by impairments in the motor behavior of knock-out mice. These results support the potential of PINK1 PD model as a basis in studying early functional symptoms to design effective and tuned treatments.

ND58 | Possible roles of amyloid-β in microglia-mediated synapse remodeling

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Microglia are critical players in neuronal plasticity and function. They are dynamic, surveilling and interacting with neighboring cells and synaptic structures. Experimental evidence shows that close contacts with synapses are driven by synaptic activity and are important for microglia-mediated synapse remodeling. A growing literature shows a link between synaptic activity and the amyloid-β (Aβ) peptide. Aβ has been widely studied in Alzheimer's disease (AD), being the major component of the extracellular plaques associated with the pathology. Interestingly, intracellular Aβ also correlates with synaptic function. Our data, supportive of high Aβ levels in the postnatal brain, led us to hypothesize that intrasynaptic Aβ might play a role in microglia-mediated synapse remodeling. Using an AD mouse model (ArcAβ) and a pharmacological approach to modulate intraneuronal Aβ level, our aim is to analyze the involvement of Aβ in microglial synaptic pruning during brain development. First, we characterized the lipidomic profiles of synaptosomes isolated from ArcAβ and wildtype littermates at postnatal day 15 (P15), identifying candidate molecules that could promote synaptic engulfment by microglia. We found that the synaptic profile in the ArcAβ hippocampus displayed alterations in both pre- and post-synaptic markers already at early time points (P15-P30). Further, to better understand the status of hippocampal synapses at P15 in the presence of mutated human Amyloid Precursor Protein (hAPP) overexpression, we assessed mitochondrial respiration in freshly prepared synaptosomes observing changes in respiratory capacities with overexpression. Furthermore, microglial density at P15 was reduced, indicating that Aβ/hAPP overexpression is associated with early changes in microglia. Overall, these findings suggest that synaptic and microglial alterations are present at early stages in the brain of an AD mouse model, likely contributing to neurodegeneration later in life.

CN09 | CCT5 variants associated with sensory and motor neuropathies: an in silico study

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Hereditary neurochaperonopathies are a group of heterogenous disorders of the nervous and neuromuscular system associated with mutations in genes encoding for molecular chaperones and chaperonins. Different genetic modifications which occur in a gene encoding for chaperones or chaperonins can lead to distinct phenotypes. Nowadays, poor information about the etiologic-pathogenic role of these molecules is available making difficult the identification of the pathological grade, e.g., how, and how much, the skeletal muscle tissue is involved, or the prognosis. The chaperonin containing TCP1 complex (CCT) is a hetero-oligomeric complex constitutively expressed by all human cytotypes. It is made up of two overlapped rings, each consisting of eight subunits (named from CCT1 to CCT8), and it is able to fold about the 15% of cytosolic proteins. Two point mutations in the gene encoding for the CCT subunit 5 (CCT5), p.(His147Arg) and p.(Leu224Val), are associated with sensory and motor neuropathies, respectively. In the present work, we discuss the clinical differences between the patients affected and show, through in silico analysis, the conformational changes and the differences in physicochemical features between CCT5 p.(His147Arg) and CCT5 p.(Leu224Val) variant, and when they are compared to the wild type subunit. The apical domain of both variants appears mainly but differently affected. The hydrogen bonds distribution and the electrostatic potential of the mutated subunits differ widely compared to the wild type molecule. We suggest that the heterogeneity observed in gene mutation, phenotype, disease onset and progression may be the mirror of the differently modified allosteric contribution of the CCT5 variants within the CCT complex. Due to the phenotypic heterogeneity of these disorders, they are hardly identified and undiagnosed, for this reason we recommend the investigation of molecular chaperone gene variant when neuromyopathies is prevalent.

CN10 | Generation of isogenic control of TBCD mutated induced pluripotent stem cells using CRISPR/Cas9 gene editing

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TBCD has been identified as a novel disease gene implicated in a previously unrecognized encephalopathy characterized by progressive early-onset encephalopathy with brain atrophy and thin corpus callosum. Affected individuals show a variable phenotype spectrum, severe psychomotor retardation with intellectual disability and seizures in the most severely affected patients. In this study, we characterized cells carrying homozygous pathogenic variant c.3365T>C (p.Pro1122Leu) in TBCD, by using non-integrating episomal reprogramming method. In particular, we studied patient-derived motor neurons by performing morphometric analyses that showed reduced efficiency in the neuronal differentiation and decreased neurites' length compared to motor neurons differentiated from iPSCs derived from healthy subject. To confirm that the observed neuronal phenotype is due to the homozygous c.3365T>C change, we generated isogenic iPSC lines by single nucleotide correction using the recently developed technology of CRISPR/Cas9. To confirm the absence of off-target effects, we sequenced the top 10 potential off-target sites (POTs) corresponding to sgRNAs and no off-target sites were found in these POTs, thus confirming the successful efficiency of the CRISPR/Cas9 gene editing methodology. Morphometric analyses on isogenic iPSCs have been performed confirming a complete rescue of the altered neuronal morphology in terms of efficiency of neuronal differentiation and neurites' length. Therefore, we have demonstrate that the altered phenotype observed in TBCD mutated motor neurons is caused by the homozygous mutation c.3365T>C in TBCD.

CN11 | Centrin 2: A Novel Marker of Mature and Neoplastic Human Astrocytes

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As microtubule-organizing centers (MTOCs), centrosomes play a pivotal role in cell division, neurodevelopment and neuronal maturation. Among centrosomal proteins, centrin-2 (CETN2) also contributes to DNA repair mechanisms which are fundamental to prevent genomic instability during neural stem cell pool expansion. Nevertheless, the expression profile of CETN2 in human neural stem cells and their progeny is currently unknown. To address this question, we interrogated a platform of human neuroepithelial stem (NES) cells derived from post mortem developing brain or established from pluripotent cells and demonstrated that while CETN2 retains its centrosomal location in proliferating NES cells, its expression pattern changes upon differentiation. In particular, we found that CETN2 is selectively expressed in mature astrocytes with a broad cytoplasmic distribution. We then extended our findings on human autoptic nervous tissue samples. We investigated CETN2 distribution in diverse anatomical areas along the rostro-caudal neuraxis and pointed out a peculiar topography of CETN2-labeled astrocytes in humans which was not appreciable in murine tissues, where CETN2 was mostly confined to ependymal cells. As a prototypical condition with glial overproliferation, we also explored CETN2 expression in glioblastoma multiforme (GBM), reporting a focal concentration of CETN2 in neoplastic astrocytes. This study expands CETN2 localization beyond centrosomes and reveals a unique expression pattern that makes it eligible as a novel astrocytic molecular marker, thus opening new roads to glial biology and human neural conditions.

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