BOOK OF ABSTRACTS

Brayn

20th OCTOBER
21st 2021
22nd PISA·ITALY

4th BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

OFFICINE GARIBALDI
Via Vincenzo Gioberti 39, Pisa, Italy

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SOCIETÀ ITALIANA DI NEUROLOGIA


ILAE
YOUNG EPILEPSY SECTION


DIPARTIMENTO DI NEUROSCIENZE
UNIVERSITÀ DI TORINO


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Viola Galligioni	Trinity College, Dublin (Ireland)
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Adrian Liston	VIB Center for Brain and Disease Research, Leuven (Belgium); Laboratory of Lymphocyte Signalling and Development, The Babraham Institute, Cambridge (UK)

Michela Matteoli	CNR Institute of Neuroscience, Pharmacology and Brain Pathology lab, Humanitas Clinical and Research Center, Rozzano, (Italy)
Michelle Monje-Deisseroth	Stanford University, Stanford (USA)
Thomas C. Südhof	Nobel Laureate • Department of Molecular and Cellular Physiology, Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford (USA)

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Dear Young Neuroscientists,

The **BraYn Association** and the **BraYn Conference** team warmly welcome you to the **4th Brainstorming Research Assembly for Young Neuroscientists**, the BraYn conference.

Inspired and organized by researchers under the age of 40 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**, to **connect**, to **collaborate**, and to **share**. We need to encourage cooperation between different research groups in order to broaden our horizons and to improve the quality of our research.

By hosting neuroscientists from all 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 built to improve future research activities. This goal was fully achieved in 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, this year we included two **new sessions** in the scientific program: **neuroimaging** and **paediatric neuroscience & epilepsy**. These sessions were added to meet the needs and the interests of researchers working in the clinical field.

Nearly 600 delegates attended the BraYn 2020 online conference. Unfortunately, because of ongoing restrictions related to the COVID-19 pandemic, it is not yet possible for us to fully and freely accept everyone registering for the BraYn conference. Nevertheless, we will continue to do our best to ensure that everyone who wishes to participate can do so.

We are looking forward to welcoming you at the 4th BraYn conference!

The BraYn Staff

BRAYN SCIENTIFIC SESSIONS

NEUROIMAGING

Neuroimaging consists in using various techniques to image the structure, function, or physiology of the nervous system. It is subdivided into two main approaches: Structural imaging, which deals with the structure of the nervous system and the diagnosis of a large-scale intracranial disease (like tumors, multiple sclerosis lesions, stroke) and injuries (like traumatic brain injury); Functional imaging, which is used to diagnose metabolic diseases (like Alzheimer) and for neurological and cognitive psychology research as well as 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), 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 the mentioned techniques, both alone and in combination, to help in understanding and/or detecting various aspects of neurological diseases.

NEUROINFLAMMATION

Neuroinflammation describes the inflammatory response initiated in the central nervous system (CNS) by resident cells or triggered by infiltrating immune cells. Furthermore, in neurodegenerative disease it is evident that neuroinflammation is a key player in central nervous system dysfunction. The neuroinflammation session is mainly devoted to basic and clinical research in multiple sclerosis (MS), Neuromyelitis Optica Spectrum Disorder (NMOSD) and other inflammatory disorders of the CNS which have a significant impact on the lives of young adults. Even though the scientific discoveries of recent decades have improved the therapeutic approach of those disease, there are still open questions. The aim of the present session will be to explore the pathogenic mechanisms, the role of immune system in the autoimmune response, the roles of genetics and environment in the development of neuroinflammatory disease and examine options within the patient-centered approach. This and other aspects will be debated in the present session.

NEUROPHYSIOLOGY & NEURAL PLASTICITY

The physiology dealing with the functions of the central nervous system and the naturally occurring adapting to anatomical and environmental changes in central nervous system will be addressed in the new scientific session of BraYn 2021. Follow the session to be updated on new research activities in the field.

NEURO-ONCOLOGY

Neuro-oncology is an emerging field of investigation that studies nervous system tumors. As many of them can cause severe nervous system damage, neuro-oncology represents a trending research area in neuroscience, which may identify the molecular mechanisms involved in tumor pathogenesis. This would ultimately lead to the development of novel therapeutic approaches for the treatment of life-threatening diseases such as glioma, medulloblastoma. These topics will be discussed in depth during the session.

PAEDIATRIC NEUROSCIENCE & EPILEPSY

Paediatric neuroscience is a branch studying neurodevelopment and its disorders. The session will focus on biological mechanisms underlying developmental and epileptic encephalopathies, including genetic disorders and their management and treatment implications.

NEURODEGENERATION

Neurodegeneration is a key aspect of a large number of diseases characterized by progressive damage of the nervous system, which leads to irreversible neuronal death such as, but not limited to, Parkinson's disease (PD) and Alzheimer's disease (AD), tauopathies, narcolepsy, depression and psychiatric disorders. 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. During the BraYn conference we will be updated on the more recent advances in the field.

BRAYN MEETS SÜDHOF

A dedicated session where to meet and discuss scientific topics with Prof. **Thomas Südhof**, winner of the **Nobel Prize in Physiology or Medicine** in 2013. On the morning of October 22nd (9:30-11:30), scheduled groups of people for a limited time (max 30 minutes) will have the chance to engage in a scientific discussion with Prof. Südhof.

OCTOBER 20th

10:45 Registration

11:45 Opening Ceremony (G. Ferrara)

12:00 **Lucia Lisa Petrilli** – Starting Grant 2020 Winner (Chairman: C. Calì)
Dissecting pædiatric high grade-glioma through single-cell mass cytometry: from tissue to cell and back

12:15 Lecture | **Laura Ferraiuolo** (Chairman: G. Nardo)
Pathways of astrocyte toxicity in ALS and precision medicine approaches

12:45 Lunch box

SESSION 1 • NEUROIMAGING • ORAL COMMUNICATIONS

Chairpersons: F. Di Lorenzo, S. Schiavi, G. Baron

13:30 **Guillem París** • *Assessing reliability of white matter metrics in diffusion MRI based on ROI variability*

13:45 **BraYn Educational Symposium • Femtonics** (Chairpersons: S. Negro, S. Schiavi)
Ivan Zsolt, *Tune in to the BraYn in 3D, SMART solutions, SMART microscopes*

14:05 **Manuela Moretto** • *Whole-brain functional dynamics in normal aging during resting conditions*

14:20 **BraYn Educational Symposium • PerkinElmer** (Chairpersons: G. D'Arrigo, S. Negro)
Fernanda Ricci (Axxam spa), *Image-based phenotypic analysis as a tool for drug discovery at the cellular and sub-cellular level in neurological disease models*

14:40 **Caterina Lapucci** • *Using the Central Vein Sign and Diffusion MRI to differentiate demyelinating from chronic vascular lesions in Multiple Sclerosis*

SESSION 2 • NEUROINFLAMMATION • ORAL COMMUNICATIONS

Chairpersons: S. Angiari, I. Prada, L. Pangrazzi

15:00 Lecture | **Adrian Liston**, *Synthetic expansion of brain regulatory T cells to prevent neuroinflammation*

15:30 **Cindy Bokobza** • *Microglial spatio-temporal heterogeneity in a perinatal inflammation mouse model – Link to Autism-like phenotypes*

15:45 **Antonella Casamassa** • *Astrocyte-microglia crosstalk promotes Ascl1-Dependent post-ischemic astrocyte plasticity through Na+/Ca2+ exchanger 1*

16:00 **Ginevra Toma** • *Electroencephalographic alterations in persons SARS-COV2 positive*

16:15 **BraYn Educational Symposium • Beckman Coulter** (Chairpersons: S. Amoretti, M. Rasile)
Valerio Chiurchiù, *Immunophenotyping of infiltrated immune cells in the CNS in health and disease*

16:35 **BraYn Educational Symposium • Campoverde-Cytek Biosciences** (Chairpersons: G. D'Arrigo, M. Rasile)
Enrico Ghersi, *Full spectrum cytometry: pushing the limits of fluorescence in a fluorochrome limited world*

16:55 Coffee Break

17:50 **Maria Cristina Mariani** • *β3-adrenergic receptor expressing stromal cells in thymus control Treg generation and release of newly generated lymphocytes*

18:05 **Francesca Corsi** • *Anti-inflammatory and anti-apoptotic activities of TSPO ligands in an in-vitro model of retinal neuro-inflammation*

18:20 **Livia Guadalupi** • *Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis*

18:35 Poster session 1 + "Lost in the protocol" session

20:00 Closing Remarks

OCTOBER 21st

SESSION 3 • NEURODEGENERATION • ORAL COMMUNICATIONS

Chairpersons: G. Nardo, B. Bettegazzi, D. Sproviero, M.Medelin

9:00 Lecture | **Konstantinos Ampatzis**, *Locomotion dependent neuron-glia interactions control neurogenesis and regeneration in the adult spinal cord*

9:30 **Edoardo Sozzi** • *Developing silk scaffold-based platform to generate functional and reproducible human bioengineered forebrain organoids*

9:45 **Monica Favagrossa** • *The intranasal administration of cholesterol as a possible therapeutic strategy in Huntington's disease*

10:00 **Anna Caretto** • *Investigating a new therapeutic role of the GHRH agonist MR409 in an experimental model of Spinal Muscular Atrophy*

10:15 Coffee Break

11:00 **Chiara Diquigiovanni** • *Biallelic variants in spart cause a severe mitochondrial dysfunction rescued by COQ10 complementation*

11:15 **Martina Gabrielli** • *Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease*

11:30 **Lorenzo Agostino Citterio** • *Expression of serum miR-223-3p and miR-7-1-5p in Parkinson's disease patients*

11:45 Lecture | **Viola Galligioni** (Chairman: S. Angiari)
In vivo research, what to factor in when planning experiments

12:15 Lunch box

SESSION 4 • NEURO-ONCOLOGY • ORAL COMMUNICATIONS

Chairpersons: G. D'Alessandro, E. Vannini, L. Lospinoso Severini

13:10 **BraYn Educational Symposium • Euroclone** (Chairpersons: M. Di Paolo, E. Stanzani)
Luca Mazzitelli, *Deciphering the Complex Biology of Brain Tumors with Single Cell and Spatial Technologies*

13:30 **Elisabetta Mori** • *Weekly systemic administration of CTX-CNF1 ameliorates motor deficits and strongly enhances survival in a mouse model of glioma*

13:45 **Gianmarco Pallavicini** • *Inhibiting microcephaly genes as alternative to microtubule targeting agents to treat brain tumors*

14:00 Lecture | **Michelle Monje-Deisseroth** (live streaming), *Neuron-glial interactions in health and disease: from cognition to cancer*

14:30 **Carmela Serpe** • *Microglia-Derived Small Extracellular Vesicles Reduce Glioma Growth by Modifying Tumor Cell Metabolism and Enhancing Glutamate Clearance through miR-124*

14:45 **Davide Ceresa** • *Myc signalling mediates clonal-wise competition dynamics during glioma progression*

15:00 **BraYn Educational Symposium • Fujifilm Visualsonics** (Chairpersons: M. Di Paolo, S. Paglia)
Valeria Grasso, *Photoacoustic imaging of Cerebral Hemodynamics: A multi-spectral approach*

15:20 Coffee Break with Poster Session 2

SESSION 5 • PAEDIATRIC NEUROSCIENCE & EPILEPSY
(curated by Young Epilepsy Section-Italy, YES-Italy, ILAE)
ORAL COMMUNICATIONS
Chairpersons: G. Balagura, S. Balestrini, G. Lignani, M. Breccia

16:40 Lecture | **Gabriele Lignani**, *From Discovery Neuroscience to Gene Therapy for Intractable Epilepsy*

17:00 **Elsa Ghirardini** • *Tackling Creatine Transporter Deficiency: new insight into cell-specific vulnerability and development of a gene therapy approach*

17:15 **Jenna Carpenter** • *Progressive myoclonus epilepsy KCNC1 (KV3.1) variant causes a developmental dendritopathy*

17:30 **Sara Carli** • *In vivo magnetic resonance spectroscopy in the brain of Cdkl5 null mice reveals a metabolic profile indicative of mitochondrial dysfunctions*

17:45 **Martina Biagioni** • *Impact of UBE3A loss on synapse development: the case of the Angelman Syndrome*

18:00 Lecture | **Thomas C. Südhof** (Nobel Laureate) (Chairpersons: G. Ferrara, S. Angiari, G. Balagura)
The molecular logic of synapse formation

19:00 Questions & Answers

20:30 BraYn Social Dinner

OCTOBER 22nd

SESSION 6 • NEUROPHYSIOLOGY & NEURAL PLASTICITY • ORAL COMMUNICATIONS
Chairwomen: E. Boda, R.C. Paolicelli, G. Calabrese, G. Nardi

9:00 Lecture | **Michela Matteoli**, *How the immune system affects synaptic function*

9:30 **Paola Pacifico** • *Human TrkAR649W and human NGFR100W impair nociception, but differentially regulate anhidrosis and cognitive abilities*

9:45 **Marco Fogli** • *Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion*

10:00 **Francesco Marrocco** • *Environmental enrichment modifies gut microbiome and metabolome enhancing memory and neurogenesis through short-chain fatty acids*

10:15 **BraYn Educational Symposium • Siemens Healthineers** (Chairpersons: P. Lippiello, S. Schiavi)
Fabrizio Fasano, *Exploring the human brain's microstructure with a "super-scanner", an Academia-Industry synergy*

10:35 Coffee Break with Poster Session 3

BRAYN MEETS SÜDHOF • Parallel Session (9:30-11:30)
For scheduled groups only (max 8 persons/group)

11:30 **Marco Rinaudo** • *Hippocampal estrogenic signaling mediates sex differences in retroactive interference*

11:45 **Katia Monsorno** • *Loss of MCT4 in microglia results in altered brain development and anxiety-like behavior*

12:00 **Ilham El Atiallah** • *Striatal dysfunction in the novel DYT25-GNAL dystonia knockout rat model*

12:15 Closing Remarks • BraYn Awards (Best Oral and Poster Presentation and BraYn Starting Grant)
(Chairpersons: E. Vannini, G. Ferrara, G. D'Alessandro, A. Musella, V. Chiurchiù, N. Iraci, C. Calì)

Neuroimaging (pages 22-24)

Guillem París
Assessing reliability of white matter metrics in diffusion MRI based on ROI variability.

Manuela Moretto
Whole-brain functional dynamics in normal aging during resting conditions.

Caterina Lapucci
Using the Central Vein Sign and Diffusion MRI to differentiate demyelinating from chronic vascular lesions in Multiple Sclerosis.

Neuroinflammation (pages 25-30)

Cindy Bokobza
Microglial spatio-temporal heterogeneity in a perinatal inflammation mouse model – Link to Autism-like phenotypes.

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Astrocyte-microglia crosstalk promotes Ascl1-dependentpost-ischemic astrocyte plasticity through Na+/Ca2+ Exchanger 1.

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Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis.

Neurodegeneration (pages 31-36)

Edoardo Sozzi
Developing silk scaffold-based platform to generate functional and reproducible human bioengineered forebrain organoids.

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The intranasal administration of cholesterol as a possible therapeutic strategy in Huntington's disease.

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Biallelic variants in spart cause a severe mitochondrial dysfunction rescued by CoQ10 complementation.

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Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease.

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Expression of serum miR-223-3p and miR-7-1-5p in Parkinson's disease patients.

Neuro-oncology (pages 37-40)

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Weekly systemic administration of CTX-CNF1 ameliorates motor deficits and strongly enhances survival in a mouse model of glioma.

Gianmarco Pallavicini
Inhibiting microcephaly genes as alternative to microtubule targeting agents to treat brain tumors.

Carmela Serpe
Microglia-derived small extracellular vesicles reduce glioma growth by modifying tumor cell metabolism and enhancing glutamate clearance through miR-124.

Davide Ceresa
Myc signalling mediates clonal-wise competition dynamics during glioma progression.

Paediatric Neuroscience & Epilepsy (pages 41-44)

Elsa Ghirardini
Tackling Creatine Transporter Deficiency: new insight into cell-specific vulnerability and development of a gene therapy approach.

Jenna Carpenter
Progressive myoclonus epilepsy KCNC1 (KV3.1) variant causes a developmental dendritopathy.

Sara Carli
In vivo magnetic resonance spectroscopy in the brain of Cdkl5 null mice reveals a metabolic profile indicative of mitochondrial dysfunctions.

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Impact of UBE3A loss on synapse development: the case of the Angelman Syndrome

Neurophysiology & Neural Plasticity (pages 45-50)

Paola Pacifico
Human TrkAR649W and human NGFR100W impair nociception, but differentially regulate anhidrosis and cognitive abilities.

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Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion.

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

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Striatal dysfunction in the novel DYT25-GNAL dystonia knockout rat model.

POSTER SESSION 1

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NIM01 | Incoming and outgoing information flows relate with node functional strength both in human and mouse resting state fMRI • Giorgia Baron

NIM02 | An investigation of the microstructural connectivity alterations in MS • Sara Bosticardo

NIM03 | Dependency of Localization Error and Spatial Spread on the regularization parameter in the EEG source reconstruction problem • Ilaria Mazzonetto

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NI01 | Pre-operative cerebral small vessels disease in old patients undergoing orthotopic liver transplantation and its impact on peri-operative neurological complications • Federica Avorio

NI02 | Nutritional overload worsens EAE severity by promoting synaptic damage and neuroinflammation • Sara Balletta

NI03 | Microglial TREM2 receptor involvement in Schizophrenia: characterization of an animal model of Maternal Immune Activation • Matteo Bizzotto

NI04 | A painless mutein of Nerve Growth Factor ameliorates neurological defects in a mouse model of Rett syndrome • Giulia Borgonovo

NI05 | Immunometabolic reprogramming by tetramerization of pyruvate kinase M2 reduces dendritic cell activation • Marta Bottero

NI06 | miR-142-3p regulates TNF-mediated synaptopathy in Multiple Sclerosis • Silvia Caioli

NI07 | In-vitro exposure to cladribine, a targeted lymphocyte-reducing drug for multiple sclerosis, affects the expression, phosphorylation status and activity of deoxycytidine kinase in activated T cells • Federico Carlini

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ND01 | In vitro validation of miR-23a-3p and miR-181a-5p targeting SNAP-25 • Simone Agostini

ND02 | Pupillometric index of Locus Coeruleus degeneration in Alzheimer disease • Silvia Animali

ND03 | The fractional Ca2+ current of human NMDA receptors as a target to reduce neuronal hyperexcitability and excitotoxic damage • Tiziano D'Andrea

ND04 | Modulation of AMPA glutamate receptors as a strategy to counteract hippocampal hyperexcitability and cognitive deficits in mouse models of cerebral amyloidosis • Laura Bellingacci

ND05 | Generation of human iPSC-derived 3D cortico-motor assembloids for disease modeling • Maria Cristina Benedetti

ND06 | Biallelic variants in LIG3 cause a novel mitochondrial neurogastrointestinal encephalomyopathy • Francesca Bianco

ND07 | Targeting neurovascular crosstalk in motor neuron disease • Ilaria Brambilla

ND08 | Nothobranchius furzeri organotypic cultures: towards a model of ex vivo brain aging • Letizia Brogi

ND09 | Human brain spheroids as a model to investigate neurotoxicity in ischemic injury • Davide Comolli

ND10 | Unravelling combined RNA interference and gene therapy in vitro and in vivo disease models as a potential therapeutic strategy for CMT2A • Roberta De Gioia

ND11 | The neuropeptide Urocortin 2 promotes peripheral nerve regeneration • Giorgia D'Este

ND12 | The role of LRRK2 G2019S on synaptic neurotransmission in Parkinson's disease • Angela Di Iacovo

ND13 | MTCH2 functionally co-operates with BID in promoting Ca2+-induced neuronal injury • Beatrice D'Orsi

ND14 | Transcriptome analysis of miRNAs and their interactors in FTD patients' small extracellular vesicles • Francesca Dragoni

ND15 | Mechanically-actuated axonal outgrowth: new perspectives in regenerative medicine • Alessandro Falconieri

ND16 | Exploiting human genetics of multiple sclerosis for drug repositioning as antioxidant redox modifiers • Alessia Formato

ND17 | miR-29a is modulated by one-carbon metabolism and involved in neurodegeneration • Tiziana Raia

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NO01 | Molecular mechanisms underlying immune evasion in glioma progression • Irene Appolloni

NO02 | Quantitative Multicomponent T2 Relaxation Showed Greater Sensitivity Than Flair Imaging to Detect Subtle Alterations at the Periphery of Lower Grade Gliomas • Pietro Bontempi

NO03 | Patient derived 3D glioblastoma-culture models: characterization and potential applications in drug screening • Alessia G. Bosio

NO04 | SALL4A promotes Hedgehog-dependent medulloblastoma by controlling HDAC1-mediated activation of GLI1 • Ludovica Lospinoso Severini

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PNE01 | Autophagy enhancement as a promising strategy for treatment for Rett syndrome • Martina Breccia

PNE02 | Pharmacological modulation of neuronal activity for the treatment of Rett syndrome • Giuseppina De Rocco

PNE03 | Investigating brain development and neuronal circuit assembly in primary immunodeficiency WHIM syndrome models • Giulia Demenego

PNE04 | Early Evidence of Reduced Myelination in the Cortex of a Mouse Model of CDKL5 Deficiency Disorder • Sunaina Devi

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NP01 | Pupil size as an index of ocular dominance plasticity • Miriam Acquafredda

NP02 | Investigating the molecular diversity of COPII-dependent transport in cortical neurons • Federica Baronchelli

NP03 | The neurobehavioral protective effect of Beta-D-Glucan dietary supplementation in a murine model of obesity and psychosocial stress • Carlotta Baroni

NP04 | The role of readiness potential in motor-induced visual suppression • Alessandro Benedetto

NP05 | ATR kinase controls neuronal function in mature hippocampal neurons • Clara Maria Cambria

NP06 | Control of endosomal membrane identity by Spastizin • Carlo C. Campa

NP07 | Electrophysiological characterization of HSV-1-based replication defective viral vectors • Stefano Cattaneo

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ORAL COMMUNICATIONS

OCTOBER 20th 13:30

Assessing reliability of white matter metrics in diffusion MRI based on ROI variability

Guillem París [1] - Tomasz Pieciak [1] - Santiago Aja-Fernández [1] - Antonio Tristán-Vega [1]

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Brain metrics computed from diffusion MRI (dMRI) acquisitions are currently being extensively studied for their capability in the assessment of several mental disorders or pathologies. However, only few studies have addressed the reliability of such metrics, that could be driven by artefacts rather than valid signals, making them clinically useless or even misleading. In this article, we study the effect of assessing the reliability of white matter measures by means of two methodologies, involving the projection, or back-projection, of the labels together with the shift of the analysis space from subject's native space to atlas' standard space. A dataset containing 30 acquisitions (5 sessions per each of the 6 subjects) was processed through MiSFIT and MAPL, and Fractional Anisotropy (FA), Propagator Anisotropy (PA) and Non-Gaussianity (NG) measures were derived. Three eroded binary masks were placed on three ROIs (regions of interest) on the Corpus Callossum (GCC, BGG, SCC) by using two registration methods (i.e. to subject space and to standard space). Estimations of inter- and intra-subject were computed and a figure of merit (FOM) was obtained as the ratio between the inter- and the intra-subject variability. By employing statistical bootstrapping, CVs (coefficients of variation) were retrieved to get insights about the FOM's overall variability. Results reveal differences among FOM values derived from each of the methodologies. GCC FOM's fall drastically when projecting the labels directly to the native space, while CVs and boxplots suggest lower FOM variability when back-projecting labels from MNI to subject space. Label back-projection results in higher FOM values (i.e. more reliable measures) and smaller FOM's CVs (i.e. more reliable FOMs), especially in the GCC.

OCTOBER 20th 14:05

Whole-brain functional dynamics in normal aging during resting conditions

Manuela Moretto [1] - Erica Silvestri [2] - Maurizio Corbetta [1] - Alessandra Bertoldo [2]

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Normal aging is associated with brain structural and functional changes. Employing functional magnetic resonance imaging (fMRI) data acquired in resting state (rs), previous studies applied static functional connectivity (FC) analysis and showed a link between the increase of inter-network connectivity and aging, thus suggesting a reorganization of resting state networks (RSN) in a more integrated topology. However, during the scanning session the brain transits in and out of different states and the FC between networks has proven to be time-varying. In this study we employed a data-driven approach, called Hidden Markov Model (HMM), to investigate brain states dynamics in the healthy aging population. We used rs-fMRI data of 88 healthy subjects, equally divided in young and old subjects. Firstly, an independent component analysis was conducted to obtain a whole brain functional parcellation of the main RSN and then the time courses of the RSN were used as input of the HMM. Six brain states were inferred and characterized in terms of FC and mean activity. A graph-based analysis applied on the six FC maps revealed that the age progression leads to a decrease in strength of the default mode network and fronto-parietal network. Moreover, an overall more integrated topology of states occupied by old subjects was observed and in particular between the dorsal attention network and other functional domains. At the single-subject level we derived the sequence of visited states and the rate of switching between them. We found that two states were mostly occupied by young subjects, whereas three states by old subjects. The transitions between states were not random, but followed preferential paths. These results suggest that HMM is able to capture the dynamic transition patterns between brain states and that the aging process has a strong impact in the reorganization of brain functional networks.

OCTOBER 20th 14:40

Using the Central Vein Sign and Diffusion MRI to differentiate demyelinating from chronic vascular lesions in Multiple Sclerosis

Caterina Lapucci [1] - Silvia Rebella [2] - Francesc Tazza [3] - Luca Roccatagliata [4] - Nicola Mavilio [4] - Giacomo Boffa [3] - Elvira Sbragia [3] - Nicolo' Bruschi [3] - Elisabetta Mancuso [3] - Maria Cellerino [3] - Simona Schiavi [3] - Matilde Inglese [5]

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The impact of vascular risk factors (VRFs) in interpreting the Central Vein Sign (CVS) in Multiple Sclerosis (MS) has been poorly investigated. The aim of the study is to evaluate VRFs impact on the percentage of CVS+/- lesions (%CVS+/-) detected on whole brain and subregions and to investigate whether diffusion MRI metrics are able to differentiate CVS+ from CVS- lesions. 120 MS pts were stratified by age in 4 groups. 3DEPI-T2*-weighted and multishell diffusion images (acquired at 3T) were analysed for the presence of the CVS. A linear regression model was used to predict VRFs impact on the %CVS+ on whole brain and subregions. DTI and NODDI metrics able to differentiate CVS+ from CVS- lesions were identify using ANCOVA. Group 1(>60 y), 2(45-60 y), 3(30-45y) and 4(18-30y) included 30 pts respectively. The median frequency of CVS+ lesions was 73,5%. The %CVS+ lesions was higher in infratentorial and periventricular areas (p=0.000), while %CVS- lesions was higher in iuxtacortical and deep subcortical (ds) white matter (WM) regions (p=0.001 and p=0.002). The %CVS- in dsWM was significantly higher than %CVS+ only in Group1 (p=0.002). The regression model showed that age was predictor of whole brain %CVS+ (R^2=0.23; p=0.000). In pts with age>45y, hypertension (HP) was predictor of %CVS- in dsWM (R^2=0.12; p=0.031). Mean diffusivity (MD) was higher in CVS+ than CVS- lesions (p=0.004). Intracellular volume fraction (ICVF) was lower and isotropic volume fraction (isoVF) was higher in CVS+ than CVS- lesions (p=0.000 for both). Age and HP showed a relevant impact on the prevalence of CVS- lesions on whole brain and dsWM. The predominance of CVS- lesions in the dsWM, especially in adult-to-elderly MS pts with VRFs, should be considered a "red flag" for concomitant causes of WM damage different from MS. MD, ICVF and isoVF differentiated CVS+ from CVS- lesions. IsoVF may reveal the presence of more pronounced inflammatory component inside CVS+ lesions.

OCTOBER 20th 15:30

Microglial spatio-temporal heterogeneity in a perinatal inflammation mouse model – Link to Autism-like phenotypes

Cindy Bokobza[1], Anne Galland[1], David Guenoun[1], Alice Jacquens[1,2], Valérie Faivre[1], Zsolt Csaba[1], Leslie Schwendimann[1], Sophie Lebon[1], Nicolas Heck[3], Claire Leconte[4], Valérie C. Besson[4], Thomas Bourgeois[1], Nelina Ramanantsoa[1], Boris Matrot[1], Jorge Gallego[1], Bobbi Fleiss[1,5], Juliette Van Steenwinckel[1]* & Pierre Gressens[1]*

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A general consensus regarding neurodevelopmental disorders including Autism Spectrum Disorder (ASD) is that they originate from early development defects in brain formation, leading to altered neuronal circuitry responsible for the pathological behavior. Despite studies on genetic implication in ASDs, a causal relationship between genomic alteration and ASD has been difficult to explain in many cases, suggesting environmental factors might be involved. In fact, preterm birth is often linked to the occurrence of inflammation and preterm infants have a ten times higher risk of developing ADS-like symptoms than infants born at term. Moreover, some clinicial studies reported ongoing neuroinflammation processes in different brain regions in autistic infants including frontal cortex, hippocampus and cerebellum. The major relay of the environmental response in the brain, including inflammatory responses, is microglia cells (MG), the brain resident macrophages that continuously survey their local environment. Moreover, during development microglia play a critical role during the synaptic pruning to contribute to the formation of the mature cerebral connectivity network. In an inflammatory context, MG are activated and participated to the local release of pro-inflammatory cytokines. Our hypothesis is, therefore, that an exposition to perinatal inflammation impacts on neurodevelopmental disorder symptoms leading to ASD. Using a mouse model of perinatal inflammation induced by IL1b injection between post natal day (P)1-5, this project demonstrates that i) there is region specific inflammation between frontal cortex, hippocampus and cerebellum determinate; ii) an impact of microglial activation on the synaptic pruning at P15 and a modulation of connectivity by UltraFast Doppler at P40; and iii) an impact of the perinatal inflammation on the onset of ASD-like phenotypes at different developmental stages by UltraSonic Vocalization (P2 and P8), Nest Odor preferency test (P8) and an adapted three-chamber test (P40). This innovative project has as objective to identify potential diagnosis markers to facilitate an early detection of ASD in premature infants based on inflammatory indicators.

OCTOBER 20th 15:45

Astrocyte-microglia crosstalk promotes Ascl1-dependentpost-ischemic astrocyte plasticity through Na+/Ca2+ Exchanger 1

Antonella Casamassa [1] - Ornella Cuomo [1] - Anna Pannaccione [1] - Pasquale Cepparulo [1] - Valeria Valsecchi [1] - Lucio Annunziato [2] - Giuseppe Pignataro [1]

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The intricate glia interaction occurring after stroke is strongly dependent by the maintenance of intraglial ionic homeostasis. Among the several ionic channels and transporters, the plasma-membrane Na^+/Ca^{2+} exchanger (NCX) represents a good candidate in the maintenance of astroglial Na^+ and Ca^{2+} homeostasis. Here, using a combined *in vitro*, *in vivo* and *ex vivo* experimental strategy we evaluated whether activated microglia may influence the morphological and the transcriptional plasticity of post-ischemic astrocytes. Astrocyte plasticity was monitored by the expression of the transcription factor Acheate-scute like 1 (Ascl1), which play a central role in the commitment of astrocytes towards the neuronal lineage. Furthermore, we explored the implication of NCX1 expression and activity in mediating Ascl1-dependent post-ischemic astrocyte remodeling. We demonstrated that: (a) in primary neonatal co-culture of astrocytes and microglia the exposure to oxygen and glucose deprivation promoted a prevalence of bipolar astrocytes, expressing Ascl1 and NCX1; (b) in *in vivo* experiments, 3 days after tMCAO, the increased expression of Ascl1 and NCX1 in the peri-lesional striatal astrocytes was accompanied by the presence of M2 microglia population; (c) in post-ischemic *ex vivo* astrocytes, Ascl1 expression was dependent by NCX1, since its silencing prevented Ascl1 expression. Collectively, the results of our study support the idea that, during brain ischemia, the coexistence of microglia and astrocytes, acting as a functional module, can influence astrocytic morphology and Ascl1 expression. This phenomenon is strictly dependent on ischemia-induced increase of NCX1 which in turn induces astrocytic Ca^{2+} elevation and Ascl1 expression.

OCTOBER 20th 16:00

Electroencephalographic alterations in SARS-CoV-2 positive persons

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Introduction - The recent severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, previously known as 2019-nCoV) is a zoonotic virus, capable of causing an acute respiratory infectious disease, primarily spreads through the respiratory tract. The current data suggest an incubation period of 1–14 days, in most cases 3–7 days. The virus is highly transmissible in humans to human and causes severe problems especially in the elderly and people with underlying chronic diseases. The ACE-2 receptors have been identified as likely infection points for the SARS-CoV-2 and they are broadly expressed in vascular endothelium, respiratory epithelium, alveolar monocytes, macrophages, neurons, and glial cells. COVID-19 patients typically present with specific, similar symptoms, such as fever, malaise, cough and neurological sign like headache, anosmia, nausea, vomiting, nystagmus, objective vertigo, convulsions. We are using EEG for assess the brain involvement in this viral pathology.

Methods - At COVID-Centre 3 of Rome, we recorded 15 EEG in patients with positive at COVID 19 (SARS-CoV-2). Their breathing in ambient air (FiO2 21%), without fever and five minutes before EEG recording, they performed an arterial blood gas analysis for testing their effective saturation, partial pressure of carbon dioxide (CO2) and oxygen (O2), PH, bicarbonate (HCO3-) and Haemoglobin level. All patients were in remission of infectious disease and the pneumonia. EEG data were recorded on individuals in a resting state with activation tests (SLI and HPN), through 19 electrodes placed on the scalp, according to the international 10/20 system (electrodes: Fp1, Fp2, F7, F3, Fz, F4, F8, T3, C3, Cz, C4, T4, T5, P3, Pz, P4, T6, O1, O2, linked ears as reference).

Results - The EEG of the 15 persons subjected to the study is result asymmetrical (and therefore is pathological), with a prevalence of signal on the left side of the brain, a condition not in relationship with the respiratory pathology or a poor cerebral blood perfusion, considering the excellent hemodynamic and saturation values of participants. The pattern most represented in this group of patients is characterized by a simultaneous presence of the irritative elements as well as a general slowing of the basic cerebral electrogenesis.

Conclusion - The presence of viruses inside the glia can drastically modify brain electrogenesis in the sense of a discontinuity of the basic rhythm. The evidences deriving from these initial observations, lead to identify in this virus a pathogenic element that strongly damages the brain structures, capable of causing an inflammation, on the one hand of altering the physiology of the basal cerebral electrogenic activity by slowing its functioning, on the other hand create an inflammatory and irritative state that is sometimes frankly visible, sometimes subclinical. Our study will continue with the acquisition of a greater number of data and the possibility of carrying out the follow ups of the persons participating, in order to assess the actual involvement of the brain in this viral pathology.

OCTOBER 20th 17:50

β3-adrenergic receptor expressing stromal cells in thymus control Treg generation and release of newly generated lymphocytes

Maria Cristina Mariani [1] - Tiziana Vigo [1] - Consuelo Venturi [1] - Erika Ricci [1] - Federico Ivaldi [2] - Nicole Kerlero de Rosbo [2] - Antonio Uccelli [1]

IRCCS San Martino Hospital, Neuroscience Lab, Genoa, Italy [1] - University of Genoa, DINOGMI, Genoa, Italy [2]

The thymus is composed of spatially discrete areas, each of which is characterized by the presence of particular stromal cells, including mesenchymal stem cells (MSC) and T lymphocyte precursors at defined maturation stage. The thymus receives extensive innervation by the sympathetic nervous system (SNS). Norepinephrine (NE) released by the SNS in thymus impacts on α and β2 adrenergic receptors (AR) expressed by thymocytes, controlling selection processes and the specification of maturing lymphocytes into CD8-positive cells. In the bone marrow, NE activates β3AR, that are selectively expressed by MSC, blocking their expression of Cxcl12 and affecting hematopoiesis. In thymus β3AR expression by stromal cells and effects of its activation on T-cell maturation has never been investigated. Here, we have speculated that SNS may promote T-cell generation through NE-mediated activation of β3AR expressed by stromal cells in thymus. To assess our hypothesis, we performed a confocal and FACS analysis of thymic stromal cells in naive mice treated or not with a selective agonist of β3AR. We demonstrate that β3AR is expressed by different subsets of stromal cells in thymus, the majority of which co-expressed the endothelial marker CD31, while a small percentage were positive for the MSC marker, stem cell antigen-1 (Sca1). A confocal analysis of the thymus revealed that β3AR+-expressing cells are present in the medulla, and form cell clusters within the external cortex. Activation of B3AR reduced the expression of Cxcl12, promoted the release of newly generated T lymphocytes into circulation and increased the frequency of regulatory T (Treg) cells in the thymus. Overall, our results indicate that the SNS can control the functionality of the thymus through a mechanism that involves β3AR-expressing stromal cells.

OCTOBER 20th 18:05

Anti-inflammatory and anti-apoptotic activities of TSPO ligands in an in-vitro model of retinal neuro-inflammation.

Francesca Corsi [1] - Emma Baglini [1] - Elisabetta Barresi [1] - Chiara Cerri [1] - Federico Da Settimo Passetti [1] - Sabrina Taliani [1] - Claudia Gargini [1] - Ilaria Piano [1]

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The 18 kDa translocator protein (TSPO) is predominantly located in the mitochondrial outer membrane, playing an important role in steroidogenesis, inflammation, survival, and cell proliferation. Its expression in the central nervous system, and mainly in glial cells, is upregulated in neuropathologies and brain injury. In this study, we investigated the anti-inflammatory and anti-apoptotic effects of a number of TSPO ligands of the N,N-dialkyl-2-phenylindole-3-ylglyoxylamide (PIGA) class, in an *in-vitro* model (661W, photoreceptor-like cell line) of LPS-induced neuroinflammation, where TSPO expression and localization had never been studied. All PIGAs tested reduced LPS-driven cellular cytotoxicity. The protective effect of PIGAs was in all cases reduced by co-treatment with the pregnenolone synthesis inhibitor SU-10603 confirming the involvement of neurosteroids in the protective mechanism of PIGAs. Here, we show for the first time, the presence of TSPO in photoreceptor-like nervous cells. Furthermore, our results indicate that PIGA TSPO ligands, reduce the inflammatory process (IL-6 and Hmox-1) and apoptosis (MitoLight and caspase-3) in 661W photoreceptor-like cells. All in all, these data suggest that these compounds, thanks to their ability to bind TSPO and induce *de novo* synthesis of neurosteroids, could represent a potential innovative therapeutic tool for slowing down retinal neurodegenerative diseases, where the inflammatory component plays a predominant role in disease progression towards cone death and complete blindness.

OCTOBER 20th 18:20

Exercise protects from hippocampal inflammation and neurodegeneration in experimental autoimmune encephalomyelitis

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Exercise training is increasingly recognized as a valuable strategy to promote wellness in people with Multiple Sclerosis (MS), a chronic inflammatory neurodegenerative and demyelinating disease. Clinical evidence and data from the animal model of MS, the experimental autoimmune encephalomyelitis (EAE), reveal that exercise can slow down disease progression and pathology. Hippocampal dysfunction represents a pathological feature of MS accounting for cognitive deficits. An inflammation-induced aberrant synaptic plasticity caused by GABAergic transmission reduction, associated with the loss of inhibitory parvalbumin-positive (PV+) interneurons, is proposed to contribute to hippocampal pathology in EAE/MS. Of note, the hippocampus is a brain area highly sensitive to the effects of exercise. Here we addressed the effects of preventive voluntary running wheel on EAE hippocampal dysfunction, evaluating behavioral, electrophysiological, biochemical and immunohistochemical outcomes. Our results show that exercise significantly improved clinical disability and corrected cognitive deficits in both presymptomatic and acute-phase EAE, as highlighted by better performance of exercise-EAE mice at novel object recognition task and nest building test, respectively. Exercise was shown to correct the EAE-induced aberrant hippocampal plasticity measured by field potential recording, by counteracting the PV+ interneuron degeneration and by attenuating inflammation. On one side, voluntary running wheel exerted a relevant neuroprotective action. On the other side, exercise reduced hippocampal microgliosis and the expression of tumor necrosis factor in microglia and, to a lesser extent, the hippocampal level of interleukin 1beta, previously shown to contribute to the aberrant synaptic plasticity in the EAE hippocampus. Overall, these data provide evidence that physical exercise improves cognitive function and prevents synaptic and neuronal damage that typically affect EAE/MS hippocampus

OCTOBER 21st 9:30

Developing silk scaffold-based platform to generate functional and reproducible human bioengineered forebrain organoids

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Three-dimensional (3D) human brain organoids have rapidly become a widely used system to study brain development in a dish. Cultured over long periods of time, brain organoids provide a unique opportunity to model mature neuronal features including cytoarchitecture and cell-cell interactions reminiscent of human brain complexity. However, conventional 3D methodology is hampered by high variability in terms of morphology, size, and cellular composition and the presence of immature differentiation in the inner core. Therefore, we established a novel technological approach, using recombinant silk protein to create a bioengineered scaffold that arranges hPSCs in an organ-like configuration while maintaining their self-organizing property. We showed that silk scaffold sustained the homogeneous differentiation into mature neurons throughout all compartments of the organoid, avoiding spontaneous differentiation of cells towards meso-endodermal fate as occasionally observed in conventionally generated organoids. Whole-cell patch clamp recordings together with calcium imaging confirmed the presence of an intricate neuronal network of functionally active neurons. Furthermore, by using optical oxygen sensors that can be easily integrated into 3D cultures, we measured the oxygen gradients in silk bioengineered and conventional organoids. Our findings showed the remarkable property of silk scaffolds to form porous microarchitectures facilitating the delivery of oxygen, nutrients, and extrinsic patterning cues, thus creating more favourable growth and differentiation conditions.

OCTOBER 21st 9:45

The intranasal administration of cholesterol as a possible therapeutic strategy in Huntington's disease

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Huntington's disease (HD) is a dominant neurodegenerative disorder characterized by neuronal dysfunction and cell loss. One of the affected pathways implicates brain cholesterol (chol) metabolism, and exogenous chol administration to HD mice ameliorates their phenotype, indicating chol as a good candidate for HD treatment. Considering that the strategies used are invasive and not easily transferable to the patients, we decided to combine the safety of the intranasal (IN) technique with the administration of liposome-loaded chol, whose formulation has already been used for commercial drugs.

WT and R6/2 mice were treated with a single dose of liposome-loaded cholesterol-D6 (chol-D6) (200 µg chol-D6/dose) during the acute trial. The LC-MS analysis confirmed the delivery of chol-D6 to the whole brain thought IN route independently from genotype by reaching a stable concentration until ten days after IN treatment (0.4 ng/mg). Chol-D6 rose in the first 24 hours in the peripheral tissues and declined ten days after IN treatment. At 42 days after IN treatment, chol-D6 level in the striatum of R6/2 mice was statistically reduced than WT mice, suggesting that exogenous chol supplied the lack of chol in R6/2 mice.

During the chronic trial, a group of 5-week-old R6/2 mice received 7 IN doses of chol-D6 (200 µg chol-D6/IN) once a week, at the same time two control groups (WT and R6/2 mice) received PBS. The LC-MS analysis confirmed the chol-D6 accumulation after IN repeated treatments in the brain areas (about 3.5 ng/mg). R6/2 mice treated with liposomes rescued cognitive decline, while their strength and the grade of neurodegeneration were statistically improved compared to R6/2 treated with PBS.

This result highlighted the accumulation of chol-D6 in the whole brain, while its excess was eliminated from the peripheral tissues. Moreover, repeated IN treatments rescued cognitive functions and counteracted partially both the muscular strength defect and the phenotype progression.

OCTOBER 21st 10:00

Investigating a new therapeutic role of the GHRH agonist MR409 in an experimental model of Spinal Muscular Atrophy

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Spinal Muscular Atrophy (SMA) is a neurodegenerative genetic disease characterized by a progressive atrophy of skeletal muscles. It is caused by a reduction of survival motor neuron (SMN) protein levels that leads to lower motor neuron (MN) loss. Nowadays the investigation of *SMN*-independent treatments is spreading ever more to bypass the limitations of the already available therapies such as difficult administration, several adverse effects and high costs. Here we evaluated the role of MR409, a growth hormone-releasing hormone (GHRH) agonist that has shown to be able in preventing apoptosis and proteolysis in an *in vitro* model of muscle atrophy. Therefore, from postnatal day 2 (P2) to P12, we daily administered vehicle or MR409 (1mg/Kg and 2mg/Kg) to SMNdelta7 mice, a model of SMA type II. We observed a progressive gain of weight, especially with the highest dose, and a significant improvement of motor performances in terms of reflexes, strength and resistance. According to these positive outcomes, histological analysis on quadriceps and gastrocnemius revealed a significant increase in the size of the muscular fibers and moreover a higher rate of neuromuscular junction maturation with an enhanced monoinnervation and a reduced denervation of the endplates. Finally, at molecular level, we observed an increased expression of several myosin heavy chain isoforms (MYH1, MYH2, MYH7 and MYH8) and of markers of myogenesis and muscular damage repairing (Myogenin and MyoD1), as well as a significant downregulation of apoptosis markers correlated with muscular atrophy (MuRF1 and Atrogin-1). Finally, the highest dose of MR409 seemed to be able in reducing MN loss in lumbar spinal cord and in decreasing astrogliosis rate with a downregulation of proinflammatory cytokine (TNFα, IL-1b and IL-6) release in the same district. Thus, our results suggest MR409 as a new promising therapeutic approach for SMA treatment, maybe in combination with *SMN*-dependent therapies.

OCTOBER 21st 11:00

Biallelic variants in spart cause a severe mitochondrial dysfunction rescued by CoQ10 complementation

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Troyer syndrome is an autosomal recessive form of spastic paraplegia resulting in lower extremity spasticity and weakness, short stature and cognitive defects, due to loss-of-function mutations in *SPART*. *SPART* encodes for Spartin, a multifunctional protein interacting with microtubules and mitochondria. We previously observed that mutant Spartin caused a mitochondrial dysfunction characterized by Complex I impairment. Performing whole-exome sequencing in a 6-years old boy with short stature, muscle weakness and developmental deley, we identified two novel compound heterozygous missense variants in *SPART (both of unknown significance, class 3), one maternally and one paternally inherited.* Immunofluorescence staining in control and patient's fibroblasts revealed a marked nuclear localization of Spartin in the mutant cells, whereas in controls it was evenly distributed in the cells. *In vitro analysis* on the patient's fibroblasts showed an altered mitochondrial network, decreased activity of the oxidative phosphorylation system and ATP levels, increased mitochondrial reactive oxygen species production, increased mitochondrial membrane potential and altered Ca^{2+} levels vs. control fibroblasts. Interestingly, re-expression of *SPART* restored both the ATP/ADP ratio and intracellular Ca^{2+} levels as in controls, providing the evidence that these observed defects were specifically caused by mutated Spartin. Moreover, we found a decreased of Coenzyme Q10 (CoQ10) compared to control fibroblasts, along with the decrease, in terms of protein expression, of COQ7 and COQ9 (two enzymes involved in the formation of Q10). Supplementing the medium of the patient's fibroblasts with a membrane permeable CoQ10 formulation, we observed a significant recovery in ATP synthesis and cell growth compared to untreated patient's and control fibroblasts. These data suggest that CoQ10 supplementation may represent an interesting therapeutic approach for in vivo treatment.

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Microglial large extracellular vesicles propagate early synaptic dysfunction in Alzheimer's disease

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Synaptic dysfunction is an early mechanism in Alzheimer's Disease (AD) which involves progressively larger areas of the brain over time. However, how it starts and propagates is unknown. We show that amyloid-beta ($A\beta_{42}$) released by microglia in association with large extracellular vesicles (Aβ-EVs) alters dendritic spine morphology *in vitro,* locally at the site of neuron interaction, and impairs synaptic plasticity both *in vitro* and *in vivo* in the entorhinal cortex-dentate gyrus (EC-DG) circuitry. 1h after Aβ-EV injection into the mouse EC, long-term potentiation (LTP) was impaired in the EC but not in the DG, its main target region, while 24h later it was impaired also in the DG, revealing a spreading of LTP deficit between the two regions. Similar results were obtained upon injection of EVs carrying Aβ naturally secreted by CHO7PA2 cells (CHO-EVs), whereas neither $A\beta_{42}$ alone nor EVs devoid of $A\beta_{42}$ were able to propagate LTP impairment. Using optical tweezers combined 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 is blocked by annexin-V coating. Importantly, when Aβ-EV motility was limited, no propagation of LTP deficit occurred along the EC-DG circuit, implicating large EV motion at the neuron surface in the spreading of LTP impairment. Our data indicate the involvement of large microglial EVs in the rise and propagation of synaptic dysfunction in AD, and suggests a new mechanism controlling the diffusion of large Aβ-EVs and their pathogenic signals in the brain parenchyma.

OCTOBER 21st 11:30

Expression of serum miR-223-3p and miR-7-1-5p in Parkinson's disease patients

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Parkinson's disease (PD) is the most common movement disorder, affecting about 6 million individuals worldwide. The etiology of PD is still poorly understood, mainly due to its multifactorial nature where genetic and environmental interaction seems to play a fundamental role. Recently, microRNAs (miRNAs) have been shown to be important biological molecules involved in diverse processes to maintain normal cellular functions. Over the past decade, many studies have reported dysregulation of miRNA expressions in PD. In particular, miRNAs extracted in circulatory fluids could represent potential biomarkers for the evaluation of the pathology. We analyzed the expression levels of miR-7-1-5p and miR-223-3p, two miRNAs involved in α-Synuclein pathway, extracted from samples of serum collected from a population of 82 subjects, including 41 PD patients and 41 healthy controls (HC), through the use of droplet digital PCR (ddPCR) in order to compare their expression level between the two enrolled groups. We also extracted miR-7-1-5p from serum exosomes, small extracellular vesicles able to cross the blood-brain barrier and with the function of facilitating intracellular communication. Serum miR-7.1.5p was significantly more expressed in PD (32.25; 5.82-71.63 copies/ng) compared to HC (0.00; 0.00-25.10 copies/ng; p=0.0006), while no differences have been found in exosomes. In the same way, the expression of serum miR-223-3p was significantly increased in the PD group (4476.19; 1981.93-8754.85 copies/ng) compared to HC (937.50; 145.19-6605.05 copies/ng; p=0.0007). An interesting correlation was also found between the expression level of serum miR-223 in PD patients and the levodopa equivalent daily dose, or LEDD (mg/die; p=0.0061). L-DOPA is the precursor of dopamine neurotransmitters and to date remains the most effective treatment for PD patients. Basing of the obtained results, we confirm the usefulness of miRNAs as potential biomarkers to investigate the etiology of PD.

OCTOBER 21st 13:30

Weekly systemic administration of CTX-CNF1 ameliorates motor deficits and strongly enhances survival in a mouse model of glioma

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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 the last few years our group has studied the effects of a recombinant molecule (CTX-CNF1) that we developed from the conjugation of two toxins: Chlorotoxin (i.e. CTX, well-known for passing the BBB and largely employed in clinics as tumor paint and/or as drug vector in glioma clinical trials) and CNF1 (recently pointed out as an antineoplastic agent and as a neuronal function keeper). The reason of fusing these two proteins lies on the fact that native CNF1, despite its potentiality to treat gliomas, is incapable of crossing the BBB and of selectively targeting glioma cells. *In vitro* studies revealed that CTX-CNF1 is effective in leading murine and human glioma cells to death through the activation of a senescence process. At the same time we found that a single *in vivo* systemic administration of CTX-CNF1 (80 nM) is able to target glioma cells with high specificity, ultimately producing a significant increase in the survival of glioma-bearing animals. To better recapitulate what happens in clinic, we also 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, 66% of glioma-bearing mice were still alive 3 months after tumor induction and no glioma mass was detected at this time point. Although our preclinical data strongly point out that CTX-CNF1 represents a very promising approach for GBM treatment, further studies need to be done to understand the underlying molecular pathways involved.

OCTOBER 21st 13:45

Inhibiting microcephaly genes as alternative to microtubule targeting agents to treat brain tumors

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Medulloblastoma (MB) and gliomas are the most frequent high-grade brain tumors (HGBT) in children and adulthood, respectively. The general treatment for these tumors consists in surgery, followed by radiotherapy and chemotherapy. Despite the improvement in patient survival, these therapies are only partially effective, and many patients still die. In the last decades, microtubule have emerged as interesting molecular targets for HGBT, as various microtubule targeting agents have been developed and tested pre-clinically and clinically with encouraging results. Nevertheless, these treatments produce relevant side effects since they target microtubules in normal as well as in cancerous cells. A possible strategy to overcome this toxicity could be to target proteins that control microtubule dynamics but are required specifically by HGBT cells. The genes mutated in primary hereditary microcephaly (MCPH) are ubiquitously expressed in proliferating cells, but under normal conditions are selectively required during brain development, in neural progenitors. There is evidence that MB and glioma cells share molecular profiles with progenitors of cerebellar granules and of cortical radial glia cells, in which MCPH gene functions are fundamental. Moreover, several studies indicate that MCPH genes are required for HGBT expansion. Among the 25 known MCPH genes, we focus on CENPE and CITK which have been found to control microtubule stability during cell division and genome stability. Inhibition of this genes lead to cell cycle block, apoptosis and proliferation arrest *in vitro* and *in vivo* models of HGBT. Our data suggest these genes are promising and specific candidates as HGBT targets.

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Microglia-derived small extracellular vesicles reduce glioma growth by modifying tumor cell metabolism and enhancing glutamate clearance through miR-124.

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Glioblastoma (GBM) is one of the most common and malignant kinds of brain cancer. An altered intercellular communication constitutes a base for the onset and the development of the disease. Extracellular vesicles (EVs) are active players in the brain homeostasis contributing to the continuous exchange of information among neurons, glial cells, and brain immune cells, namely microglia. The major non-neoplastic cell population in GBM microenvironment is represented by tumor-associated macrophages/microglia (TAMs), which can constitute up to 40% of the tumor mass. There are two subtypes of EVs, the medium/large EVs (m/lEVs) and small EVs (sEVs). sEVs released by microglia play an important role in brain patrolling both in physiological and pathological processes. In this work, we analysed the effects of microglia-derived sEVs in GBM by using *in vitro* and *in vivo* models (murine glioma cells and injection of tumor cells in C57BL6/N mice). Our findings indicated that sEVs carry messages to cancer cells that modify glioma cell metabolism, reducing lactate, nitric oxide (NO), and glutamate (Glu) release, all molecules important in supporting tumor growth. Particularly, sEVs affect Glu homeostasis, increasing the expression of Glu transporter Glt-1 on astrocytes. We demonstrated that this effect is mediated by miR-124 contained in microglia-released sEVs. Furthermore, the *in vivo* benefit of microglia-derived sEVs results in a significantly reduced tumor mass and an increased survival of glioma-bearing mice, depending on miR-124.

OCTOBER 21st 14:45

Myc signalling mediates clonal-wise competition dynamics during glioma progression

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Glioma progression is a long process in which a neural cell, after a first genomic lesion, clonally expands accumulating mutations and gaining malignancy. Most clonal analyses of glioma progression originate from retrospective studies, which reconstruct clonal dynamics of glioma evolution based on phylogenetic analysis of cells within progressed tumors. This approach, however, could be blind to clonal extinction events, failing to completely depict the whole glioma progression dynamics. We decided to take advantage of a well-established mouse model of gliomagenesis induced by in-vivo overexpression of PDGFB gene, a known driver of glioblastoma pathogenesis, to directly observe the clonal dynamics of glioma progression from the earliest possible stage. Strikingly, we observed a strong and continuous clonal purification, which lead to monoclonal full-blown gliomas. Transplantation assays and in-silico analyses strongly suggest that this clonal purification could be due to cell-cell competition events underlying glioma evolution. When analyzing gene expression of gliomas at different stages of clonal purifications, we observed that Myc signalling and Myc gene itself are highly expressed in more purified gliomas. This evidence agrees with recent understanding of the role of Myc in the induction of supercompetitive phenotype in mammalian embryo. Remarkably, single-cell RNAseq showed the same Myc signalling modulations among clones of different sizes within same tumors, corroborating the idea of a clonal-wise cell-cell competition mediated by long term fluctuation of Myc expression.

OCTOBER 21st 17:00

Tackling Creatine Transporter Deficiency: new insight into cell-specific vulnerability and development of a gene therapy approach

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Creatine Transporter Deficiency (CTD) is an X-linked neurodevelopmental disorder caused by mutations in the Creatine Transporter (CrT) gene presenting with cerebral creatine depletion, intellectual disability, behavioural problems, and epilepsy. To these days there is no cure for CTD, and the pathogenic mechanisms of the disease remain elusive, hampering the identification of good therapeutic targets. Achieving a better understanding of the bases of CTD and searching for therapies are therefore challenges that need to be addressed in parallel. We generated a mouse model which faithfully recapitulates the symptoms observed in patients. Based on this tool, we studied how creatine depletion affects the different cell populations of the brain. By combining single-cell RNA sequencing, electrophysiological techniques, and behavioural studies we found that creatine depletion alters gene expression in specific cell types, with a major impact on parvalbumin inhibitory neurons, causing structural and functional alteration in these cells. Creatine depletion in parvalbumin neurons is sufficient to cause cognitive impairment and increased susceptibility to epilepsy, indicating a fundamental role for these cells in the pathogenesis of CTD. We are also evaluating gene therapy as a possible treatment. We used Adeno-Associated Viral vectors to deliver a functional CrT gene (AAV/CrT) to newborn CTD mice. AAV/CrT administration resulted in the expression of transgenic CrT, increasing brain creatine levels and improving cognitive performance. However, toxicity was observed with high titres of the vector. We are currently optimising the vector dosage and design to obtain a widespread, physiological expression of CrT reducing the toxicity caused by creatine overload.

OCTOBER 21st 17:15

Progressive myoclonus epilepsy KCNC1 (K_V3.1) variant causes a developmental dendritopathy

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Progressive myoclonic epilepsy (PME) is a rare and severe monogenic epilepsy syndrome characterised by the core symptoms of myoclonus, epilepsy and progressive neurological dysfunction, usually in the form of dementia and ataxia. Recently, a recurrent, *de novo* p.Arg320His mutation in *KCNC1* was discovered as a novel cause of PME without dementia, since termed 'Myoclonic epilepsy and ataxia caused by mutation of K^+ channel' or (MEAK). *KCNC1* encodes the voltage-gated potassium channel, K_V3.1, which facilitates high-frequency firing in interneurons. Previous biophysical studies have found the R320H mutation to be a loss-of-function with a dominant negative effect, however, until now, the effects of the mutation on neuronal function had not been investigated. We introduced the R320H mutation into K_V3.1b and expressed the channel in cortical interneurons *in vitro* using lentiviruses. Electrophysiological recordings performed in mature interneurons revealed that mutant channels significantly reduced interneuronal excitability compared to K_V3.1b wild-type and GFP controls. The expression of $K_V3.1b^{R320H}$ mutant channels in immature, developing interneurons was additionally found to elicit an unexpected and profound impairment in neurite development and neuronal survival, which could not be rescued by blocking K_V3 currents. Electrophysiological recordings of oocytes expressing $K_V3.1b^{R320H}$ channels confirmed a dominant negative loss-of-function effect due to slowed channel activation. Further electrophysiological recordings of engineered, non-conducting alpha-pore variants of $K_V3.1b^{WT}$ and $K_V3.1b^{R320H}$ channels ruled out toxic H^+-carried gating pore currents as a possible pathogenic mechanism. Overall, our data suggest that, in addition to the regulation of high-frequency firing, K_V3.1 plays a hitherto unrecognised role in neuronal development. MEAK may be described as a developmental dendritopathy.

OCTOBER 21st 17:30

In vivo magnetic resonance spectroscopy in the brain of Cdkl5 null mice reveals a metabolic profile indicative of mitochondrial dysfunctions

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CDKL5 deficiency disorder (CDD) is a severe neurodevelopmental disorder caused by mutation in the X-linked *CDKL5* gene. Principal features are early onset seizure, autistic-like behaviours and intellectual disability. No biomarker is available to evaluate the efficacy of a pharmacological intervention in an objective way. To fill this gap of knowledge, we used different techniques of Magnetic Resonance Imaging (MRI) in a *Cdkl5* mouse model. *Ex vivo* Manganese Enhanced MRI (MEMRI) was performed to select brain areas showing major dysfunctions, while *in vivo* proton-MR Spectroscopy (^{1}H-MRS) measured neurochemical signature of the chosen brain regions. Then, different approaches of molecular biology were used to validate the mitochondrial defects unveiled through MRS. MRI experiments were conducted on a 7-Tesla MRI scanner for rodent. LCMODEL was used to measure metabolite concentrations from MRS spectra in P70 *Cdkl5* KO mouse and corresponding WT. Hippocampi from *Cdkl5* KO mice and WT controls were used for western blots (WB), qPCR and CellTiter Glo. No difference in brain morphology was found both *in vivo* and *ex vivo* MRI analyses. MEMRI revealed increase manganese uptake in the whole brain, which was particularly prominent in the hippocampus, confirming the involvement of this brain area in *Cdkl5* phenotype. ^{1}H-MRS highlighted a strong reduction in metabolites involved in energy metabolism and mitochondrial functions in the KO mouse. Accordingly, ATP levels were reduced while the number of mitochondria remained the same, indicating a defect in the ATP production, maybe related to electron transport chain (ETC) deregulation. Of note, WB showed decreased protein levels of the ETC complex IV and AMPK phosphorylation, a master regulator of energy homeostasis. All in all, our results indicate the existence of a mitochondrial deregulation that might participate to the development of the CDD.

Impact of UBE3A loss on synapse development: the case of the Angelman Syndrome

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The *UBE3A* gene codes for an E3 ubiquitin ligase and is critical to ensure a proper brain function. Indeed, perturbations of *UBE3A* dosage or function result in pathological phenotypes. Loss of *UBE3A* causes the Angelman Syndrome, a severe neurodevelopmental disorder characterized by intellectual delay, motor deficits and seizures, while increased levels or activity of *UBE3A* are associated with Autism. Importantly, duplications and triplications of *UBE3A* chromosomal locus are the most common cytogenetic events associated with autism. Although considerable efforts have been put to dissect the molecular underpinnings of *UBE3A* function in neurons, the pathogenic mechanisms of *UBE3A*-associated neurodevelopmental disorders are still poorly understood. In this project, we study the effects of *UBE3A* loss (thus mimicking the genetic alterations of the Angelman syndrome) on the regulation of synaptic development at single cell level *in vivo*. To this aim, we combine cortex-directed *in utero* electroporation to inactivate *UBE3A* in sparse layer 2/3 pyramidal neurons with confocal and super-resolution Stimulated Emission Depletion (STED) microscopy to investigate the consequences of *UBE3A* loss on the functional organization of excitatory and inhibitory postsynaptic compartments at synaptic and sub-synaptic resolution. As already suggested by other groups, our results indicate that *UBE3A* critically regulates the formation of excitatory synapses. Strikingly, our data also suggests that *UBE3A* controls the assembly and the maturation of specific subtypes of inhibitory synapses, namely those that are located in the perisomatic region and in the axon initial segment. Together, our preliminary results suggest for the first time that the *UBE3A* gene may be critical to set the number of excitatory and inhibitory synaptic connections at the single-cell level, thus contributing to regulate the ratio between excitation and inhibition through cell-autonomous mechanisms.

OCTOBER 22nd 9:30

Human TrkAR649W and human NGFR100W impair nociception, but differentially regulate anhidrosis and cognitive abilities

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Nerve growth factor (NGF) and its high-affinity tyrosine kinase receptor TrkA are key molecules in generating and maintaining the pain states in mammals. This physiological function of NGF-TrkA axis is affected in patients with Hereditary Sensory and Autonomic Neuropathies type IV and V (HSAN IV and V). HSAN IV and V, caused by mutations in TrkA or NGF genes respectively, are mainly characterized by loss of response to noxious stimuli. Additional symptoms such as anhidrosis and variable degrees of mental retardation are present exclusively in HSAN IV patients. Understanding how mutations in TrkA or in NGF can lead to different clinical phenotypes remains necessary to explicate these two syndromes. Previously, our laboratory generated a knock-in mouse line harbouring the R100W mutation in the human NGF as animal model of HSAN V. NGF$^{R100W/m}$ mice lack to respond to noxious stimuli preserving cognitive abilities. Here, we show that the R649W mutation in TrkA tyrosine kinase domain affects the ability of TrkA to respond to NGF without modifying the total amount of the protein. To verify whether TrkA^{R649W} influences *in vivo* the physiology of dorsal root ganglia (DRG) sensory neurons and the pain sensitivity, we generate a knock-in mouse carrying the mutation R649W in the human TrkA. TrkA^{R649W} alters the expression of nociceptive markers in DRG and the skin innervation, resulting in an impaired nociception (thermal and chemical noxious stimuli). Using a sweat assay, we observe that anhidrosis is strictly related to TrkA^{R649W} while no sweating deficits are detected in NGF$^{R100W/m}$ mice. Lastly, we show that cognitive abilities linked to spatial-working memory, anxiety and sociability are impaired only in TrkA$^{R649W/m}$ mice. Our results provide extensive insights into the molecular and behavioral bases of HSAN IV and V diseases and show that both TrkA$^{R649W/m}$ and NGF$^{R100W/m}$ mice accurately reproduce the clinical manifestations of patients.

OCTOBER 22nd 9:45

Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion

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In the adult brain, subsets of astrocytes act as neural stem cells in two anatomically defined neurogenic niches: the sub-ventricular zone and the hippocampal dentate gyrus. Surprisingly, after excitotoxic lesion striatal astrocytes acquire stem cell properties and generate a large amount of neuroblasts for at least six months. Yet the presence and organization of striatal neurogenic niches and the spatio-temporal dynamics of striatal astrocytes activation and lineage progression remain unclear. To address these issues here we employed genetic lineage-tracing, BrdU birth-dating analyses and 3D reconstructions coupled with mathematical modelling and computer simulations. Neurogenic astrocytes are scattered throughout the striatum and expand locally, generating clusters of clonally related cells that we defined as striatal niches. Striatal astrocytes activate at a constant rate resulting in the continuous addition of new striatal niches with time. These niches live for about 8/10 days and are continually turned over indicating that continuous striatal neurogenesis emerges from the asynchronous activation of scattered neurogenic astrocytes. The analyses of cellular composition revealed that striatal niches are initially composed of activated astrocytes and transient amplifying progenitors that further divide and differentiate into proliferating neuroblasts. Both cell types stochastically undergo symmetric divisions that are uncoupled from cell differentiation. Conversely, the differentiation rate of transit amplifying progenitors and proliferating neuroblasts deterministically increases in an exponential manner. Finally, post-mitotic neuroblasts accumulate in the cluster before dispersing as individual cells. Overall, these data suggest that the neurogenic potential is widespread among striatal astrocytes, and that the striatal parenchyma is largely permissive for de-novo establishment of neurogenic niches.

OCTOBER 22nd 10:00

Environmental enrichment modifies gut microbiome and metabolome enhancing memory and neurogenesis through short-chain fatty acids

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Gut microorganisms and their products thoroughly affect both host behavior and brain development and function. Since improvement of brain plasticity and cognition have been demonstrated with enriched housing condition with prolonged motor, sensorial and social stimuli, we hypothesized that gut microbiota and metabolome could be modulated by environmental enrichment, providing part of the missing link among environmental signals and brain effects. Metagenomic and metabolomic analyses of mice housed in standard or enriched environment, highlight environment-specific microbial community, and metabolic profiles. We observed that mice housed in an enriched environment showed a reduction of gut microbial richness and diversity indexes and were characterized by a metabolomic fingerprint with the increase of two short chain fatty acids (SCFA) formate and acetate and the decrease of bile salts. Moreover, we demonstrated that mice treated with a mixture of formate and acetate improved hippocampal neurogenesis, neurotrophins expression and cognitive behavior recapitulating some effect of environmental enrichment. These data showed us that SCFA could be molecular effectors of enriched environment in the brain.

OCTOBER 22nd 11:30

Hippocampal estrogenic signaling mediates sex differences in retroactive interference.

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Memory loss is the distinctive trait of different neurodegenerative diseases. However, memory removal is a physiological function and little is known about its molecular and cellular underpinnings. One mechanism for removing information stored in the brain is retroactive interference, a phenomenon in which newly acquired information overwrites or interferes with the retrieval of already stored information. We have observed that, in a different version of the novel object recognition test in which a new couple of objects unrelated to the training couple is experienced prior to the test phase, adult male C57bl/6 mice suffer from retroactive interference and are unable to discriminate the novel object from the old object. On the other hand, age matched C57bl/6 female mice show resistance to the same interference protocol. Modulation of estrogenic signaling within the dorsal hippocampus during the interference paradigm renders female mice susceptible to interference, suggesting estrogen involvement. Western blot analysis revealed a higher level of activatory phosphorylation of ERK1/2 at Thr202/Tyr204 in the hippocampus of female mice compared to male mice in response to the interference protocol. Analysis of *c-fos* expression within the dorsal hippocampus showed higher activation of the dentate gyrus (DG) in female mice. Finally, injection of an ERK1/2 inhibitor into the dorsal hippocampus of female mice prior to the interference procedure renders females susceptible to the interference-mediated memory loss. Collectively, our data suggest that hippocampal estrogenic signaling may contribute, through ERK1/2 and DG activation, to a pattern separation mechanism that reduces object-related retroactive interference in female mice.

OCTOBER 22nd 11:45

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

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Microglia are the tissue-resident macrophages of the brain. Beyond their innate immunity roles, they are implicated in a variety of physiological processes required for proper brain development, including removal of apoptotic neurons and synapse remodeling. Not surprisingly, dysregulation of microglial function is linked with the onset of neuropathology. 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 increases in response to neuronal activity, was shown to regulate inflammatory responses in peripheral immune cells. However, the physiological role for lactate in modulating microglial function is still unexplored. In order to address this question, we generated a microglia-specific conditional *knock out* (cKO) mouse model for the monocarboxylate transporter 4 (MCT4), which we describe to be specifically upregulated in microglia upon lactate exposure and which is implicated in lactate transport. We analyzed key microglia features during postnatal development, and we found alterations in microglial density and in CD68+ endosomal/lysosomal structures in the hippocampus of two-week-old cKO mice. This was associated with alterations in the levels of presynaptic markers and changes in excitatory post-synaptic currents, indicating that microglia-specific depletion of MCT4 is sufficient to affect neuronal development and function. Additionally, adult cKO mice present an anxiety-like phenotype. In summary, this study highlights the importance of microglial MCT4 for correct brain maturation, emphasizing how metabolic flexibility, and in particular lactate transport, could be functionally coupled to microglial regulation. Given the established role of microglia in neuropathology, a mechanistic understanding of lactate-dependent modulations may be relevant for targeting microglia in brain diseases.

OCTOBER 22nd 12:00

Striatal dysfunction in the novel DYT25-GNAL dystonia knockout rat model

Ilham El atiallah [1] - Giulia Ponterio [1] - Annalisa Tassone [2] - **Paola Imbriani** [2] - Giuseppe Sciamanna [2] - Antonio Pisani [3]

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Dystonia is the third most common movement disorder, characterized by involuntary and sustained muscle contractions resulting in repetitive twisting and abnormal postures. To date, 27 forms of dystonia (DYT) have been identified. DYT25 is an adult-onset isolated dystonia caused by loss-of-function mutations in the *GNAL* gene. *GNAL* encodes the olfactory type G-protein alpha olf subunit (G_{alpha}olf), highly enriched in the striatum, where it positively couples both dopamine D1 (D1R) and adenosine A2A (A2AR) receptors to adenylyl cyclase, to activate cyclic adenosine monophosphate signalling. In the present work, we characterized a newly generated heterozygous GNAL knockout rat model. $GNAL^{+/-}$ rats exhibit an altered behavioural phenotype, showing decreased locomotor activity and impaired motor coordination. Since it is well known the involvement of the striatum, which is part of the basal ganglia network, in motor learning and memory, we investigated its molecular and electrophysiological alterations. We found an impairment of striatal synaptic plasticity, with loss of dopamine-dependent Long-Term Depression (LTD) in $GNAL^{+/-}$ rats. Further electrophysiology experiments showed that striatal LTD was partially rescued by a combination of both dopamine D1R and D2 receptor (D2R) agonists. Additionally, LTD was fully rescued by antagonism of either adenosine A2AR or type 5 metabotropic glutamate receptor (mGlu5R). Both A2AR and mGlu5R exert antagonistic actions on striatal D2R. Therefore, the full rescue of LTD was likely obtained by relieving the inhibitory action of these receptors on D2R. Electrophysiology and immunoblotting experiments are ongoing in order to characterize the molecular mechanisms of both LTD impairment and its pharmacological rescue by A2AR and mGlu5R antagonists. These data will provide important insights on the pathophysiology of the debilitating and incurable DYT25 dystonia, and on new pharmacological targets for novel and more effective therapies.

POSTER SESSION

1

NIM01 | Incoming and outgoing information flows relate with node functional strength both in human and mouse resting state fMRI

Giorgia Baron [1] - Danilo Benozzo [1] - Elvina Gindullina [1] - Ludovico Coletta [2] - Mattia Zorzi [1] - Alessandro Gozzi [2] - Maurizio Corbetta [3] - Alessandro Chiuso [1] - Alessandra Bertoldo [1]

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Brain network analysis with resting state functional Magnetic Resonance Imaging (fMRI) data commonly uses a network description based on Functional Connectivity (FC), i.e. the statistical dependence between brain regions. This type of connectivity metrics lacks in measuring directed interactions and is biased by the presence of spurious interactions. A much richer description can be obtained through Effective Connectivity (EC). Indeed, EC allows to account for the direction of propagating information, which is interpreted in terms of causal interaction among brain areas. Moreover, Dynamic Causal Modelling (DCM), which is considered the state-of-the-art method to infer EC, provides a biophysical model of the fMRI signal by decomposing it into the underlying neuronal signal and the hemodynamic effect. However, little is known about how FC- and EC-based whole-brain networks relate with each other. In this work, we employed both human and mouse data to apply a recently proposed sparse version of DCM developed for resting state fMRI, focusing on connectivity network properties at single node level. Firstly, particularly in humans, we observed that most EC links are short-range with the exception of the homologous inter-hemispheric interactions. Then we found that the incoming information of each node positively correlates with the mean node FC strength, while a negative correlation was observed between the incoming and outgoing EC information, meaning that on average strong receivers are weak senders and vice versa. On the contrary, FC seems not to relate to outgoing EC. Specifically for humans, cerebellum consistently shows a negative FC correlation with cortical nodes and behaves as an inhibitory outgoing EC hub.

NIM02 | An investigation of the microstructural connectivity alterations in MS

Sara Bosticardo [1] - Simona Schiavi [1] - Sabine Schaedelin [2] - Po-Jui Lu [3] - Muhamed Barakovic [3] - Matthias Weigel [3] - Ludwig kappos [3] - jens Kuhle [2] - Alessandro Daducci [1] - Cristina Granziera [2]

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The map of brain structural connections can be modeled as a graph where nodes correspond to gray matter (GM) regions and edges to the structural connections between them. This formalism allows to extract network metrics to capture pathology-related alterations. In general, connection strength is computed by counting the number of streamlines (NOS) connecting pairs of GM regions. However, recent works have highlighted that this method is not quantitative. In this study we weighted the connections using diffusion-based microstructural maps to investigate the structural changes in multiple sclerosis (MS) patients' networks, as well as the assessment of the correlations between these changes and clinical disability. We performed the analyses in a group of 66 MS patients (39F, 43.9±14.5 yrs) and 64 healthy controls (38F, 36.9±12.8 yrs). The networks were built using deterministic-like tractography; the GM was segmented in 85 regions using T1 weighted images, and the connections strength was computed by averaging along the streamlines paths the value of the microstructural maps. From each connectome we extracted 5 global metrics: Density (ratio between actual and possible connections); Efficiency (inverse characteristic path length); Modularity (network segregation); Clustering coefficient (degree on which nodes tend to cluster together); Mean strength (average of edge weights connected to a node). We employed a robust linear model using age, sex and density as covariates. The patients' connectomes weighted with intra-cellular maps showed a significant reduction in global efficiency, clustering coefficient and mean strength as well as increased modularity w.r.t. controls (all the p-values are below 0.03). Moreover, the increased modularity of patient networks was related to the worsening of motor disabilities (p-values < 0.03). Network properties assessed with NOS were neither sensitive to MS pathology nor correlated with clinical measures of disability in MS patients.

NIM03 | Dependency of Localization Error and Spatial Spread on the regularization parameter in the EEG source reconstruction problem

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High density electroencephalography (EEG) combined with source reconstruction techniques has nowadays become a powerful brain imaging tool. Accuracy of EEG source reconstruction depends on several factors such as the degree of approximation considered to build the head model, and the inverse solution method adopted. Since the inverse problem is ill-posed, a regularization procedure is essential. To best of our knowledge, no study has investigated the accuracy of the source reconstruction depending on the choice of the regularization parameter when solving the inverse problem. To tackle this issue, we used simulated EEG data with a signal to noise ratio equal to 5, 10, 15. Firstly, we built a realistic head model based on the segmentation of a structural image considering 256 channels and 40,000 sources homogeneously distributed in the gray matter cortex. Combining information from the head model, channel positions and dipole locations, we computed the leadfield matrix using the simbio Finite Element Method. Finally, the simulated EEG potentials were obtained by projecting each source onto the scalp sensors using the leadfield matrix and adding Gaussian white noise. For each source the inverse problem was solved using the Weighted Minimum Norm Estimation method with 30 different regularization parameters (λ) logarithmically spaced between 10^{-5} and 10^{1}. Performances of source reconstructions were quantified by means of the localization error and spatial spread. Our analyses revealed that: i) with greater λ, sources are localized with higher precision; ii) very low and very high levels of regularization yielded more widely distributed solutions; iii) with both metrics, noisier data require more regularization to achieve the same performance as for cleaner data. The choice of the regularization parameter should therefore be made considering the amount of noise affecting the data.

NI01 | Pre-operative cerebral small vessels disease in old patients undergoing orthotopic liver transplantation and its impact on peri-operative neurological complications

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Background: Age-related cerebral small vessel disease (CSVD), most prevalent among the elderly, may led to stroke, dementia and motor impairment. For this reason we decided to include the CSVD screening in the evaluation of liver transplant eligibility in cirrhotic patients over 65 years old. Aim: to evaluate whether CSVD is a risk factor for post-operative adverse outcomes, focusing on perioperative acute neurological complications (NCs). Methods: We collected data derived from a retrospective medical chart review. Inclusion criteria were: deceased donor liver recipients, age ≥ 65-year-old, preoperative brain Magnetic Resonance Imaging (MRI) available. The vascular lesion burden on MRI scan was computed using the Fazekas score. All the post-operative acute NCs e non-NCs, occurred during the hospital stay, were collected and analyzed. Results: n°22 patients fulfil the inclusion criteria. The major findings were: the prevalence of NCs, occurred during the peri-operative period, was 18.1%, with toxic-metabolic encephalopathy as the most frequent diagnosis; severe CSVD was associated with a higher risk of seizures (*p 0.036*), longer hospital stay (*p 0.029*) and disability (*p 0.013*); hepatic encephalopathy (*p 0.029*) and ascites (*p 0.027*) were found to be predictors factors of NCs. Discussion and conclusions: though not confirmed by stronger statistical methods for the small sample size, the CSVD severity was found to be a predictor of post-operative seizure occurrence, longer overall hospital stay, and disability in elderly liver recipients. After these preliminary results, a more extensive prospective study will be designed in order to finally establish if a neurological screening in older age liver transplant candidates is useful to stratify the peri-operative risks.

NI02 | Nutritional overload worsens EAE severity by promoting synaptic damage and neuroinflammation

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Multiple sclerosis (MS) is the main neurodegenerative autoimmune disease of the central nervous system in young adults. Growing evidence indicates that chronic inflammation promoted by obesity contributes to MS susceptibility and disease severity, although the reason for these phenomena is still not completely understood. Recent studies suggest that the "metabolic pressure" induced by nutritional overload could set the basis for an exaggerated immuno-inflammatory response to self, leading to chronic inflammation/autoimmunity in subjects with autoimmunity risk factors. In MS and in its mouse model experimental autoimmune encephalomyelitis (EAE) inflammatory molecules and downstream mechanisms cause 'synaptopathy', a reversible synaptic dysfunction that later on can cause excitotoxic damage and neuronal death. The aim of this study was to identify the relationship of nutritional overload with neuroinflammation and synaptic damage in EAE, in order to understand the influence of obesity on the pathological mechanisms that control the disease course. We explored the impact of high fat diet (HFD) compared to standard diet (SD) in EAE and control mice (n=25 for each experimental group) by monitoring clinical score and by performing behavioural, electrophysiological and molecular experiments. Our results indicate that HFD caused significant increase of both excitatory transmission and inflammation within the striatum of control mice. As expected, HFD- obesity prompted a worsen EAE clinical deficits by increasing clinical score and weight loss dependent on EAE induction. Interestingly, during the acute phase of the disease the HFD exacerbated the EAE striatal synaptopathy, strongly increasing the duration and the frequency of glutamatergic currents. In parallel, the striatal neuroinflammatory status of EAE mice fed with HFD was significantly enhanced compared to EAE mice fed with SD. Overall, we demonstrated that high-fat diet strongly contributes to the pathogenesis of EAE by altering glutamate signaling and neuroinflammation, the potentially reversible mechanisms that control MS severity.

NI03 | Microglial TREM2 receptor involvement in Schizophrenia: characterization of an animal model of Maternal Immune Activation

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Schizophrenia (SZ) is a complex neurodevelopmental disorder. Symptoms of SZ occur late in adolescence and the pathology is defined by cognitive deficits and brain abnormalities. Microglia have been described to play major roles during development and in synaptic pruning process. Microglia express the Triggering Receptor Expressed on Myeloid cells 2 (TREM2), which is involved in phagocytosis, survival, metabolic processes and synapses elimination. Since SZ is characterized by neuronal abnormalities, the hypothesis that microglial dysfunctions may lead to defective neuro-glia signaling is under investigation. Infections in pregnant mothers increase the risk of SZ in the offspring, as observed in animal models of Maternal Immune Activation (MIA), in which immunogenic substances, such as polyriboinosinic-polyribocytidilic acid (Poly-IC) are administered to the pregnant dams. In this project, we aim at understanding the interplay between environmental factors such as pre-natal immune challenge and the microglial TREM2 receptor in the onset of SZ. Our preliminary data showed that PolyIC treatment of WT female mice decreased *Trem2* mRNA levels 16 hours after the injection. These findings were supported by our *in vitro* data on WT primary murine microglia showing reduced *Trem2* mRNA expression after PolyIC treatment. Surprisingly, the same decrease in TREM2 protein was detected in the offspring of pregnant dams treated with PolyIC as compared to controls. Since TREM2 is involved in synaptic engulfment, we tested whether the decreased receptor expression induced by PolyIC can lead to microglial diminished pruning capacity. By immunohistochemistry, confocal analysis and 3D reconstruction, we observed reduced PSD-95 staining inside CD68 positive structures in microglia in the offsprings from PolyIC treated dams. These data support the hypothesis that TREM2 and its interactors might be involved in SZ and open a new scenario about the mechanisms leading to the disease.

NI04 | A painless mutein of Nerve Growth Factor ameliorates neurological defects in a mouse model of Rett syndrome

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Rett syndrome (RTT) is a rare genetic neurodevelopmental disease, affecting 1 over 10,000 females born worldwide and caused by sporadic mutations in the X-chromosome-located methyl-CpG-binding protein 2 (MeCP2) gene. In the last decade, a growing body of reports has highlighted astrocytes and microglia as important contributors to RTT pathogenesis and progression. Moreover, the neurotrophin Nerve Growth Factor (NGF) has been linked to RTT since its levels are reduced in patients' brains and blood. In our lab, we demonstrated a potent neuroprotective and anti-inflammatory activity of NGF, an effect mediated by glial cells. Thus, we tested the therapeutic potential of a painless mutein of NGF (human NGF painless, hNGFp), via a non-invasive intranasal delivery in female MeCP2+/- mice. We performed (1) a short-term 30-days-long treatment, to evaluate the reversal of established symptoms and (2) a long-term treatment (up to a humane endpoint), to assess the amelioration of symptoms and evaluate survival. In both cases, in hNGFp-treated RTT mice we found rescued phenotypic and motor deficits, and a significantly increased life expectancy. In addition, we observed a reversal in glial morphological alterations upon hNGFp treatment: the asthenic phenotype of astrocytes was rescued, and the microglial phenotype reversed to resting levels, both in the hippocampus and cortex. Lastly, a general tendency to downregulation of inflammatory cytokines in RTT mouse brains and a rescue of some of them after hNGFp administration were detected. As a parallel analysis, we counted cholinergic neurons in the medial septum, since these neurons (whose survival depends on NGF) are impaired in RTT mice and patients. This revealed a significant deficit in MeCP2+/- mice, fully rescued by hNGFp. The overall conclusion is that hNGFp delivered intranasally can ameliorate and delay symptoms in the MeCP2+/- model of RTT via its pleiotropic activity on both neurons and glia.

NI05 | Immunometabolic reprogramming by tetramerization of pyruvate kinase M2 reduces dendritic cell activation.

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The last step of the metabolic pathway that converts glucose into pyruvic acid (glycolysis) is regulated by the enzyme pyruvate kinase (PK). In mammals, four isoforms of PK have been identified, and recent studies highlighted the peculiar activity of the isoform PK muscle 2 (PKM2). The enzymatic activity of PKM2 is dependent on its oligomerization state, including active tetramer, less active dimer, and inactive monomer. PKM2 displays both metabolic and non-metabolic functions: on one hand in the cytoplasm PKM2 catalyzes the production of pyruvate and on the other hand PKM2 in the nucleus may regulate the transcription of several genes, directly or by affecting the functionality of other transcription factors. Dendritic cells (DCs) play a crucial role in immune system activation and during inflammation, immature DCs become activated expressing molecules required for DC migration, antigen presentation and T-cell activation. Upon activation per se and in experimental autoimmune encephalomyelitis (EAE), glycolysis is increased in DCs, supporting a metabolic switch from oxidative phosphorylation (resting DCs) to glucose intake. These observations indicate that metabolic changes occurring in immune cells have a crucial role in their effector responses; accordingly, targeting immune-metabolism is considered an anti-inflammatory strategy. Therefore, the aim of this project is to reduce the activation of DCs using a PKM2 activator, leading to a reduced activation of T cells and, consequently, of the encephalitogenic response in EAE. To explore the role of PKM2 in DC activation, we have used an activator, TEPP-46, which stabilizes PKM2 in its tetrameric form. To evaluate the role of PKM2 in DC activation, we analyzed PKM2 expression in DCs and we observed that PKM2 is increased at mRNA levels upon lipopolysaccharide (LPS)/IFNγ stimulation. Moreover, at mRNA level TEPP-46 reduces the expression of pro-inflammatory markers Cd40 and Il1b upon LPS/IFNγ stimulation of DCs and inhibits the production of IL-12 and TNFα pro-inflammatory cytokines, as measured by ELISA. In addition, FACS analysis indicated that PKM2 activator seems to drive a reduced expression of the pro-inflammatory surface markers, CD40, CD80, CD86 and MHCII, by DCs upon LPS/IFNγ, but did not promote the up-regulation of anti-inflammatory markers PD-L1 and MerTK. Our preliminary data suggest that metabolic reprogramming of DCs through PKM2 tetramerization could reduce their pro-inflammatory activation.

NI06 | miR-142-3p regulates TNF-mediated synaptopathy in Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS) triggered by an aberrant immune response against myelin. Recent preclinical and clinical studies have demonstrated that diffuse synaptic dysfunction and loss, known as excitotoxic synaptopathy, are a hallmark of MS pathophysiology. Proinflammatory cytokines, like TNF and IL-1β, contribute to the neuronal excitotoxic damage also by inducing synaptopathic small noncoding RNAs (miRs) in both MS and its mouse model, the experimental autoimmune encephalomyelitis (EAE). MiRs are new modulators of gene expression circulating in the cerebrospinal fluids (CSF), which have been recently proposed as diagnostic and prognostic biomarkers for MS. Specifically, we observed that miR-142-3p is increased in the CSF of MS patients as well as in cerebellum of EAE mice, where it causes synaptopathy-driven excitotoxic damage. Moreover, high miR-142-3p levels associate with a worse disease progression and therapeutical response. Coherently, miR-142 knock-out mice are totally resistant to EAE. In this research, we used transgenic heterozygous miR-142 mice as a good tool to investigate miR-142-3p role in EAE striatal synaptopathy since they show reduced miR expression in both the CNS and in the periphery compared to wild-type mice. By performing electrophysiological, immunohistochemical and molecular experiments, we demonstrated that low miR-142-3p levels provide a full protection from TNF-driven synaptopathy in the presence of EAE striatal neuroinflammation and EAE symptoms. Furthermore, in a cohort of MS patients, we found a positive correlation between TNF levels in CSF and MS progression, as for miR-142-3p. Interestingly, we observed that the patients with high CSF levels of both TNF and miR-142-3p show the most severe disease progression index, suggesting that TNF needs high miR-142-3p levels to exert its worst detrimental effects. Further mechanistic studies are still ongoing.

NI07 | In-vitro exposure to cladribine, a targeted lymphocyte-reducing drug for multiple sclerosis, affects the expression, phosphorylation status and activity of deoxycytidine kinase in activated T cells

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Activation of cladribine (2CdA), a drug approved for multiple sclerosis, is driven by a high ratio of deoxycytidine kinase (dCK)/5'nucleotidase. In view of their high dCK content, lymphocytes are preferential target for 2CdA. We demonstrated that the 2CdA-induced apoptosis in stimulated T cells is correlated with enhanced dCK expression and activity. Up to 16 dCK phosphorylation sites have been described to date but little is known about how they affect dCK activity. Our objective was to assess the differential composition of post-translational dCK isoforms in healthy donor T cells activated or not with anti-CD3/CD28 antibodies in presence/or absence of 2CdA. We used Phos-tag™ electrophoresis, which traps phosphorylated proteins thereby reducing their migration according to their phosphorylation status. Cell lysates treated with alkaline phosphatase were used to define the control band corresponding to de-phosphorylated dCK and this latter was much reduced in unstimulated cells. Lysates from activated T cells showed five separate areas of phosphorylated dCK isoforms. Areas were fewer in lysates from activated T cells exposed to 2CdA, with a profile that appeared specific to the treatment. As areas 4 and 5 were consistently observed in all samples tested and could be reliably measured, we focused our analysis on these two areas. Our data suggest that exposure to 2CdA results in a shifted composition of phosphorylated dCK isoforms, which might be related to the activity of the enzyme and thereby influence the susceptibility of activated T cells to the drug. Further analysis of dCK phosphorylation status and activity in lymphocytes from 2CdA-treated multiple sclerosis patients will help understand the impact of 2CdA on pathological immune responses related to central nervous system autoimmunity.

ND01 | In vitro validation of miR-23a-3p and miR-181a-5p targeting SNAP-25

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SNAP-25 protein is a key component of the SNARE complex, involved in synaptic vesicles fusion with plasma membranes and neurotransmitter release, fundamental for the neural plasticity. Our recent paper showed that the concentration of three specific miRNAs – miR-27b-3p, miR-181a-5p and miR-23a-3p – are associated with a specific *SNAP-25* polymorphism (rs363050). Target prediction *in silico* analysis showed that all the three miRNAs target *SNAP-25*, but the binding between these miRNAs and the 3'UTR region of SNAP-25 mRNA was never demonstrated. For this reason, here we verified *in vitro* whether these three miRNAs are able to bind and to modulate the expression of SNAP-25. Co-transfection of Vero cell line with the miRNAs mimic or inhibitor and luciferase reporter plasmid containing SNAP-25 3'UTR showed that miR-181a-5p ($p \leq 0.01$) and miR-23a-3p ($p < 0.05$), but not miR-27b-3p, can modulate the luciferase signal, confirming the interaction of these two miRNAs with SNAP-25 3'UTR region. Next, human oligodendroglial cell line (MO3.13) was transfected with miR-181a-5p and miR-23a-3p, confirming that the two miRNAs are able to regulate the SNAP-25 gene and protein expression. Interestingly, the two miRNAs modulate in an opposite way SNAP-25, as miR-181a-5p significantly increases ($p < 0.0005$), whereas miR-23a-3p decreased ($p < 0.0005$) its expression. In conclusion, these results verify for the first time that miR-181a-5p and miR-23a-3p can modulate the SNAP-25 expression; considering the important role of SNAP-25 on synaptic function and plasticity, and that its deregulation has been associated with different diseases (i.e. autism, psychiatric disorders, dementia and sarcopenia), these data highlight the importance of studying these miRNAs as potential biomarkers or therapeutic targets.

ND02 | Pupillometric index of Locus Coeruleus degeneration in Alzheimer disease

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The cognitive impairment and pathological brain alterations occurring in the Mild Cognitive Impairment (MCI)-dementia continuum of Alzheimer's disease (AD) are paralleled by a progressive degeneration of the pontine noradrenergic nucleus Locus Coeruleus (LC). Thus, the search of non-invasive indices of LC integrity in humans is a primary interest in the research on AD. Pupillometry stands as a promising option, given that pupil dynamics and LC activity appear to be closely coupled. We performed pupillometry in 30 subjects, for whom an estimate of LC integrity was obtained by post-hoc analysis of neuromelanin-sensitive magnetic resonance imaging (MRI) obtained by 3Tesla scan. At the time of MRI acquisitions, all patients were classified as MCI and had comparable cognitive performance. At the time of pupillometry, 15 patients had developed dementia due to AD (converters) and the remaining 15 had maintained their MCI diagnosis (non-converters). Pupillary responses were recorded in the context of an auditory oddball task, with infrequent target tones eliciting pupil dilation stronger than both infrequent distracters and frequent standard tones. We found that pupillary responses accurately differentiated the two groups of patients, with stronger dilations to target tones in the non-converter than in the converter group. Crucially, the magnitude of pupillary responses was positively correlated with LC integrity measures specifically in the converter group. This suggests that the pupillary oddball response may be a solid, non-invasive proxy of the LC degeneration in AD. It also suggests a strong link between structure and function in the pupil control system, whereby less degenerated LC nuclei support stronger pupillary oddball responses.

ND03 | The fractional Ca2+ current of human NMDA receptors as a target to reduce neuronal hyperexcitability and excitotoxic damage.

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Neurodegenerative diseases (e.g., Alzheimer's Disease, Parkinson's Disease, ALS) share some core molecular mechanisms, including the neuronal damage induced by glutamate-derived excitotoxicity. The excitotoxicity is mainly caused by an excess of Ca^{2+} influx into the cell, leading to the apoptotic signaling cascades. A key step in this process is an excessive increase of the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), due to an overactivation of the highly Ca^{2+} permeable ionotropic glutamatergic NMDA receptors. The high Ca^{2+} permeability of the NMDA receptors makes them an important target for treatments able to reduce P_f without altering the Na^+ current, and consequently without affecting the normal glutamatergic neurotransmission. Such a therapeutic strategy could reduce the excitotoxic damage in several relevant neuropathologies with less or no adverse effects. To evaluate the efficacy of treatments able to reduce Ca^{2+} entry in neurons, we quantify the Ca^{2+} permeability in terms of the fractional Ca^{2+} current (P_f, the percentage of the total current carried by Ca^{2+} ions). To these days, highly reliable but extremely slow electrophysiological techniques have been used to measure P_f. This project aims to develop a new two-fluorophores fluorescence microscopy technique allowing to simultaneously visualize the intracellular Ca^{2+} and Na^+ concentration changes due to the activation of the NMDA receptors. This new experimental approach could allow to rapidly and efficiently study the NMDA receptor role in neuropathologies and neurodegenerative diseases, as well as the effects and the mechanisms of action of potentially therapeutic molecules.

ND04 | Modulation of AMPA glutamate receptors as a strategy to counteract hippocampal hyperexcitability and cognitive deficits in mouse models of cerebral amyloidosis

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Background. Pathological accumulation of A**β** oligomers has been linked to neuronal networks hyperexcitability, potentially underpinned by glutamatergic AMPA receptors (AMPARs) dysfunction. The aim of our work was to investigate if the modulation of AMPARs may counteract the alteration of hippocampal epileptic threshold and synaptic plasticity linked to A**β** oligomers accumulation. **Methods.** Field-excitatory postsynaptic potentials (fEPSPs) were recorded from hippocampal dentate gyrus in an acute model of A**β** oligomers induced neurotoxicity. The *in vitro* models of epileptic-like activity were induced with bath-application of either bicuculline or 4-aminopirydine (4-AP). Long-term potentiation (LTP) was induced by high frequency stimulation. Seizure susceptibility to bicuculline and 4-AP was also evaluated in an *in vivo* model of amyloidosis obtained by stereotaxic injection of A**β** oligomers in the dentate gyrus. Injected mice were also challenged to hippocampal based behavior and cognition with the Morris water maze, passive avoidance and novel object recognition tasks, to assess possible cognitive deficits associated with oligomers accumulation. Forced swimming test and elevated plus maze were used to asses depression and anxiety-like behaviors, respectively. **Results.** Aβ induced *in vitro* hyperexcitability was counteracted by mild AMPARs non-competitive antagonism which, *per se*, does not affect physiological synaptic transmission. In parallel, the reduced *in vivo* epileptic threshold found in A**β** oligomers-injected mice was restored by mild modulation of AMPARs. AMPARs antagonism also restored A**β**-induced impairment of hippocampal LTP *in vitro* and significantly improved hippocampal-based cognitive performances of Aβ-lesioned mice. No differences were detected in the forced swimming test and elevated plus maze. **Conclusions.** Targeting glutamate AMPARs might be a strategy to reduce hippocampal networks hyperexcitability and synaptic plasticity deficits induced by A**β** oligomers accumulation.

ND05 | Generation of human iPSC-derived 3D cortico-motor assembloids for disease modeling

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Movement is controlled by a wide network of nerve cells that involves all levels of the nervous system, from the cortex to the spinal cord. The complexity of this system makes it challenging to identify the etiopathology of diseases affecting the cortico-spinal motor pathways, due also to the lack of appropriate human models. The use of human induced pluripotent stem cells (iPSCs) has made it possible to study single components controlling movement, alone or in combination. Furthermore, the development of 3D organoids has allowed to generate more complex and physiological tissue models. Recently, the generation of cortico-motor assembloids has shown that cortical neural projection could control muscle contraction via activation of motor neurons. The aim of my project is to reproduce this complex network by fusing human cortical organoids (hCOs) with neuromuscular organoids (NMOs). NMOs self-organize to reproduce neuromuscular junction, formed by both spinal cord and musculoskeletal cells. Using human iPSCs, we have generated and characterized hCOs and NMOs by morphological and molecular analysis, showing their ability to recapitulate the complexity of cortical and NMJ system over time. Their combination will provide deeper insight into the descending pathway that generate movement in health and disease to better understand the contribution of each cell types to the altered phenotype. Thus, 3D assembloids can be used as a platform for disease modelling and drug screening in order to develop new therapeutic approaches. In particular, this system will be used for modelling GNAO1 disorder, a rare genetic disease affecting psychomotor development with high clinical heterogeneity. We are generating four iPSC lines individually carrying different mutations in the GNAO1 gene by CRISPR/Cas9 system. These lines will be used to generate assembloids, in order to dissect molecular mechanisms underlying the disease heterogeneity.

ND06 | Biallelic variants in LIG3 cause a novel mitochondrial neurogastrointestinal encephalomyopathy

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Mitochondrial encephalomyopathies can be characterized by leukoencephalopathy due to mitochondrial dysfunction and severe abnormality of gut motility, such as chronic intestinal pseudo-obstruction, an impairment of gut propulsion. Mitochondrial neurogastrointestinal encephalopathy (MNGIE) is caused by mutations in *TYMP* or POLG or mitochondrial DNA (mtDNA) itself, but a number of patients are still unresolved. We aimed to identify the genetic defects in seven patients from three independent families showing severe gut dysmotility and neurological abnormalities, including leukoencephalopathy, epilepsy, migraine, stroke-like episodes, and neurogenic bladder. None of the patients carried mutations in *TYMP*, *POLG* or mtDNA. Whole exome sequencing was performed on the DNA extracted from peripheral blood. Dermal fibroblasts were obtained from patients' and controls' skin biopsies and grown in standard culture media. Functional *lig3* ablation in zebrafish was performed via morpholino analysis and/or CRISPR/Cas9 gene editing. We identified heterozygous variants in a new disease gene, named *LIG3*. The *LIG3* gene encodes the only mtDNA ligase and plays a pivotal role in mtDNA repair and replication. *In vitro* assays in patient-derived cells showed a decrease in LIG3 protein levels and ligase activity. We demonstrated that the *LIG3* gene defects affect mtDNA maintenance, leading to mtDNA depletion. A decrease in the number of myenteric neurons, and increased fibrosis and elastin levels were the most prominent changes in the gut. Muscle pathology of decreased cytochrome c-oxidase (COX) staining was also observed. Disruption of *lig3* in zebrafish reproduced the brain alterations and impaired gut transit *in vivo*, and was rescued by the wild-type human LIG3 isoform, but not the mutant one. We identified biallelic variants in the *LIG3* gene that result in a novel mitochondrial phenotype characterized by predominant gut dysmotility, leukoencephalopathy, and neuromuscular abnormalities.

ND07 | Targeting neurovascular crosstalk in motor neuron disease

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Amyotrophic Lateral Sclerosis (ALS) is a late-onset neurodegenerative disease characterized by degeneration of both upper and lower motor neurons in the brain and spinal cord. The pathogenic mechanisms responsible for the selective loss of motor neurons in ALS are largely unknown, but it has been shown that both cell autonomous and non-cell autonomous factors are involved. Among the many cell types present in the microenvironment surrounding motor neurons, we became interested in the possible contribution of endothelial dysfunction to ALS pathogenesis, since vascular abnormalities have been detected in ALS patients and animal models. To begin unravelling the crosstalk between vascular endothelium and motor neurons, we are employing Translating Ribosome Affinity Purification (TRAP) technology to profile the *translatome* of endothelial cells from the spinal cord of ALS mutant mice at different stages of disease progression. Bioinformatic analysis of deep-sequenced TRAP samples confirmed enrichment in endothelial-specific translated mRNAs and depletion of other parenchymal markers. A discrete set of genes was found deregulated in the endothelial *translatome* of mutant mice at disease onset. The number of differentially expressed transcripts increased markedly at post-symptomatic stages, revealing a significant association with pathways related to inflammation and metabolism. We are currently analysing candidate factors to identify the molecular determinants of vascular abnormalities in ALS pathogenesis, and probe their diagnostic and therapeutic potential.

ND08 | Nothobranchius furzeri organotypic cultures: towards a model of ex vivo brain aging

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Organotypic culture of brain slices is an ex-vivo technique used to investigate long-term neuronal survival. Organotypic cultures maintain a three-dimensional organization and mimic the *in vivo* development of cells and synapses. The absence of the blood-brain barrier allows direct access of small molecules to the culture. Also, organotypic cultures allow to study the effects of age on brain in isolation without the influence of the systemic milieu. The ex-vivo model has been widely used in rodents for conducting molecular, pharmacological, and physiological studies. To our knowledge, no long-term culture system for fish brains is established. The short-lived annual fish *Nothobranchius furzeri* shows extremely short life span and accelerated expression of age markers and a long-term culture system would enable the study of brain aging ex-vivo. We thus established organotypic cultures from brain slices of *N. furzeri*. The brains were extracted from MZCS-222 fish of 5, 12, 30 weeks after hatching from which we cut 500 µm slices of various brain regions. The brain slices were incubated on porous membranes in an ad-hoc medium for at least of 5 weeks. Slices were incubated with EdU for the first three days to label new-born cells. One week after EdU treatment, we observed neurogenesis in all slices indicating that adult neurogenesis is retained ex-vivo even in slices from old fish. as well as *in vivo*. In addition, we specifically tested the viability of noradrenergic neurons labelled with TH and we observed that these neurons persist for at least five weeks *in vitro*. Our future aims are to prolong the culture period to test whether brain aging markers become expressed *in vitro* and finally test drugs and nutraceutical compound.

ND09 | Human brain spheroids as a model to investigate neurotoxicity in ischemic injury.

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Cerebral ischemic stroke is defined as the infarction of the brain, retina or spinal cord caused by blood vessels occlusions and resulting blood flow reduction. *In vitro* and *in vivo* models have been developed to study the injury's pathophysiology and investigate potential drug targets. Here we used a human 3D-induced pluripotent stem cells (iPSC)-derived culture system (human brain spheroids, hBS), to characterize the ischemic injury caused by oxygen-glucose deprivation (OGD). IPSC are initially differentiated into neural progenitor cells (NPCs) using dual SMAD inhibition. Then, hBS were generated by plating NPC-single cells suspension under defined neural maturation media conditions in constant shaking for sixty days. Fully matured spheroids were placed into a hypoxic chamber for 2 or 8 hours with oxygen and growth factors-deprived culture media. Our results showed a massive cell death induced by OGD in hBS. The propidium iodide (PI) incorporation showed diffusion of PI signal from central core to the periphery correlated to the severity of the injury. Lactate dehydrogenase (LDH) release in conditioned media, measured after 24 and 48 hours from OGD, showed an overall cell death, according to the OGD period length. Moreover, the quantification of light neurofilaments (NFL) and GFAP in the conditioned media, with a highly sensible immunoassay (Simoa Quanterix™), revealed a neurons/astrocytes-specific injury, whose severity depends on OGD length. The RT-PCR revealed a variation of glial and neuronal mRNA levels and confirmed a direct effect on these cell-types. The OGD condition was also able to impair the dendritic structure and the neuron/astrocyte ratio, as revealed by whole-mount immunostaining analysis on hBS. We characterize a human iPSC-based *in vitro* model of ischemic injury, where increasing neurons and astrocytes degeneration was observed, depending on the injury severity. This represents a promising platform for testing strategies for disease treatments.

ND10 | Unravelling combined RNA interference and gene therapy in vitro and in vivo disease models as a potential therapeutic strategy for CMT2A

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Charcot-Marie-Tooth type 2A (CMT2A) is an inherited sensory-motor neuropathy caused by missense mutations in the MFN2 (Mitofusin2) gene. MFN2 mutations appear to induce the disease with a dominant-negative mechanism, where the wild-type MFN2 allele expression is negatively regulated by the mutant protein. Gene therapy for dominant inherited diseases uses RNA interference (RNAi) to selectively inhibit expression of the mutant allele, which results in a toxic protein. Since this approach can also reduce the expression of the wild-type functional allele, wild-type allele restoration, in combination with mutant allele silencing, could improve the therapeutic effects. Here, we propose this double strategy as a possible CMT2A new therapeutic approach. Indeed, we tested the effective silence of the endogenous MFN2 (both mutant and wild-type MFN2 alleles) and the its replacement with an exogenous copy of the wild-type MFN2 gene in CMT2A human induced pluripotent stem cells (iPSCs)-differentiated motor neurons (Rizzo et al 2016) and in Mitocharc1, a mouse model of CMT2A (Cartoni et al., 2010). To evaluate the amelioration of the disease phenotype after this strategy, we will analyze key motoneuronal features relevant to CMT2A, observing an enhancement in mitochondrial distribution and function, beyond in apoptotic and autophagic parameters. Our data confirm the feasibility of combined RNAi and gene therapy approach as potential therapeutic strategy for treating CMT2A and other similar genetic neurological disorders.

ND11 | The neuropeptide Urocortin 2 promotes peripheral nerve regeneration.

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The neuromuscolar junction (NMJ) is a synapse composed by the motor axon terminal, peri-synaptic Schwann cells (SCs), and the muscle fibre, separated by a basal lamina. It has retained throughout evolution the ability to regenerate, at variance from central synapses, but the search for molecules involved in the process is still open. We performed a transcriptome analysis at the NMJ using a model of degeneration based on a spider-derived neurotoxin, α-latrotoxin (α-LTx). This is a controlled method to induce an acute, localized and reversible nerve terminal degeneration not blurred by inflammation, thus helping to identify the mediators involved. Among the differentially expressed transcripts urocortin II is strongly up-regulated four hours after intoxication, when the pre-synaptic nerve terminal is degenerated and then decreases during the regeneration, thus underlying a possible involvement in the process. Urocortin2 (Ucn2) is a neuropeptide belonging to the corticotropin-releasing factor peptide family, involved in the physiological response to stress. It exerts its peripheral function by binding selectively to a G protein coupled receptor, Corticotropin Releasing Hormone Receptor 2 (CRHR2). We found CRHR2 expression on SCs, who are key players in the regenerative process and are activated by exogenous Ucn2 itself. Moreover, CRHR2 localizes on the axon tip of MNs and Ucn2 administration promotes axon growth; a selective antagonist of the receptor abolishes this effect. On our in vivo model of peripheral damage, we observed the involvement of CRHR2 in the regenerative process: the administration of the selective antagonists Astressin2B delays the functional and structural recovery. Lastly, exogenous Ucn2 administration accelerates functional and structural recovery of degenerated NMJ. These findings reveal a pro-regenerative action of Ucn2 in vivo that suggests it as a candidate molecule for the treatment of peripheral neurodegenerative conditions.

ND12 | The role of LRRK2 G2019S on synaptic neurotransmission in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative syndrome characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta*, with consequent reduction of striatum projections. Recently, *Leucine-rich repeat kinase 2* (LRRK2) has been discovered to play a role in both monogenic and sporadic forms of PD. Several LRRK2 mutations are observed in PD patients, and among them, the substitution Gly2019Ser is the most common. It has been reported that a gain of function of the mutated kinase activity affects synaptic transmission; in particular, it is known an influence on the glutamatergic pathway. Conversely, the role of LRRK2 on GABAergic transmission is poorly understood. In order to gain insights on the relation among the G2019S LRRK2 mutation and $GABA_A$ receptors functionality, we assessed electrophysiological experiments using microtransplantation technique. Membranes from mouse striatum tissues of LRRK2-associated PD model were injected into *Xenopus laevis* oocytes and excitatory and inhibitory currents were characterized using two-electrode voltage clamp. In G2019S striatum tissues, we find an enhanced glutamate evoked currents, according to the hypothesis of altered glutamatergic transmission in PD disease. Interestingly, the data show a significant reduction of GABA evoked currents amplitude in the LRRK2 G2019S condition. The ratio of Glutamatergic and GABAergic currents confirms the impact of mutated LRRK2 on excitatory/inhibitory imbalance in the pathological tissue. To investigate the cause of GABA current reduction, we tested whether chloride homeostasis was altered in oocytes injected with LRRK2 G2019S membranes. The results demonstrate that the reduction of GABA current amplitude was not associated with a change in GABA reversal potential (E_{GABA}). In conclusion, our preliminary data show a reduced GABAergic transmission in LRRK2 G2019S mouse tissues, raising fundamental issues on the role of LRRK2 in the modulation of neurotransmission.

ND13 | MTCH2 functionally co-operates with BID in promoting Ca2+-induced neuronal injury

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The BH3 interacting-domain death agonist (BID) is a pro-apoptotic member of the Bcl-2 protein family. While proteolytic processing of BID links death receptor-induced apoptosis to the mitochondrial apoptosis pathway, we previously showed that full length BID also translocates to mitochondria during Ca^{2+}-induced neuronal cell death. Moreover, mitochondrial carrier homolog 2 (MTCH2) was identified as a mitochondrial protein that interacts with BID during cell death. We started our studies by investigating the effect of *Mtch2* silencing in a well-established model of Ca^{2+}-induced mitochondrial permeability transition pore opening in non-neuronal HCT116 cells. We found that silencing of *Mtch2* inhibited mitochondrial swelling and the associated decrease in mitochondrial energetics, suggesting a pro-death function for MTCH2 during Ca^{2+}-induced injury. Next, we explored the role of BID and MTCH2 in mediating Ca^{2+}-induced injury in primary cortical neurons triggered by prolonged activation of NMDA glutamate receptors. Analysis of intracellular Ca^{2+} transients, using time-lapse confocal microscopy, revealed that neurons lacking *Bid* showed markedly reduced Ca^{2+} levels during the NMDA excitation period. These Ca^{2+} transients were further decreased when *Mtch2* was also silenced. Collectively, our data suggest that BID and MTCH2 functionally interact to promote Ca^{2+}-induced neuronal injury.

ND14 | Transcriptome analysis of miRNAs and their interactors in FTD patients' small extracellular vesicles

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Extracellular vesicles (EVs) cargo has been evaluated in neurodegenerative disorders, especially concerning their microRNAs content. Fronto-Temporal Dementia (FTD) is characterized by aggregation of proteins (TDP-43 and Tau) in the frontal and temporal lobes with microvacuolation and relevant deregulation of RNA-binding proteins (RBPs). We investigated miRNA cargo of small EVs (SEVs) derived from plasma of FTD patients and healthy controls for evaluating deregulated miRNAs in patients to highlight new peripheral biomarkers. Moreover, we aimed to identify mRNA targets involved in FTD pathogenesis. SEVs were isolated from plasma of 9 FTD patients and 9 healthy volunteers by differential centrifugation and characterized by Nanosight. MicroRNA libraries were generated using Small RNA-Seq Library Prep Kit (Lexogen) and sequenced on a NextSeq 500/550 (Illumina). Interaction prediction was carried out on TarBase v.8 database. We found a total of 197 Differentially Expressed (log Fold Change (FC) >1 and <-1) microRNAs, 99 up-regulated and 98 down-regulated. Then, we looked for directly validated mRNA targets of the most deregulate microRNAs (5 up and 5 down-regulated) in our analysis. Interestingly, hsa-miR-522-5p, down-regulated in our profiling, targets RTN3 that in turn interacts with and modulates BACE1, and the up-regulation of hsa-miR-203a-3p may have an impact on TNF and IL-12 levels, reduced in CSF of FTD patients. Moreover, hsa-miR-181c-5p was up-regulated and its role was already linked to a negative feedback network of TDP43. We also evaluated the deregulation of microRNA already associated to other dementia types and we found a down-regulated microRNA in common with Alzheimer's disease, hsa-miR-1260b, involved in Wnt pathway. In conclusion, our data highlight the importance of microRNAs cargo examination in EVs of FTD patients. In fact, their potential is exploitable both for biomarkers discovery and for study of gene expression alteration in FTD pathogenesis.

ND15 | Mechanically-actuated axonal outgrowth: new perspectives in regenerative medicine

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Axon regeneration is a hot topic to the community of neuroscientist. This regeneration is a complex phenomenon, which passes through the elongation of the axons that try to reach their target. Recent discoveries have shown that mechanical forces can stimulate axonal elongation, a phenomenon known as stretch-growth (SG). We exploited two technologies, magnetically-actuated, to apply exogenous forces to mice primary neurons, magnetic nanoparticles (MNPs) and magnetic microposts. Both of them allow to exert mechanical tension by manipulating the neurites with magnetic fields. We found that our magnetically-actuated technologies are biologically compliant with the model and promoted axonal elongation, as well as the ability of axons to form branches. We observed no reduction of axon caliber, accumulation of endoplasmic reticulum cisternae and the block of SG by treatment with an inhibitor of protein synthesis, the cycloheximide. All these evidences suggest that the observed elongation is related to a real mass addition. We have also seen that SG altered intracellular Ca^{2+} transient. It follows that our mechanical forces could increase the elongation rate. Our findings on neurons cultured *in vitro* suggest that magnetically-actuated microposts and MNPs could be used on living organisms (as no adverse effects were observed) and that they induce SG by the addition of axonal mass. The use of external magnetic fields has already been approved for human therapies, as well as the administration of MNPs. On the other hand, the scaffold in which magnetic microposts are enclosed could be exploited in translational medicine. It could open new interesting scenarios, as the use of our magnetically-actuated technologies could allow to find new therapeutic targets in the treatment of damaged or sick neurons following injury or disease.

ND16 | Exploiting human genetics of multiple sclerosis for drug repositioning as antioxidant redox modifiers

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Multiple Sclerosis (MS) is the most common chronic inflammatory and progressively disabling disease of the Central Nervous System in young adults characterized by demyelination, oligodendrocyte loss and neuroaxonal degeneration in the white and gray matter of the brain and spinal cord. There is significant evidence that the sustained inflammatory phase of MS creates an imbalance between Reactive Oxygen and Nitrogen Species generation and the antioxidant defense systems causing oxidative/nitrosative stress (OS/NS) which has potential role in MS-specific damage. Thus, the development of drugs able to effectively support the maintenance of redox homeostasis represents a rational approach to treat this disease. Our idea is to identify genes and/or gene products involved in MS that are linked to antioxidant pathways and repurposable drugs acting as modulators of these targets. To this aim, first we identified 698 different MS genetic variants from Genome-Wide Association Studies (GWAS) Catalog. To assign the most reliable gene target to each associated variant, molecular Quantitative Trait Loci (QTLs) were searched for each hit in public databases. In parallel, we selected 22 OS-related pathways by the Reactome database and extracted all possible proteins which have been successively overlapped with MS genetic results. Among the 91 common targets identified, we are selecting gene targets that are known to be modulated by at least one approved or in clinical trial drug by means of four different drug databases. With the purpose of selecting the best drugs, we will perform in silico ADME-Tox (Absorption Distribution, Metabolism, Excretions and Toxicology) studies, assigning a higher priority to orally administrable compounds expected to cross the Blood Brain Barrier. This strategy could promote the development of successful regenerative therapies for MS using existing drugs capable of modulating biomarkers of OS.

ND17 | miR-29a is modulated by one-carbon metabolism and involved in neurodegeneration

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Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia in the elder population. It is characterized by the loss of neurons involved in cognitive functions due, among other factors, to the accumulation of beta-amyloid (Aβ) which, in turn, could be due to the loss of epigenetic control in the expression of genes involved in AβPP (amyloid-β protein precursor) processing. Some of these genes are controlled by their promoters methylation, a process related to "one carbon metabolism", leading to the production of S-adenosylmethionine (SAM), the main endogenous methyls donor. microRNAs (miRNAs) are associated to several diseases, including AD. To investigate if the modulation of the methylation pathway, induced by B-vitamin deficiency and SAM-supplementation, could change their expression, miRNAs have been assessed by total RNA extraction, specific retrotranscription and Real-time PCR. miR-29a was selected for its involvement in methylation processes and AD after a screening in human SK-N-BE neuroblastoma cells, cultured in control and B-deficient medium with or without SAM-supplementation. miR-29a was also analyzed in mice (under the described B-deficient and SAM-supplemented conditions) and in brain samples from healthy subjects and patients. Then we studied in vitro the effects of miR-29a silencing/over-expression by assessing its specific targets. miR-29a was repressed in B-deficiency (hypomethylation), over-expressed with SAM (hypermethylation) both in cells and in mice brain and down-regulated in post-mortem AD brains. This demonstrates that miRNAs expression is associated to DNA-methylation both directly and indirectly, suggesting that one carbon metabolism can interfere with the AD pathogenesis not just through gene-specific methylation. miR-29a silencing/over-expression experiments have the purpose to evaluate its role in AD models, claiming for using it as an epigenetic biomarker and a new treatment approach of the disease.

NO01 | Molecular mechanisms underlying immune evasion in glioma progression

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The concept of a cross-talk between tumor and immune system leading to a mutual reshape of their phenotypes and supporting the malignant progression is a known phenomenon but, the mechanisms governing it, especially in gliomas, are not fully elucidated. We used a well-characterized glioma model, based on somatic gene transfer of PDGF-B, that recapitulates glioma progression. This model induces low-grade gliomas (LG) that are not able to orthotopically graft and this positively correlates with an immunostimulatory phenotype. On the contrary, high-grade gliomas (HG) induce secondary tumors when transplanted *in vivo* and show an immune infiltrate with a M2 pro-tumorigenic phenotype. We demonstrated, interestingly, that LG cells are able to graft in immunodeficient NOD/SCID mice. To evaluate the ability of progressed gliomas to reshape the immune system phenotype we co-culture HG cells with splenocytes from mice bearing immunogenic LG. Our results show that HG cells induce an increase in the percentage of $CD8^+$ lymphocytes and NK cells, but drastically reduce their cytotoxic activity as shown by the decrease of Granzyme-B expression. Moreover, a proliferative analysis with CFSE show that $CD8^+$ cells do not actively proliferate indicating that their relative increase is likely due to a decrease in other immune subpopulations rather than to a CD8 stimulation. Interestingly, we noticed that HG cells assist the grafting of LG cells following an orthotopically co-injection, suggesting that the immunosuppressive environment induced by HG cells could be sufficient to sustain also immunostimulatory LG cells. To dissect which subpopulation of the immune system counteracts the growth of gliomas in the early stages of tumor progression, we orthotopically transplant LG in mice depleted for specific immune population (CD4+, CD8+, NK cells) and we show that mice depleted for $CD4^+$ lymphocytes sustain the grafting of LG cells *in vivo.*

NO02 | Quantitative Multicomponent T2 Relaxation Showed Greater Sensitivity Than Flair Imaging to Detect Subtle Alterations at the Periphery of Lower Grade Gliomas

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Non-invasive characterization of brain water can provide valuable insights for a better understanding of pathologic conditions affecting the central nervous system (CNS). In a prospective study that enrolled patients who received proton irradiation to the CNS, we investigated the potential of multicomponent T2 relaxometry in radiation oncology. The data showed that decomposing T2 can be more sensitive than conventional FLAIR imaging for detecting subtle tissue alterations in the peri-tumoral region. Six patients affected by lower-grade non-enhancing gliomas underwent T2 relaxation and FLAIR imaging before a radiation treatment by proton therapy (PT) and were examined at follow-up. The T2 decay signal obtained by a thirty-two-echo sequence was decomposed into three main components, attributing to each component a different T2 range: water trapped in the lipid bilayer membrane of myelin, intra/extracellular water and cerebrospinal fluid. The T2 quantitative map of the intra/extracellular water was compared with FLAIR images. Before PT, in five patients a mismatch was observed between the intra/extracellular water T2 map and FLAIR images, with peri-tumoral areas of high T2 that typically extended outside the area of abnormal FLAIR hyper-intensity. Such mismatch regions evolved into two different types of patterns. The first type, observed in three patients, was a reduced extension of the abnormal regions on T2 map with respect to FLAIR images (T2 decrease pattern). The second type, observed in two patients, was the appearance of new areas of abnormal hyper-intensity on FLAIR images matching the anomalous T2 map extension (FLAIR increase pattern), that was considered as asymptomatic radiation induced damage. Preliminarily results suggest that quantitative T2 mapping of the intra/extracellular water component is more sensitive than conventional FLAIR imaging to subtle cerebral tissue abnormalities, deserving to be further investigated in future clinical studies.

NO03 | Patient derived 3D glioblastoma-culture models: characterization and potential applications in drug screening.

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In the last decades, glioblastoma (GBM) therapy, despite remarkable research efforts, has not achieved significant improvement in patient survival, also due to the lack of appropriate models to study the role of cellular heterogeneity and microenvironment in its growth and invasiveness. 2D cultures of patient-derived cancer stem cells (CSCs), one of the key players in GBM relapse, have been fundamental to deeply study GBM development and drug resistance. Though, this model lacks mimicking cell-cell and cell-microenvironment interactions. Recently, several 3D culture models have been developed to overcome the weaknesses of monolayer cultures. Here, we present a 3D culture model obtained from patient-derived CSCs embedded in Matrigel, a mouse sarcoma-derived extracellular matrix. In these conditions, GBM CSCs organized themselves in a tissue-like structure, and continued to grow for more than 30 days. We identified a spatial localization of proliferating, Sox2+ and Olig2+ cells (likely CSCs) in the external layer, while non-proliferating and GFAP+ cells (differentiated GBM cells) were in the inner region of spheroids. By qRT-PCR, we show that 3D growth induced CD44 expression, which was low in monolayer cultures. Metformin and novel biguanide analogues reduced proliferation rate, in 3D as well as 2D cultures. Lastly, we developed a new 3D model in which minced GBM specimens are embedded in Matrigel (tumoroids). In these conditions tumor fragments are able to grow for several months and invade the surrounding matrix. By immunofluorescence, we identified βIII-tubulin, Sox2, GFAP, Olig2, CD31 and IBA1 positive cells within tumoroids, indicating that these culture conditions maintain the viability of different cell subpopulations composing GBM. In conclusion, 3D culture models, both from CSCs or minced GBM specimens, recapitulate the in vivo cell-cell interaction and tumour cell hierarchy and could represent a model for drug screening with high translational validity.

NO04 | SALL4A promotes Hedgehog-dependent medulloblastoma by controlling HDAC1-mediated activation of GLI1

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Medulloblastoma (MB) comprises a heterogeneous group of embryonal tumors of the cerebellum; it occurs mostly in childhood and is associated with very poor prognosis. The current availability of -omics data allowed to classify MB in four molecular subgroups. The Sonic Hedgehog variant (HH-MB) is characterized by aberrant activation of HH signaling, an essential pathway involved in developmental and tumorigenic processes. Although HH-MB subgroup is the best genetically understood, the molecular mechanisms involved in HH signaling deregulation are still unclear. We previously identified the tumour suppressor *REN*^{KCTD11} as a key negative regulator of the HH pathway. *REN*^{KCTD11} localizes on chromosome 17p, a genomic region frequently lost in human HH-MBs, and encodes for a Cul3/E3-ubiquitin ligase. The identification of REN^{KCTD11} interactors is important to elucidate new molecular events whose deregulations can contribute to MB onset. Among REN^{KCTD11} interactors identified by mass spectrometry, we focused on Spalt-like transcriptional factor 4A (SALL4A), a crucial stemness-related factor involved in the maintenance of pluripotency and self-renewal features of embryonic stem cells. In adult tissues, SALL4A expression is mainly inhibited in the post-natal period, but it is reactivated in different cancers and is often related to worse prognosis and lower survival rate. We found that SALL4A is a binding partner and substrate of REN^{KCTD11}, which induces its poly-ubiquitylation and proteasome-mediated degradation. We demonstrated that SALL4A binds GLI1 (the final effector of the HH signaling) and enhances its activity working in complex with HDAC1, a well-known HH activator. Of note, we observed that genetic depletion of SALL4A inhibits HH-dependent tumor growth both *in vitro* and *in vivo*. Our findings identify SALL4A as a previously unknown player of the HH pathway and indicate SALL4A as an interesting target in tumor biology.

PNE01 | Autophagy enhancement as a promising strategy for treatment for Rett syndrome

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Mutations in the X-linked *MECP2* gene cause Rett syndrome (RTT), a neurodevelopmental disorder representing the first cause of female intellectual disability worldwide. Affected girls show an apparently normal development until 6 to 18 months of life, when most of the acquired motor and cognitive skills are lost. *MECP2* gene encodes for a multifunctional protein involved in many fundamental cellular processes inside and outside the nucleus, however its direct targets are still not fully identified and causative molecular pathways remain mainly unknown. Autophagy is the primary catabolic process exploited by cells for degradation of not functional organelles and macromolecules. Depletion of autophagy related genes in animal models frequently results in growth retardation, seizures and abnormal limb clasping. Moreover, neurons appear immature, showing decreased dendritic complexity. Interestingly, these defects overlap with several phenotypes observed in RTT patients and animal models. First evidence of autophagy impairment was recently observed in erythrocytes and fibroblasts from RTT patients, however its role in central nervous system, where *MECP2* is mainly expressed, has not been further investigated. Here, we dissected the autophagy signalling in *Mecp2*-null (KO) mice neurons and brain cortices. Our analysis revealed a significant accumulation of autophagosomal marker proteins LC3II and p62, suggesting the presence of a blockage in autophagy flux. *In vitro* administration of trehalose, an autophagy enhancer, rescued defective morphology of KO neurons. Moreover, intraperitoneal injections of trehalose ameliorated spontaneous and non-spontaneous motor skills of KO mice and their general condition. Taken together, these data reinforce the hypothesis that autophagy dysfunction participates to RTT pathogenesis and that its modulation might represent a promising strategy to ameliorate RTT manifestations.

PNE02 | Pharmacological modulation of neuronal activity for the treatment of Rett syndrome

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Mutations in the *MECP2* gene cause Rett syndrome (RTT), a severe neurodevelopmental disorder that typically affects females. Early developmental defects have been reported, but their contribution to the pathogenesis is still not understood. In line with the role of *Mecp2* as a master regulator of gene expression, transcriptional maturation is affected in null samples both *in vivo* and *in vitro,* as well as the ability of null neurons to respond to external *stimuli*. We tested the possible causative link between immaturity and reduced neuronal activity by pharmacologically stimulating null neurons within early time windows of differentiation. By treating NPCs derived neurons with Ampakine CX546, a positive modulator of AMPA receptor, we ameliorated null neurons transcription, morphology, and activity, highlighting the contribution of defective mechanisms of development to typical RTT phenotypes. Preliminary results suggested a positive result of an early treatment also *in vivo.* Although the selected time window of treatment suggested a prolonged benefic effect on *Mecp2* null mice, it was devoid of translational value. We thus decided to test *in vivo* later time points. To identify the best therapeutic window for intervention, we selected two pre-symptomatic (P3-P9 and P15-21) time points, an early symptomatic (P28-34) and a late symptomatic (P55-61) phase. First results were collected administrating two different ampakines at P28-P34. The efficacy of the treatment was tested by evaluating the lifespan, the phenotypic score commonly used for RTT mice, and performing some behavioral tests at different time points.

PNE03 | Investigating brain development and neuronal circuit assembly in primary immunodeficiency WHIM syndrome models

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Warts Hypogammaglobulinemia Immunodeficiency Myelokathexis (WHIM) syndrome is a rare juvenile immunodeficiency characterized by recurrent infections and leukocyte retention in the bone marrow. Other than recurrent infections, WHIM patients present with anxiety, depression and motor coordination disorders, together with an abnormal orientation of the cerebellar folia observed by brain MRI. All causative autosomal dominant mutations that lead to WHIM syndrome affect the CXC chemokine receptor 4 (CXCR4) and are gain-of-function by up-regulating the response to its unique ligand stromal cell derived factor-1 (SDF-1, also called CXCL12). The role of CXCL12/CXCR4 axis in regulating immune cell homeostasis, trafficking and chemotaxis is well established and this molecular signaling has also been shown to be relevant in neuronal cell migration and brain development. As the immunological signs of WHIM syndrome have been thoroughly investigated through a genetic mouse model, in this project we aim to analyze its neurological phenotype during development. Our behavioural analysis suggest that WHIM mice present an anxiety-like phenotype and have defects in their vestibular input and sensorimotor coordination, both strongly linked to the cerebellum. Indeed, we found some morphological and molecular cerebellar alterations, including shortening of lobule III and reduced number of Purkinje cells. As CXCR4 is also involved in the interneuron radial migration in the developing cerebral cortex, we are currently investigating their migratory behavior and terminal lamination. It is compelling that the psychological and structural assessment of WHIM patients correlates with our experimental results. Thus, the WHIM mouse model is proving to be valuable in the study of neuronal phenotype and will ultimately help us identifying specific therapeutic approaches for WHIM syndrome in humans.

PNE04 | Early Evidence of Reduced Myelination in the Cortex of a Mouse Model of CDKL5 Deficiency Disorder

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CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disease without a cure that is caused by mutations in the gene cyclin-dependent kinase-like 5 (*CDKL5*) and it is characterised by early-onset epilepsy as well as severe cognitive, sensorimotor and intellectual disabilities. CDKL5 is a serine/threonine kinase that is expressed early during postnatal development in neurons where it phosphorylates epigenetic factors (MeCP2, DNMT1), elements of both axonal and dendritic compartment (Shootin1, NGL1), and microtubule associated proteins (MAP1S, EB2) which are crucial in nucleation and assembly of microtubules (MT). Along with neurons, CDKL5 is also expressed in glial cells, including oligodendrocytes (OL) and OL precursor cells (OPCs), underlying myelination process. Although growing evidence indicate that the organization of myelin sheath can be severely compromised in the autism spectrum and in other neurodevelopmental disorders weather myelin is affected by *CDKL5* mutation is still unknown. To start addressing this issue, we evaluated the extent of myelination, its developmental trajectory, and the expression of molecules modified by myelin deposition or axonal injury – i.e.: MBP (myelin basic protein) and NF (neurofilaments) – in *Cdkl5* KO mice, an established model of CDD. By using both immunofluorescence and western blot analysis of young (P15) and adult mice (P56), we found that mutant mice show a reduction of both MBP and phospho-NFs expression in primary somatosensory and visual cortices compared to WT animals, whereas no changes were detected in total NFs expression. The analysis of myelinated axons using transmission electron microscopy showed that the g-ratio was increased in mutants indicating that myelin sheath in CDKL5 KO mice is reduced compared to controls. In conclusion, our data indicate that cortical areas in CDD animals exhibit a global reduction/distortion of myelination thus disclosing a novel role of *Cdkl5* in the CNS.

NP01 | Pupil size as an index of ocular dominance plasticity

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Recent studies have shown that two hours of monocular deprivation alter the dynamics of binocular rivalry in favor of the deprived eye, by transiently boosting the strength of the deprived eye representation and suppressing the one of the non-deprived eye – an effect that is also captured by physiological indices of V1 (primary visual cortex) activity such as VEP (visually evoked potentials) and BOLD (blood oxygenation level dependent) responses. Here we investigate the effect of depriving one eye on the sensory representations of the rivalling visual stimuli with an objective measure of stimulus strength: pupillometry. Ten participants tracked the perceptual dynamics of binocular rivalry, while we measured pupil diameter with an Eyelink. Stimuli were white and black disks, seen through a four-mirror stereoscope, allowing each eye to see one of the disks. Four trials were administered before and after monocular deprivation, achieved by applying a translucent patch on the dominant eye for two hours. Across trials, we varied the eye to which black and white stimulus were presented. In line with previous studies, we found that subtle pupil size oscillations tracked alternations between exclusive dominance phases of the black or white disk. After monocular deprivation, the amplitude of pupil oscillations changed, but not across all conditions and not consistently across delays after patch removal. Our results show that pupillometry might index deprivation effects; however, the pattern is not immediately predictable from a change of effective contrast and might be mediated by more complex mechanisms.

NP02 | Investigating the molecular diversity of COPII-dependent transport in cortical neurons

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Neurons are the most highly compartmentalized and morphologically complex cells of the body. In particular, synapses, the functional unit of the brain, are highly heterogeneous and are composed by a unique repertoire of molecules. For this reason, their correct development and function require sophisticated mechanisms to target cargoes in their site of action in the right amount at the right time. While the local insertion and removal of proteins in and out from synapses is relatively well characterized, the role of the first stations of the secretory pathway, namely the Endoplasmic Reticulum (ER) and the Golgi Apparatus (GA) in the transport and precise targeting of newly synthetized neuronal proteins to their final destination is not known. Here, we investigate the role of COPII-dependent transport from the ER to the GA. In particular, we focus on the functional relevance of the molecular diversity of SEC24, a component of the inner coat of COPII vesicles involved in cargo selection. To this aim, we first developed a proximity-dependent biotinylation assay followed by a proteomic screen to identify the early secretome associated with specific SEC24 isoforms. Our data obtained from heterologous cells and primary cultures of cortical neurons indicate that SEC24 proteins fused to the biotin ligase are enriched at the ER exit sites, interact with protein cargoes and efficiently biotinylate a number of proteins upon biotin administration. Second, we are also investigating the activity-dependent regulation of the early secretory pathway. Using a pharmacological strategy to increase the overall activity of network, we observe an overall increase in the expression of SEC24 proteins and a partial redistribution of the early secretory pathway in proximal dendrites. Together, our approaches may contribute to dissect the role of the early secretory pathway during neurodevelopment and unravel novel mechanisms of synaptic development and function.

NP03 | The neurobehavioral protective effect of Beta-D-Glucan dietary supplementation in a murine model of obesity and psychosocial stress

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Lifestyle-related risk factors, mainly obesity and work-related stress, hugely affect brain health, contributing to the onset of serious diseases such as stroke, dementia, mood and anxiety disorders. The in-depth study of the mechanisms that trigger these pathogenetic processes, aimed to identify prevention and treatment strategies, requires a translational animal model capable of mimicking dysfunctions and helping validate neuroprotective non-invasive interventions, such as nutraceuticals compounds. To fill this gap, a murine neurobehavioral dysfunction model (NDM) was developed by subjecting 10wks-old C57BL/6J mice to High-Fat Diet (HFD) for 18wks and psychosocial stress (PS) via Resident Intruder Paradigm during the last 2wks of diet. Behavioral assessment was carried out through Y-maze and Elevated Plus Maze, while morpho-functional analyses were performed on perfused hippocampal slices. The NDM characterization highlighted hippocampal remodeling and Brain-Derived Neurotrophic Factor depletion. HFD+PS animals also exhibited anxiety-related traits, depressive-like behavior and spatial memory decay. The effect of a nutraceutical treatment was evaluated by adding 3% of barley Beta-D-Glucan (βGlucan) from the 8th wk of HFD. The βGlucan-treated mice (HFDβ+PS) were compared with the NDM (HFD+PS) and with the control group, fed with standard diet and not stressed (SD). βGlucan determined spatial memory recovery and normalization of anxiety-related traits in the HFDβ+PS group. Of note, hippocampal immunohistochemistry found levels of synaptic plasticity (PV+ interneurons), neurogenesis (BrdU+ cells) and astrogliosis (GFAP) comparable to the SD group and statistically different from HFD+PS mice, while dentate gyrus volume (Hoechst) was not restored in the treated animals. Overall, our data reveal the neuroprotective activity exerted by βGlucan in subjects at high cerebrovascular risk and that the NDM has a high valuable potential to be employed for future investigations.

NP04 | The role of readiness potential in motor-induced visual suppression

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Visual suppression of sensitivity often occurs at the time of a voluntary action, such as saccadic eye-movements, hand-movements, speech, etc. It has been suggested that the suppression results from a corollary discharge signal from the pre-motor cortex, which informs sensory processing of the upcoming movement. Here, we investigate whether motor-induced suppression of visual processing involves predictively the pre-motor activity of the readiness potential. We recorded EEG activity from 18 human volunteers and estimated motor-induced visual suppression in a spatial-frequency discrimination task (visuomotor task). Participants made a voluntary button press, following which two brief gratings with slightly different spatial frequencies were presented, after a delay chosen randomly from 18 possible stimulus onset asynchronies (SOAs) ranging from 16 to 800 ms. Participants had to locate the grating with the higher spatial frequency by verbally responding ‘up’ or ‘down’. In a separate block, they were simply asked to press a button at their own pace, without any visual stimulation (motor-only task). Results show that (i) discrimination of stimuli presented close to the button press (visuomotor delay < 150 ms) was less accurate than those presented long after action-onset (visuomotor delay > 600 ms), suggesting visual processing was suppressed by motor-related mechanisms. (ii) The magnitude of the motor-induced suppression correlated across subjects with the magnitude of the EEG readiness potential. (iii) A similar correlation was observed between the same behavioural effects in the visuomotor task, and the amplitude of the readiness potential estimated in the motor-only task without visual stimulation. Taken together, our results point to an automatic and predictive link between the readiness potential and visual processing and suggests that the signal from the (pre-)motor to the sensory brain areas is mediated by the readiness potential.

NP05 | ATR kinase controls neuronal function in mature hippocampal neurons

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ATR (ATM and Rad3-related) is a serine/threonine protein kinase, belonging to the phosphatidylinositol 3-kinase superfamily and known for its role as DNA repair protein. In undifferentiated cells ATR activates in response to genomic stress, but recently it has been discovered that ATR plays fundamental functions also in postmitotic neurons where it regulates the synaptic vesicles recycling and neuronal firing. Suitably, we decided to investigate if ATR may be involved in the correct development of the nervous system and in learning and memory processes. To address this issue, we treated cultured hippocampal neurons with a selective ATR kinase inhibitor. Following the treatment, we first analysed the excitatory-to-inhibitory switch of GABA by Ca^{2+} imaging experiments in developing neurons. Secondly, we measured neuronal properties and plasticity by electrophysiology in mature cells. Ca^{2+} imaging data show that ATR inhibition does not affect the GABAergic system development, whereas electrophysiological and immunofluorescence experiments indicate that both acute and chronic treatments with the ATR blocker generate an unbalanced excitatory/inhibitory (E/I) ratio. Moreover, chronic ATR blockade affects neuronal plasticity processes, as indicated by electrophysiological analysis of long-term potentiation (LTP) and depression (LTD). In parallel, in vitro real time PCR data suggest that the ATR kinase activity controls transcription factors directly involved in the control of both plasticity processes and spontaneous neurotransmitter release. Finally, we inhibited the ATR activity in vivo by exploiting the intranasal route and performed in hippocampal tissues RNA sequencing analysis. We found that most of the differentially expressed genes (DEGs) are downregulated in hippocampi of treated animals. Altogether our data suggest that ATR acts as a transcriptional activator of factors directly involved in the control of neuronal function, extending ATR significance in neuronal physiology.

NP06 | Control of endosomal membrane identity by Spastizin

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The control of membrane identity of cell compartments relies on molecules governing spatially and, timely correlated, functional interactions. Switches represent the prototype of a functional class of molecules that digitally respond to a number of external stimuli and, therefore, they are employed in controlling molecular recognition events as they exhibit reversibility and programmability. Here, we investigate equilibrium dynamics of nanometre-scale biomolecular condensate formed by Rab GTPase biochemical switches adsorbed on the surface of an endocytic intracellular vesicle. We illustrate that endocytic structures possess finite-number of boundaries which divide distinct biological condensates characterized by different Rab switches, namely Rab5 and Rab11. We found that condensate segregation entails dependence between switching activities and spatial confinement, providing information about both dynamics and consolidation of colloidal-like grains on endocytic structures. Such low-diffusing particles are originated by a regulatory sequestration feedback mediated by Spastizin, a protein involved in hereditary spastic paraplegia. Spastizin imposes a functional cross-talk between the different Rab condensates providing evidences for a biological multipath propagation system through collective switching activities. Our results provide key principles that ensure maintenance over time of molecular identity even without physical direct interaction between their molecular constituents.

NP07 | Electrophysiological characterization of HSV-1-based replication defective viral vectors

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Gene therapy approaches are promising to treat central nervous system (CNS) disorders, but their current use is limited by the little payload capacity of the pioneering viral vectors such as Adeno-Associated and Lentiviral vectors. Herpes Simplex virus-1 (HSV-1) based viral vectors, which have a natural neuronal tropism, are emerging as potential tools to treat CNS disorders. Two are the most promising classes: HSV-1 replication-defective and amplicon vectors. However, safety concerns are still an issue for viral vectors derived from HSV-1, which is naturally cytotoxic, and growing evidence is pointing to HSV-1 as a potential element contributing to the development of neurodegenerative diseases, such as Alzheimer's disease. Amplicon vectors are considered safest because the genome packed into the particles does not carry any residual viral gene. Indeed, we showed that amplicon vectors do not alter neuron physiology and might be exploited to achieve safe and long-lasting transgene expression in the CNS. We recently characterized a new generation of HSV-1 replication defective viral vectors, that are able to persist in the rat brain up to 6 months. Although no evidence of overt cell toxicity or induction of inflammation was observed, here we characterize their influence on cell physiology. We show that mutations in the envelope glycoprotein B (gB), which is responsible for viral entry and cell fusion, might arise during viral vector production. The resulting particles carrying mutated form of gB are able to increase neuron firing frequency, while reducing both input resistance and resting membrane potential of transduced neurons. Moreover, we show that such spontaneous activity is tightly related to the fusogenic properties of HSV-1, which is naturally able to form syncytia. Altogether, these data suggest that further genetic engineering is needed in order to develop safe HSV-1 replication defective vectors dedicated to the treatment of CNS disorders.

NP08 | Top-down influences and visual perceptual learning

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Perceptual learning is a form of procedural memory referring to any change in perceptual ability as a result of experience. Perceptual learning occurs in all sensory modalities, in response to a variety of perceptual tasks including, in the visual system, grating, texture, hyperacuity or stereoscopic discrimination. Visual perceptual learning (vPL) is currently supposed to rely on still poorly characterized cellular mechanisms that occur at both early sensory processing stages and higher order visual areas. Here, we developed a mouse model to study the potential contribution of top-down connections to vPL. We first implemented a vPL task based on a modified version of the Prusky water maze test: mice were required to discriminate between two simple sinusoidal gratings, differing only for their spatial frequencies, that were made progressively more similar to each other until the animal performance reached a steady plateau. Using this task, we demonstrated the possibility to induce robust and reliable vPL in mice through a highly orientation-dependent process that strongly points toward a key involvement of the primary visual cortex. Then, we exploited chemogenetic tools to dissect the role of higher sensory processing stages in vPL. We found that temporary inactivation of the secondary visual cortex (lateromedial cortex, LM) leads to a marked impairment in the vPL process. These results suggest that top-down connections may convey key information during vPL.

NP09 | Role of the autosomal dominant IVS10+16 mutation in hiPS-derived cortical organoid development and maturation

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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, together with a prominent neuroinflammatory state located in the medial temporal lobes. The alternative splicing of MAPT(microtubule-associated protein Tau) gene on chromosome 17 leads to the expression of six different tau isoforms in the adult human brain. The expression of the different isoforms is developmentally regulated with the 0N3R isoform mainly expressed in neurons at embryonic stages while the 2N4R expression occurs once matured. Although the correct splicing seems to be necessary to keep neurons functional, the unbalanced 3R/4R tau ratios are linked with neurodegenerative disorders such as FTD. The presence of several MAPT autosomal dominant missense mutation, which promote to the inclusion of exon 10, appear to be sufficient to trigger disease. The IVS10+16 mutation, which characterize FTDP-17 shows a dominant pattern of inheritance. Although mouse models and 2D cell cultures have been largely employed for over a century to study neurodegeneration, both disease models lack to fully recapitulating the molecular mechanisms that underlie the neurodegenerative processes occurring in Tauopathies. Herein, since Tauopathies like FTDP-17 are genetically inherited, we propose to investigate the role of the IVS10+16 Tau mutation during brain development and maturation using patterned cortical organoids generated exploiting a commercial-available iPSC line that carry an intronic mutation that facilitate the inclusion of *MAPT* exon 10.

NP10 | Microglia modulate hippocampal synaptic transmission and sleep duration along the light/dark cycle

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Microglia, the brain's resident macrophages, actively contribute to the homeostasis of cerebral parenchyma by sensing neuronal activity and supporting synaptic remodeling and plasticity. While several studies demonstrated different roles for astrocytes in sleep, the contribution of microglia in the regulation of sleep/wake cycle and in the modulation of synaptic activity in the different day phases has not been deeply investigated. Using light as a zeitgeber cue, we studied the effects of microglial depletion with the colony stimulating factor-1 receptor antagonist PLX5622 on the sleep/wake cycle and on hippocampal synaptic transmission in male mice. Our data demonstrate that almost complete microglial depletion increases the duration of NREM sleep and reduces the hippocampal excitatory neurotransmission. The fractalkine receptor CX-3CR1 plays a relevant role in these effects, because *cx3cr1*$^{GFP/GFP}$ mice recapitulate what found in PLX5622-treated mice. Furthermore, during the light phase, microglia express lower levels of *cx3cr1* and a reduction of *cx3cr1* expression is also observed when cultured microglial cells are stimulated by ATP, a purinergic molecule released during sleep. Our findings suggest that microglia participate in the regulation of sleep, adapting their *cx3cr1* expression in response to the light/dark phase, and modulating synaptic activity in a phase-dependent manner.

NP11 | Behavioral and physiological effects of a probiotic supplementation on a mouse model of CDKL5 deficiency disorder: a promising strategy of intervention to ameliorate CDD clinical symptoms

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In the past few decades, the increasing interest of the scientific community in understanding the interaction between gut microbiota and the brain has brought to novel and important insights into the role of intestinal bacteria in modulating host neural function and development, also in terms of behavioral outcomes. Based on such evidences, a modulating role of the gut microbiota has been also recently demonstrated for a variety of neuropsychiatric and neurodevelopmental disorders, whose etiogenesis remains mostly unknown. Cyclin-Dependent Kinase-Like 5 (CDKL5) deficiency disorder (CDD) is a rare X-linked developmental encephalopathy caused by pathogenic variants of the CDKL5 gene. It can manifest in a broad range of clinical symptoms and severity including gastrointestinal dysfunctions, a condition shared also by other neurodevelopmental and neuropsychiatric disorders that may be related to alterations in the intestinal microbiota composition. Since nobody has ever investigated aspects related to the gut-brain axis in mouse models of Cdkl5, neither in humans, this study aims at evaluating whether probiotic supplementation (via Vivomixx®) is able to ameliorate behavioral and neuronal deficits in a CDD murine model. Our results demonstrate a significant amelioration in the nesting performance of Vivomixx-treated knock-out (KO) mice compared to the respective untreated KO, and the same trend was also observed in the Y-maze task. The intrinsic optical imaging analysis also revealed an amelioration in the cortical response to the visual stimulation of the treated KO mice in comparison to the untreated ones. The overall picture emerging from this preliminary study indicates a general amelioration of behavioral and visual deficits in probiotic-treated mice, supporting a potential clinical role for probiotic intervention as a non-invasive way to ameliorate symptoms in CDD patients.

NP12 | FXS-patient derived cortical organoids integrating microglia as 3D model system to dissect the neurodevelopmental roots of the disease

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Fragile X syndrome (FXS) is the most common inherited form of human mental retardation, and it is caused by expansion of CGG repeat in the FMR1 gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein (FMRP) with defects in the regulation of dendritic spine morphology and synaptogenesis. FXS is widely studied into 2D cell culture differentiating human iPSCs into neuronal population to characterize the disease phenotype taking advantages of molecular and functional analysis. However, conventional 2D cell culture fails to recapitulate the complex neural environment revealing itself as a not reliable in vitro model system to fully characterize the pathology. In this direction novel 2D and 3D model systems have been proposed for dissecting the molecular and cellular processes underling FXS. Several 3D protocols are available to better mimicking the cell complexity and architecture of the brain tissue, however the lack of non- neural cell types such as microglia still hinders their exploitation for the study of the neuro-immune axis in neurodevelopmental diseases. The aim of our study is to create an in vitro 3D model based on patient-specific induced pluripotent stem cells (iPSCs) with the purpose of deciphering the neurobiological phenotypes associated with FXS. Specifically, we propose to co-culture iPSC-derived cortical organoids and isogenic iPSC-derived microglia to generate a disease-relevant and tailored platform for the investigation of neuro-immune interaction during brain development. Indeed, microglia plays a prominent role in shaping synaptic circuitries during neurodevelopment and its presence might unveil possible neural-immune interplay at the basis of FXS and the establishment of a mature synaptic transmission.

NP13 | Glutamate signaling in schizophrenia

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Schizophrenia is a disorder of synaptic plasticity and aberrant connectivity in which a major dysfunction in glutamate synapse has been suggested. However, a multi-level approach tackling diverse clusters of interacting molecules of the glutamate signaling in schizophrenia is still lacking. We investigated in the post-mortem dorsolateral prefrontal cortex (DLPFC) and hippocampus of schizophrenia patients and non-psychiatric controls, the levels of neuroactive D- and L-amino acids (L-glutamate, D-serine, glycine, L-aspartate, D-aspartate) by HPLC. Moreover, by quantitative RT-PCR and western blotting we analyzed, respectively, the mRNA and protein levels of pre- and post-synaptic key molecules involved in the glutamatergic synapse functioning, including glutamate receptors (NMDA, AMPA, metabotropic), their interacting scaffolding proteins (PSD-95, Homer1b/c), plasma membrane and vesicular glutamate transporters (EAAT1, EAAT2, VGluT1, VGluT2), enzymes involved either in glutamate-dependent GABA neurotransmitter synthesis (GAD65 and 67), or in post-synaptic NMDA receptor-mediated signaling (CAMKIIα) and the pre-synaptic marker Synapsin-1. Unexpectedly, univariable analyses revealed that none of the investigated molecules was differently represented in the post-mortem DLPFC and hippocampus of schizophrenia patients, compared with controls.

NP14 | Neurophysiological investigation of numerosity adaptation

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Visual adaptation is a phenomenon that occurs whenever the prolonged presentation of a stimulus biases the perception of the subsequent ones. This property of the visual system has been known for a long time (since the report of the waterfall illusion) and it is often exploited in psychophysical research to investigate brain perceptual mechanisms. One of the latest visual features that has been reported to be susceptible to adaptation is stimulus numerosity. As a consequence of a sustained exposure to a highly numerous array of dots, a subsequent array is grossly underestimated (and viceversa). Despite the robustness of this perceptual illusion (changes in perceived numerosity up to 50%), the brain mechanisms responsible for numerosity adaptation are still far from being fully understood. In the present study we attempt to fill in this gap by leveraging on an electrophysiological approach. Previous evidence has found that non-symbolic numerosity perception modulates both early (N1) and late (P2p) components of the EEG signal. Here we applied EEG to a well-established numerosity adaption paradigm to investigate whether short-term numerosity plasticity relies on the early or late component of numerical processing. Subjects were required to perform an active numerosity estimation task both in neutral and high-numerosity adapting conditions. To prevent any possible effect induced by other factors, non-numerical characteristics of the stimuli were carefully controlled for. Our preliminary results revealed a modulation of P2p induced by high- numerosity adaptation with this effect being more prominent for stimuli presented in the left hemifield. Overall, these results suggest that numerosity adaptation is a phenomenon occurring at the later processing stages of the visual hierarchy likely involving visual areas beyond the primary visual cortex.

POSTER SESSION 2

NIM04 | A whole-brain approach to map the individual impact of gliomas on brain function

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Neurosurgical resection is the first line therapeutic approach to the treatment of brain tumors, and a gross total resection is associated with a prolonged survival. Nevertheless, the benefits of a larger resection must be balanced against the risks of significant decrements of the quality of life. Current strategies employ intra-operative stimulation or specific task/rest state functional mapping to delineate areas to be preserved. One main limitation of these approaches is in that they overlook distal regions or networks that could be functionally impaired by the tumor. Here we developed a novel method to detect whole brain resting-state networks (RSNs) alterations at the individual level for a safer surgery planning, then we applied such method to investigate the impact of brain gliomas on RSNs. Functional and structural images of 28 patients with de novo gliomas (13F/15M; age 59.6±15.8y) were acquired on a 3T Siemens Biograph mMR scanner at the University Hospital of Padova. From a publicly available dataset of controls (HCs), we derived a high-resolution RSNs template using independent component analysis (ICA) and extracted the same RSNs at the patient level by means of the group-information guided ICA. Next, the alteration of single RSNs were detected computing the cosine similarity between the spatial map of each patient's RSN as compared to a group of HCs. Moreover, we calculated the overlap between altered RSN maps and the tumor extent to investigate the spatial relationship between altered RSNs and tumor location. Tumors caused broad alterations of RSNs topography that occurred mainly in structurally normal regions outside of the tumor and edema region. On the contrary, cortical regions near the tumor often showed normal synchronization. Gliomas have a critical functional impact on remote regions and networks. A whole-brain functional mapping approach entirely performed at rest could provide helpful information for tumor surgery planning.

NIM05 | Investigating the impact of different FreeSurfer approaches on cortical thickness estimation

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Cortical thickness is one of the most important structural parameters. Among a variety of other anatomical features, it can be successfully used to study the relationship between cortical anatomy, disease and cognition. One of the most common tools for the automatic estimation of the cortical thickness is FreeSurfer, now available in version 6 (FS6) and 7 (FS7). This software requires as input a T1-weighted (T1w) image and, when available, a T2-weighted (T2w) image. To the best of our knowledge, no studies have investigated the impact of the different versions or the use of different inputs on cortical thickness estimation. In order to do so, T1w and T2w images from 129 minimally processed healthy subjects (68/61 M/F, from 36 to 86 years old) from the HCP-Aging project were submitted to the FreeSurfer main pipeline. Four different approaches were implemented: FS6 with T1w (FS6T1), FS6 with T1w and T2w (FS6T1T2), FS7 with T1w (FS7T1) and FS7 with T1w and T2w (FS7T1T2). Cortical thickness was extracted from 148 regions of interest, according to the Destrieux atlas. Statistical differences between the approaches were evaluated by a two-way repeated measures ANOVA and post-hoc tests. Results show significant effects (p<0.001) due to software version (FS6 vs FS7, F(1)=127.1) and image used (T1w vs T1wT2w, F(1)=779.4) in cortical thickness estimation. Post-hoc tests (p<0.01 after correction for multiple comparisons) reveal that: i) adding the T2w image as input of the pipeline increases cortical thickness value; ii) results obtained with FS6 are higher than FS7 ones. Our study proves that different approaches bring to different cortical thickness estimations. Therefore, when comparing values of cortical thickness from different studies it is important to consider both the software version and the images used.

NIM06 | A connectomic approach to investigate structural brain connectivity in Fabry Disease

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Fabry disease (FD) is a rare and progressive systemic X-linked lysosomal storage disorder. With reference to central nervous system involvement, widespread microstructural alterations affecting the normal appearing white matter have been demonstrated. We analyzed brain structural connectivity of 46 FD patients (28F, 42.2±13.2yrs) and 49 healthy controls (HC, 21F, 42.3±16.3yrs). Diffusion-weighted magnetic resonance images were processed using probabilistic tractography and *Convex Optimization Modeling for Microstructure Informed Tractography* (COMMIT). To build quantitative connectomes, we employed a modified Automated Anatomical Labeling parcellation with 100 regions and weighted each connection by the total signal fraction associated to the corresponding bundle of streamlines. From each brain network we extracted five global metrics: *density* (as an index of how much the network is connected), *mean strength* (showing how much these connections are strong), *global efficiency* (as a measure of the ability to exchange information), *clustering coefficient* (indicating how well a node is connected to its neighbors) and *modularity* (showing the degree of segregation). Between-group comparisons were performed by means of robust linear model, considering age and gender as confounding factors. We found statistically significant differences in both *global efficiency* ($p=0.01$) and *mean strength* ($p<0.001$) distributions. These results confirmed the mild, but widespread, microstructural damage occurring in FD, which reduces the effectiveness of fiber connections to efficiently exchange information. Moreover, since for a subset of 11 subjects neuropsychological tests were available, correlations between them and the adjusted global metrics were probed, to evaluate the clinical impact of these structural changes. Significant correlation emerged between the mean strength and *Rey Auditory Verbal Learning Test* score ($r=0.721$, $p=0.03$), further highlighting the relevance of these findings.

NI08 | Microglia lacking C9orf72 mediate aberrant synaptic pruning in vitro

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The hexanucleotide repeat expansion (HRE) in the C9ORF72 gene is responsible for the 25% of familial Frontotemporal Dementia (FTD). The HRE number reduces the expression of C9orf72 and triggers a proinflammatory phenotype and lysosomes accumulation in adult mouse microglia. During development, microglia is responsible for beneficial synaptic pruning, i.e. phagocytosis of aberrant synapses which are tagged by complement factors (C1q/C3). Excessive complement-mediated pruning is activated under neurodegenerative conditions, causing pathological synaptic loss. However, whether and how C9orf72 downregulation may influence synaptic pruning is unknown. To address this question, we first characterized the phenotype of perinatal microglia from C9orf72 knock out (ko) mice. Real time PCR analysis for homeostatic and inflammatory genes revealed that perinatal C9orf72 ko microglia already show a more reactive state, suggesting a 'primed' phenotype. C9orf72 ko microglia released more extracellular vesicles (EVs) and complements factors (C1q and C3) associated to EVs compared to wt cells, as revealed by qNano and WB analysis respectively. This suggested that C9orf72 ko microglia may deliver excessive C1q/C3 to the synapse via EVs and cause detrimental synaptic pruning. Accordingly, in microglia ko cocultured with mature hippocampal neurons for 24h we found greater co-localization of the post-synaptic marker Shank-2 and the lysosomal marker CD68, an index of the capacity of microglia to phagocyte and degrade synaptic material. Furthermore, analysis of the density of Bassoon positive and Shank-2 positive pre- and postsynaptic puncta along dendrites close to C9orf72 ko microglia revealed much lower density of synaptic puncta compared to neuron-wt microglia cocultures. We are currently investigating how inhibitors of EV biogenesis or EV supplementation to the microglia-neuron co-culture impact microglial synaptic pruning to directly prove the key involvement of EVs in the process.

NI09 | Cognitive and visual function, a simultaneous decline in neuro-inflammation

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The earliest phase in most of the age-related neurodegenerative diseases is an undercurrent and chronic neuroinflammation whose mechanisms and mediators might be shared with the specific pathogenesis. Despite most of neurodegenerative diseases have no cure, an early diagnosis might enhance therapeutic strategies slowing down the disease. Unfortunately, this preliminary stage of chronic neuroinflammation is not easily identifiable, especially in the brain. One of the most accessible and sensitive part of the central nervous system is the retina which might play a reporter role on the brain healthiness. However, specific correlation between visual function and neuroinflammation is not completely understood. Here we characterize an animal model of lipopolysaccharide (LPS)- induced neuroinflammation in order to identify functional changes of the retina which can be predictive for an early diagnosis of brain dysfunctions. 6 months old C57/BL mice were intraperitoneally injected for five days with 0.25 mg/Kg of LPS. Electroretinogram (ERG) recordings and novel object recognition test (NOR) were performed at different time points up to 10 days from the last injection. ERG analysis describes a severe and irreversible reduction in the "a-" and "b- wave" amplitude till 10 days of recovery. In particular, decline of the scotopic response precedes the photopic one. Analogously, NOR test results underline a significant cognitive impairment with no recovery up to 10 days. These results show a link between cognitive decay and retinal function in neuroinflammatory condition confirming the essential role of the retina as a window of brain in pathological condition.

NI10 | Mitochondrial impairment in lymphoblasts derived from Aicardi-Goutières Syndrome' patients

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Aicardi-Goutières Syndrome (AGS) is an uncommon childhood disease affecting the brain, immune system, and skin. 9 AGS gene mutations cause a buildup of endogenous nucleic acids (NAs) that the organism misidentifies as foreign NAs of viral origin, resulting in an aberrant Interferon-alpha (IFN-)-mediated immunological response. Mitochondrial abnormalities can result in the release of mtDNA and the production of IFN-, which can stimulate immunological pathways. Aim of this work was to examine mitochondria in AGS patient cells and determine if they play a role in the disease' pathogenesis. The study employed lymphoblasts (LCLs) from *RNASEH2A* and *RNASEH2B* mutant AGS patients and one healthy control. The morphological alterations, ROS generation, and membrane potential changes of these organelles were studied by transmission electron microscopy and flow cytometry. The SeaHorse Analyzer was used to investigate the metabolic changes, while immunofluorescence was utilized to study mtDNA oxidation and the VDAC oligomerization. The release of mtDNA was investigated using qRT-PCR. Both mutant LCLs showed morphological and structural changes in mitochondria when compared to controls, while *RNASEH2A* LCLs showed a lack of physiologic membrane potential. ROS generation was enhanced in both mutant LCLs, although it was considerably greater in *RNASEH2B* LCLs. According to these findings, *RNASEH2B* LCLs had a higher levels of 8-oxoG, a marker of mtDNA oxidation, and a stronger signal generated from VDAC protein oligomerization, indicating the development of a mitochondrial pore that might enable the release of mtDNA into the cytoplasm. In *RNASEH2B* LCLs, there was a significant increase in cytoplasmic mtDNA content. The damage to these structures in both mutant cell lines was also verified by metabolic changes. Our findings support the existence of mitochondrial abnormalities in LCLs from AGS patients, indicating that these organelles could be involved in the disease's etiology.

NI11 | New visions of biased agonism through orthosteric and allosteric dual targeting of the cannabinoid receptor type 2

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In the classical two-state model, G protein-coupled receptors (GPCRs) are considered to exist in equilibrium between an active and an inactive conformation. However, more recent observations led to a revisitation of this traditional concept, rather indicating that GPCRs are dynamic proteins that can adopt multiple active states responsible for distinct functional outcomes following by the binding of both allosteric and orthosteric ligands, so determining the signaling 'bias' phenomenon. A novel route to engender signaling pathway selectivity in the actions of orthosteric ligands at GPCRs consists of the development of dualsteric/bitopic ligands by linking the orthosteric and allosteric pharmacophoric units using specific spacers. This strategy offers access to GPCR modulators with a unique receptor-subtype and signaling selectivity profile and, consequently, to drugs with fewer side effects. We have decided to direct our efforts on the development of dualsteric/bitopic ligands targeting the subtype cannabinoid receptor CB_2R. We have chosen CB_2R due to its lack of adverse psychotropic effects along with its wide therapeutic application in pathologies such as neuroinflammation where CB_2R activation appears to prevent or decrease microglial activation. Here, we describe the design, synthesis, and biological evaluation of bitopic ligands at CB_2R, which were obtained linking, through different linkers, the pharmacophoric portion of the CB_2R positive allosteric modulator (PAM), previously identified by our research group, with that inspired by the class of the 1,8-naphthyridin-2(1*H*)-one-3-carboxamide derivatives, previously identified as CB_2R orthosteric agonists with a remarkable affinity. Our results showed that most of these compounds displayed a significant bias towards activation of the cAMP pathway over recruitment of β-arrestin. Moreover, the best compounds were also evaluated for their modulation on cytokines production using the human microglial HMC3 cell line.

NI12 | Pharmacological and Epigenetic Regulators of the NLRP3-Inflammasome Activation in Alzheimer's Disease.

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Background: Activation of the NLRP3 inflammasome-complex results in the production of IL-18, Caspase-1 and IL-1β. Although these cytokines have a beneficial role in promoting inflammation, an excessive activation of the inflammasome and the consequent constitutive inflammatory status plays a role in a number of human pathologies including Alzheimer's Disease (AD). MicroRNAs (miR-) target the 3'UTR region of NLRP3, preventing the activation of the inflammasome, thus inhibiting IL-18, Caspase-1 and IL-1β production. Because Stavudine (D4T), an antiretroviral drug, was recently shown to inhibit inflammasome activation, we verified whether its effect is mediated by the modulation of miR-7, miR-22, miR-30 and miR-223, miRNAs that bind the same *NLRP3*- mRNA-UTR region and interfere with protein translation, reducing NLRP 3activation.
Methods: PBMC of twenty AD patients and ten sex-matched healthy controls (HC) were stimulated with Lypopolisaccaride (LPS)+Amyloid-beta ($A\beta_{42}$) in the absence/presence of D4T. Expression of genes within the inflammasome complex and of miR- was evaluated by RT-PCR; cytokines and caspase-1 production was measured by ELISA.
Results: NLRP3, ASC, IL-1β and IL-18 expression, as well as IL-18, Il-1β and caspase-1 production, were significantly augmented ($p< 0.05$) in LPS+$A\beta_{42}$-stimulated PBMC of AD patients. D4T reduced the expression of inflammasome genes and cytokines production ($p< 0.005$). miR-7, miR-22, miR-30 and miR-223 expression was significantly increased in LPS+$A\beta_{42}$-stimulated PBMC of AD patients and was not modulated by D4T.
Conclusion: These results suggest that the inhibitory effect of mRNAs on NLRP3 inflammasome is lost in AD patients and indicate that the ability of D4T to reduce the activation of the NLRP3 inflammasome is not mediated by mRNAs modulation.

NI13 | Activation of the MET receptor as therapeutic tool in multiple sclerosis: a new protective mechanism involving the glutamatergic system

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Multiple sclerosis (MS) is an autoimmune disease of central nervous system (CNS) characterized by immune-mediated inflammation, demyelination, and axonal damage. Among the new investigated pathological mechanisms involved in MS, glutamate excitotoxicity exerts crucial role. MS patients show increased glutamate levels in brain and cerebrospinal fluid in comparison to healthy controls and up-regulation of glutamate receptor (NMDAR) in brain lesions. Interestingly, the inhibition of NMDAR in the murine model of MS, represented by the experimental autoimmune encephalomyelitis (EAE), ameliorates the neuropathological signs characteristic of the model. Recently, the Hepatocyte Growth Factor (HGF) and its receptor MET, have emerged as critical molecules able to modulate the glutamatergic synapse. It is known that the over-expression of HGF in neurons induces reduction of infiltrating cells in the CNS and protective action through a pro-tolerogenic response in the EAE model. Evidence suggests also that HGF/MET axis promotes survival of oligodendrocytes and neurons, but the mechanisms are yet unknown. Our unpublished data indicate that HGF and MET activating monoclonal antibody (METamAb) mitigate the NMDAR-induced calcium influx and induce resistance against neuronal death in vitro. Further studies are needed to understand the possible interaction between Met and NMDAR. Since HGF binds and is sequestered by the extracellular matrix, its use as a therapeutic molecule is difficult. Thus, here we evaluated whether METamAb alters the EAE disease course in term of disability and neurological damage. EAE mice were intravenously injected with METamAb or vehicle starting before onset (about 6 day post immunization, dpi). Doses were decided starting from pharmacokinetics. Interestingly, we observed delayed EAE onset and reduced cumulative clinical disability score in the group of METamAb treated EAE mice in comparison to vehicle group. Accordingly, the neuropathological analysis at 21 dpi revealed a mild reduction in the number of inflammatory infiltrates and microgliosis. No differences in demyelinated areas and astrogliosis were highlighted. We provide new insights about the function of MET receptor in development of EAE and bases for the development of new drugs targeting MET or NMDAR to counteract MS.

NI14 | Immune system dysfunction and pro-inflammatory changes in the brain of two mouse models of autism spectrum disorders

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Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders associated to social communication deficits and repetitive sensory–motor behaviors. These symptoms 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 with ASD. This condition is often linked with a strong inflammatory state, which contributes to neurodegeneration and impairments in synaptic plasticity. Cntnap2$^{-/-}$ and Shank3b$^{-/-}$ mice have widely been considered robust animal models of ASD. In the current study, we analyzed the expression of classical pro-inflammatory molecules in the cerebral cortex, hippocampus and cerebellum of mutant mice. mRNA and protein expression of IL-6, TNF, IFNγ and IL-1β were increased in the cerebellum of Cntnap2$^{-/-}$ and Shank3b$^{-/-}$ mice, in comparison with their WT littermates. In addition, increased levels of the same molecules were found in the blood of both mutant mice. Finally, a link could be identified between inflammation within cerebellum and impaired social and motor behaviors (common ASD-related features) in these mice. Taken together, these results suggest that cerebellar inflammation may support ASD-like behaviors in autism.

ND18 | Multi-OMICs approach to elucidate the role of candidate modifiers in C9orf72-ALS patient-derived spinal cord organoids

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Amyotrophic lateral sclerosis (ALS) is a rare neuronal disorder involving motor system that is characterized by upper and lower motor neuron (MN) loss, resulting in progressive muscle paralysis that eventually costs the life of the patient. No therapy is able to effectively slow, halt or reverse disease progression. Therefore, expanding our knowledge of ALS pathophysiology is imperative to develop novel treatment strategies. 3D models represent a challenging tool that can recapitulate the complexity of tissue framework, unlike limited 2D cultures. The primary goal of this proposal is to 1) characterize spinal cord organoids to maximize their reproducibility and reliability; 2) outline the ALS phenotype in this model with omic approaches; 3) identify and validate candidate genes/pathways related to the pathophysiology of the disease.
On this basis, we generated spinal cord organoids and we verified the presence of neural progenitors, post-mitotic neurons, MNs, and glia. Organoids were harvested, fixed, and cryosectioned at three time points (day 30, 45, 80) and evaluated for their morphology and neurodevelopmental features by IHC and qPCR. Specifically, day 80-organoids expressed markers such as MAP2, DCX, OLIG2, PAX6, SMI32, TUBB3, and GFAP. Terminally differentiated spinal cords underwent mass spectrometry to reveal proteins differentially expressed in ALS samples compared to controls. We reported around 250 significantly dysregulated proteins that we are currently validating with western blot and qPCR. Preliminary gene ontology analysis depicted alterations associated with cytoskeleton, energy metabolism, and astrocyte reactivity. Finally, organoids at day 80 were dissociated and cryopreserved to investigate possible aberrant transcriptomic profile related to ALS pathogenesis through single-cell RNA sequencing.
Overall, this project might allow the assessment of novel candidate genes linked with C9orf72-ALS pathogenesis and their potential as therapeutic targets.

ND19 | Astrocytic differential modulation of mTOR signaling and localization during ischemia and starvation

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During an ischemic event, the brain suffers a critical shortage of oxygen and nutrients that affects metabolism and leads to cell death and, in some cases, subsequent neurodegeneration. Mammalian target of rapamycin (mTOR), a serine/threonine kinase, triggers different cellular responses focused on maintaining the balance between anabolism and catabolism. Generally, nutrients and oxygen maintain mTOR active, which is usually assembled into mTOR complex1/2 (mTORC1/2). In fact, the supply of growth factors, amino acids, oxygen and glucose are essential inputs for the translocation of mTORC1 from the cytoplasm to the lysosomal surface, whereas mTORC2 is commonly membrane associated. Over the lysosome, mTORC1 is activated by Rheb via PI3K/Akt and can modulate protein synthesis through the phosphorylation of two main targets: P70S6K and 4EBP1. Additionally, mTORC1 activation leads to the inhibition of processes such as autophagy or apoptosis. In contrast, in a pathological situation such as an ischemia the supply of nutrients and oxygen may affect mTORC1 activity and, finally, the integrity of the cell. Here, we recreate ex vivo two pathological models after oxygen-glucose (OGD) or amino acids (AA) deprivation. We use primary cultures of cortical astrocytes to study the relationship between mTORC1 localization and activity. Our results suggest a different output of the mTORC1 signaling pathway during OGD and AA deprivation. The inhibition of mTORC1 activity inferred from the phosphorylation levels of its targets is distinct from its pharmacological inhibition. Surprisingly, mTORC1 activity seems to not be entirely related with its localization and depends on the initial signal. The individual analysis of AA deprivation and OGD could provide fine tuning information of mTORC1 signaling in the healthy and pathological brain. Particularly, the study of glial cells offers an interesting point of view for the understanding of ischemic stroke and neurodegenerative diseases.

ND20 | Colocalization and interaction study of neuronal JNK3, JIP1,Beta-arrestin2 together with PSD95

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The c-Jun-N-terminal kinases (JNKs) are a family of stress-activated serine threonine protein kinases belonging to the Mitogen-Activated Protein Kinases (MAPKs). Mammals express three different JNK isoforms: JNK1, JNK2 and JNK3. While JNK1 and JNK2 are widely expressed in all body tissues, JNK3 is selectively expressed in the central nervous system, cardiac smooth muscle and testis. JNK3 is also the JNK isoform most responsive to stress stimuli in the brain and is involved in synaptic dysfunction and general neurodegenerative processes. JNK3 pathway is organized, as other MAPKs, in a cascade of signaling amplification in which the signal transduction occurs by a phosphorylation mechanism. Since different MAPKs shared common upstream activators, the pathway specificity is guaranteed by scaffold proteins. In this contest, JIP1 and b-arrestins2 are two of the most important regulators of the JNK signaling and they are highly expressed in the brain. Due to the strong involvement of JNK3 in synaptic dysfunction, we investigated whether these interactions occur in the whole brain homogenate as well as at the level of the dendritic spines performing hippocampal in-vitro culture and isolating the post-synaptic enriched protein fraction from brain lysate. We biochemically studied the interaction of JNK3 with JIP1 and/or b-arrestin2 in C57bl6/J mice total brain homogenates and in the post-synaptic compartment. We found by immunoprecipitation that the proteins can be found interacting together with PSD95, a post synaptic density marker. We next took advantage of super-resolution microscopy to demonstrate the co-localization among JNK3-PSD95-JIP1 and JNK3-PSD95-b-arrestin2 in cultured hippocampal neurons. Targeting JNK3 taking advantage of its interaction with scaffold proteins could represent a therapeutical tool against many brain diseases, since it mediates stress-pathways, neuronal death and synaptic injury.

ND21 | LRRK2-related Parkinson's disease mutation impairs glutamate transporter trafficking

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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 knockin 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, causing its intracellular relocalization 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.

ND22 | MiRNAs Shuttled by Exosomes Produced by Mesenchymal Stem Cells Ameliorate the Astrocyte Phenotype in Late Symptomatic SOD1G93A Mouse Astrocyte Primary Cultures

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by muscle wasting, weakness, and spasticity, due to progressive degeneration of cortical and spinal motor neurons. Both familiar and sporadic ALS has been described. Despite the notable growth of genetic studies, the genesis of most sporadic cases remains unknown. Currently, epigenetic research involving miRNA studies shows some promising aspects.

We previously reported that intravenous administration of mesenchymal stem cells (MSCs) in the $SOD1^{G93A}$ ALS mouse model significantly improved disease progression and modulated the astrocyte and microglia reactive phenotype. We proposed that MSC effects were paracrine, possibly involving exosome-mediated cell communication. Indeed, unpublished results substantiate the positive impact of MSC-derived exosomes on spinal cord primary astrocyte cell cultures prepared from late symptomatic 120-day-old $SOD1^{G93A}$ mice.

Here, we investigated the effects of nine miRNA, which were found up-regulated in IFNγ-primed MSCs and shuttled by MSC-derived exosomes. For this purpose, we transfected $SOD1^{G93A}$ astrocytes with the single synthetic miRNAs and analyzed their effect on the astrocyte phenotype. Seven out of nine miRNA mimics significantly decreased the overexpression of GFAP, IL1β, and TNFα, detected by confocal microscopy, in $SOD1^{G93A}$ astrocytes.

Four of these miRNAs (466q, 467f, 466m5p, 466i3p) were over-expressed in MSCs and in exosomes. We selected in-silico their relevant pathways (p38, TNFα, and NFKB) that have been validated by determining the miRNA effects on MAP3K8, MAPK-APK2, MAPK11, and TRAF6 by qPCR. 466q and 467f strongly reduced MAPK11 mRNA expression, thus inhibiting TNFα formation.

Our results suggest that the amelioration of the reactive phenotype of spinal cord $SOD1^{G93A}$ astrocytes, brought about by in-vivo MSC treatment, operates through exosome-shuttled specific miRNAs.

ND23 | Neuroprotective effect of (R)-(-)-linalool on hydrogen peroxide-induced oxidative stress in PC12 cells

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Oxidative stress is an imbalance between production of reactive oxygen species and antioxidant agents, which plays an important role in neurodegeneration, pain and inflammation. (R)- (-)-linalool (LIN), a monoterpene compound present in essential oils from aromatic plants, is known to possess neuroprotective, anti-nociceptive and anti-inflammatory properties. The aim of the study was to investigate the hypothesis that LIN's neuroprotective, antinociceptive and anti-inflammatory properties descend from its ability to act as antioxidant. The study challenges this hypothesis by evaluating whether LIN is able to counteract hydrogen peroxide (H_2O_2)-induced oxidative stress in PC12 cells, a well-known neuronal model. The effect of (-)-linalool on cell viability and on damage of plasma membrane was first investigated, respectively by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) release assays. Then intracellular levels of reactive-oxygen-species (ROS), apoptosis and cell cycle distribution were investigated by flow cytometry. The experimental results revealed that LIN protects PC12 cells from H_2O_2-induced cell viability reduction. Furthermore, LIN showed a protective effect by preventing the H_2O_2-dependent release of LDH and counteracting intracellular ROS overproduction. Finally, LIN reduced apoptotic cells, and also the H_2O_2-induced quiescent cells in the G2/M phase. In conclusion, these results demonstrate that LIN possesses antioxidant activity against H_2O_2-induced oxidative damage in PC12 cells. The antioxidant LIN effect can justify its neuroprotective, anti-nociceptive and anti-inflammatory actions. On the basis of these findings, the use of LIN as a potential therapeutic agent for the management of pain mediated by oxidative stress could be suggested.

ND24 | Dissecting the molecular mechanism linking GCase deficiency with the onset of neurodegeneration in Gaucher and Parkinson's diseases

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β-glucocerebrosidase (GCase) is a lysosomal glycohydrolase encoded by GBA gene, responsible for the catabolism of the sphingolipid glucosylceramide (GlcCer). Deficiency of this enzyme causes the lysosomal accumulation of GlcCer, leading to the onset of GCase-related pathologies, characterized by neurological impairment and neurodegeneration, which comprise Gaucher Disease (GD) and GBA-dependent Parkinson's disease (GBA-PD). Nevertheless, the relation between GCase loss of function and neurodegeneration is not understood so far. To dissect the possible molecular mechanism linking GCase deficiency and the consequent GlcCer accumulation with the onset of neuronal damage occurring in GCase-related pathologies, we developed an *in vitro* human model of the neuronal form of GD represented by hiPSCs-derived dopaminergic neurons obtained from healthy subjects' fibroblasts treated with 500 µM conduritol B epoxide (CBE), a specific GCase inhibitor. CBE-treated neurons present a progressive and time-dependent accumulation of GlcCer. Moreover, they recapitulate the neurodegenerative phenotype of GCase-related pathologies, presenting a significantly decreased expression of neuronal markers such as Tau, MAP2, Neurofilament H and PSD95. We also observed that GCase deficiency causes an enhanced lysosomal biogenesis and exocytosis, which leads to the extracellular release of uncatabolized GlcCer and to its accumulation also at the plasma membrane (PM) level. Interestingly, at the PM level GlcCer accumulates in specific signalling microdomains called "lipid rafts", altering their sphingolipid pattern and protein content. In particular, we identified a reduction of complex gangliosides together with an enrichment of the active form of the non-receptor tyrosine-kinase c-Src. These data let us to speculate about the existence of a lysosome-PM axis responsible for the alteration of the PM architecture that can leads to the neuronal damage occurring in GCase–related pathologies.

ND25 | Identification of novel biomarker for Alzheimer's Disease early-stage diagnosis

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Alzheimer's disease (AD) is the most common neurodegenerative disorder and the first cause of dementia in the elderly. To date, no treatments are available to cure or slow down the pathology. Synaptic Dysfunction has been identified as the first neurodegenerative event in AD, therefore it represents a mandatory target to the development of pharmacological strategies. One of the key players in this process is c-Jun N-terminal kinase (JNK). JNK3, the brain specific JNK isoform, is the most responsive to stress-stimuli in animal models. Therefore, the main aim of this project is to characterize synaptopathy and JNK activation in AD human samples.
We found that AD patients shown JNK activation in the frontal cortex compared to control patients, in line with increased phosphorylation of JNK elective target c-Jun. This brain area also showed increased levels of phosphorylated APP in T668, JNK main phosphorylation site. In the post-synaptic enriched fraction (TIF) JNK was also highly activated in AD compared to controls. Furthermore, Drebrin, a marker for mature dendritic spines, and NMDA receptors levels were severely reduced in AD as expected, indicating spine pathology.
Now we are currently measuring JNK3 levels in CSF and olfactory mucosa (OM), exploiting its potentiality as a new biomarker. Preliminary results indicate that JNK3 levels increased in AD patients compared to controls, confirming JNK3 as a potential diagnostic biomarker.

ND26 | The repositioning of the antibiotic Moxifloxacin as a novel approach for Spinal Muscular Atrophy treatment: therapeutic effects in SMNΔ7 mice.

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease affecting children, characterized by motor neuron (MN) impairment, skeletal muscle atrophy and premature death. SMA is due to the mutation/deletion of the Survival Motor Neuron 1 (*SMN1*) gene; even if spared in case of disease, its human-specific copy (*SMN2* gene) fails in rescuing SMA phenotype, since it produces a low amount of functional SMN protein. Improving therapeutic strategies aimed at increasing *SMN2* function is still a hot topic in the SMA field. Recently, by performing a screening of FDA-approved drugs, the antibiotic Moxifloxacin demonstrated to exert positive effects on *SMN2* exon 7 splicing, increasing SMN protein level on several SMA models (a Drosophila-based reporter system and SMA patients-fibroblasts). Here, we tested the effects of the Moxifloxacin administration in SMAΔ7 mice (a SMA type II murine model). The drug was daily injected into mice subcutaneously, from postnatal day 2 (P2) to P12. Weight assessment, behavioural and molecular analysis respectively showed a significant increase in body weight, motor skills and SMN protein levels in different tissues (≥34% in the spinal cord, ≥91% in the quadriceps) in treated mice compared to the untreated ones. In addition, stereological and immunohistochemical analyses performed on lumbar spinal cord sections showed a delay in MN degeneration and a significant reduction in the levels of the apoptotic marker cleaved caspase 3 (≤46%) in treated mice. Moreover, concerning effects on neuroinflammation, astrogliosis (GFAP signal) was significantly reduced (≤48%), as well as a different degree of microglia ramification/activation was morphologically assessed in treated mice. Finally, histological analysis of skeletal muscles showed a significant increase in fibers area and Feret's diameter in treated mice in comparison with untreated pups. Overall, the results demonstrated that Moxifloxacin can be potentially repositioned for the SMA treatment.

ND27 | Age-dependent BBB damage favours brain iron deposits, activation of the Hepc/Fpn1 pathway and astrocytic-neuronal crosstalk

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During aging, iron levels increase in the brain and accumulates in regions that are vulnerable to age-dependent neurodegeneration: the cerebral cortex and the hippocampus. However, the mechanism of iron regulation in the brain remains scarce. Here, we demonstrate for the first time the involvement of the Hepcidin/Ferroportin1 (Hepc/Fpn1) pathway in the metabolism of iron in the brain. We measured a remarkable reduction of Zonula occludens1 (ZO-1), a tight junction protein of the Blood-Brain Barrier (BBB), indicating an increased permeability to iron in old mice; the alteration of iron homeostasis and its deposition in the brain drives neuroinflammation and oxidative stress. We found that Hepc is upregulated by the increase of iron content and it acts as inhibitor of the iron exporter Fpn1. Interestingly, both in the cerebral cortex and hippocampus Fpn1 colocalize specifically with astrocytes, while the iron storage protein ferritin light-chain colocalize with neurons. This differential distribution within neuronal tissue suggests that astrocytes drive iron shuttling in the brain and that neurons are unable to metabolize it. Moreover, we observed an increase of the ferritinophagy inductor Nuclear Receptor Coactivator 4 (NCOA4) which selectively degradates the ferritin heavy-chain (Ft-H) protein promoting in turns the increase of the ferritin light chain (Ft-L) heteropolymers, which are more effective for iron storage. Altogether, these data highlight for the first time the involvement of the Hepc/Fpn1 axis and NCOA4 in brain iron increase during mice aging as a response to a higher iron flux in Central Nervous System consequent to a BBB alteration.

ND28 | C. elegans helps to decipher the mechanism underlying tau toxicity in tauophaties

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Abnormal tau phosphorylation and aggregation into bundles of filaments in the central nervous system is a common feature of a heterogeneous group of pathologies called tauopathies. Similarly to other misfolded proteins, tau oligomers more than fibrillar assemblies, have been suggested to be the main responsible of toxicity. Hyperphosphorylated tau is able to spread in the brain exerting its toxic function through a *non-cell-autonomous* mechanism. With hypothesis that abnormal tau conformers play a causal role in driving toxicity, we conceived an original, integrated approach involving the use of recombinant human wild-type (WT) tau or tau carrying P301L mutation, cells overexpressing tau P301L, brain homogenates from WT or transgenic mice overexpressing tau P301L, and *C. elegans*. Cerebral homogenates from chronic traumatic brain injured (TBI) mice, showing widespread tau pathology, were also employed. We found that recombinant tau oligomers, but not monomers, induced functional deficits in *C. elegans* consisting on neuromuscular impairment and altered synaptic transmission. Results were similar when worms were exposed to brain homogenates from P301L or TBI mice. Harsh protease digestion to eliminate the protein component of the brain homogenates from TBI or P301L mice, preincubation with anti-tau antibodies or tau depletion by immunoprecipitation, abolished the toxicity indicating a pivotal role of abnormal tau conformers. These findings indicate that *C. elegans* represents a tractable model to investigate *in vivo* the toxicity of misfolded/ aggregated tau, assessing its impact on neuromuscular function. This *C. elegans*-based platform can be successfully employed to test the ability of anti-tau compounds to interfere with the consequences of tauopathies.

ND29 | Role of SOX2 in the neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion

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After excitotoxic lesion, parenchymal astrocytes in the striatum undergo a spontaneous neurogenic activation and locally generate neuroblasts. Starting from neurogenesis onset, during the third week post-lesion, striatal astrocytes continuously and asynchronously transit from quiescence to a neurogenic active state giving rise to sparse independent niches (Fogli et al. unpublished observations). Yet, the mechanisms that drive this response are still unclear. The TF SOX2 is physiologically involved in the maintenance of stem cell populations and its overexpression is sufficient to induce neurogenic activation of striatal astrocytes. Conversely, the abrogation of SOX2 in cortical astrocytes greatly reduces their reactivity in response to traumatic brain injury and the severity of the damage. The deletion of SOX2 in about half of the striatal astrocytes before excitotoxic lesion do not affect the lesion size nor the volume of the spared striatum while it completely abolished the neurogenic response. Conversely the neurogenic response was only partially impaired by the loss of SOX2 between the early post-lesion astrocyte reactivity and neurogenesis onset. Interestingly in these latter animals, SOX2 positive and negative astrocytes provide a comparable contribution to the establishment of neurogenic niches indicating that after an early phase post-lesion the loss of this TF does not hinder the neurogenic expansion at the single cell level while it may play a non-cell autonomous inhibitory role on the neurogenic response at the population level. These observations indicate that the expression of SOX2 within an early post-injury time window is critically required to prime the subsequent neurogenic response. Overall these results support a model in which the awakening of striatal astrocyte neurogenic competence and the transition to a neurogenic active state are dissociable components of a complex multi-step process.

ND30 | In-vivo targeting GPR17 by Montelukast affects survival and disease progression in a gender dependent manner in SOD1G93A Mice

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Amyotrophic lateral sclerosis (ALS) is a multifactorial neurodegenerative disease leading to motor neurons death. Among the different pathological mechanisms, oligodendrocyte degeneration (OL) and OL precursor cell (OPC) maturation dysfunction contribute to ALS, guiding to myelin deterioration. An important regulator of OPC differentiation and survival is GPR17. However, at a specific stage, GPR17 needs to be downregulated for OPC maturation completion. We have previously shown that GPR17 expression is abnormally increased in the lumbar spinal cord (SC) of $SOD1^{G93A}$ mice and in SC-derived OPCs. Of note, a market available drug, montelukast (MTK)-a GPR17 antagonist-successfully restored the differentiation defects in primary OPC cultures from $SOD1^{G93A}$ mice. Here we investigated the *in-vivo* effects of the MTK treatment in $SOD1^{G93A}$ mice. Two different MTK doses were used (10 or 30 mg/kg/24h), starting at the early symptomatic phase of the disease (90 days of life). Survival probability was determined by the Kaplan Meier analysis. Behavioral tests have been performed three times per week to examine the progression of motor coordination (Rotarod, Beam balance tests); motor skills (Gait, Extension reflex tests); muscle strength (Paw-grip endurance, Grip strength meter tests). The low MTK dose neither increased survival probability nor ameliorated disease progression. Accordingly, immunohistochemical analyses in lumbar SC did not highlight MTK-induced modifications of OL differentiation, myelin integrity, neuroinflammatory readouts. On the contrary, the high MTK dose positively affected survival probability and strongly delayed weight loss in $SOD1^{G93A}$ female mice. Significant amelioration was also registered in behavioral tests. In contrast, $SOD1^{G93A}$ males were not significantly affected by MTK treatment. Our results suggest that *in-vivo* blocking GPR17 by MTK positively affects ALS progression in a gender-specific way and identify GPR17 as a novel pharmacological target.

ND31 | 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 which requires supportive ventilation. Currently there are no effective therapies available but only palliative treatments, and only few research on this regard. Recently, adeno-associated virus 9 (AAV9)-mediated gene therapy showed promising results in preclinical models. To refine this approach, we compared the efficiency of two AAV9-*IGHMBP2* vectors, carrying different promoters, by administering them intracerebroventricularly (ICV) in SMARD1 mice model (*nmd*), during the presymptomatic phase of the disease at post-natal day 1. Expression analysis demonstrated a significant increase in the IGHMBP2 protein expression level compared to *nmd* mice. Treatments resulted in an extended survival time, higher body weight and improvement of the motor behaviours. Histopathological analysis on mice muscles showed an increased innervation of the neuromuscular junctions and a recovery of fibers' diameter, in addition on spinal cords we also observed an increased number of motoneurons associated to a reduced astrocyte gliosis. To support the translatability of the therapy, we confirmed the lack of a significative alteration of the toxicity biomarkers after the treatment, especially of the hepatic enzymes, usually. These data confirmed the efficacy of local administered gene therapy with a lack of relevant toxic effects. Although further investigations are needed on the possibility to expand the therapeutic window, up to now these results provided a promising starting point for future application in clinical practice, paving the way for the development of an effective treatment for SMARD1 patients.

ND32 | Evaluation of the metabolic profile of fibroblasts carrying the L145F mutation in the superoxide dismutase 1 gene

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by the loss of upper and lower motor neurons (MNs) at the spinal or bulbar level. MNs loss determines skeletal muscle paralysis and leads to patients' death, mostly by respiratory failure, 3-5 years after symptoms onset, and no effective treatment is available. ALS is a multifactorial disease caused by both environmental and genetic factors. The majority (90-95%) of ALS forms are classified as sporadic (sALS), while about 10% of cases are associated with mutations in specific genes (familial, fALS). Among the numerous defective genes associated with ALS, SOD1 (Cu/Zn superoxide dismutase-1) is the first and the most extensively studied gene. Mutant SOD1 can adopt multiple misfolded conformations, loose the correct coordination of metal binding, decrease the structural stability and form aggregates. Thus, the characterization of common conformational alterations of ALS-associated mutant SOD1 should be particularly challenging. In our work we focused our attention on the L145F mutation, typical of Mediterranean countries, sharing peculiar clinical features. Even though SOD1 mutations usually lead to motor neuron degeneration through a gain of toxic function, it has been shown that this mutation causes a reduced SOD1 activity; moreover, it affects anti-oxidative defense. In this preliminary study of the mutation, we analyzed phenotypic peculiarities in fibroblasts derived from a 48-year-old female patient, by evaluating the impact on protein conformation, alterations of proliferation rate, oxidative stress response and bioenergetic metabolism by using Seahorse Analysis.

ND33 | Evaluation of ApoE e4 polymorphism on the acetylcholine pathway in a cholinergic neuron-like cell model.

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Alzheimer's Disease (AD) is clinically characterised by progressive memory/cognitive impairment and cerebral cortical atrophy, mostly due to alterations of synaptic functions, i.e. loss of acetylcholine (ACh) receptors and neurotransmission deterioration leading to the relative accumulation of acetylcholinesterase (AChE) at neuronal synapses. The apolipoprotein E (ApoE) e4 allele is the main genetic risk factor to develop AD. Although its involvement in the amyloid beta (Ab) clearance/accumulation in neurons is widely demonstrated, its putative association with alterations of ACh production/degradation as well as cholinergic receptors activation has so far only poorly been investigated. Thus, the current project aims to study the role of ApoE polymorphism on neuronal cholinergic pathways and particularly on ACh homeostasis considering its production/degradation together with the activation of cholinergic receptors. To this purpose, a cholinergic neuron-like cell model was developed. We demonstrated that this cell model well recapitulates the differentiation of central cholinergic neurons, expressing ApoE isoforms. To evaluate ACh homeostasis, the expression of ChAT (choline acetyltransferase)/VAChT (vesicular acetylcholine transporter)/AChE genes was assessed, along with the ChAT protein expression. The production and release of ACh were also detected. The inositol triphosphate and intracellular calcium levels are being assessed to investigate the efficacy of ACh postsynaptic receptors activation. Overall, the obtained results could highlight possible alterations of ApoE isoform-dependent ACh homeostasis, involving both the regulation of genes expression implicated in ACh production/degradation and cholinergic receptor activation. This would be a promising starting point to assess the influence of ApoE polymorphism on the cholinergic pathway, which could contribute to elucidate new pathogenic mechanisms that occur in AD, opening the way to new interventions.

ND34 | Functional and -omic analysis of a cellular model of Amyotrophic Lateral Sclerosis (ALS)-related neuronal damage

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Amyotrophic lateral sclerosis (ALS) is a lethal neurodegenerative disorder affecting upper and lower motor neurons (Hardiman et al., 2017). Multiple evidence suggests that ALS is a dying-back neuropathy characterized by axonal degeneration which occurs earlier than motor neuron loss (Fischer et al., 2004). Several ALS-related mutations affect RNA-binding proteins, including TDP-43, impairing transport of mRNAs along axons and their local translation; thus, altered RNA metabolism seems to be one of the key mechanisms underlying this disease (Butti and Patten, 2019). Mutations in the *TARDBP* gene, encoding TDP-43, represent 5% of familial ALS patients while 97% of patients, both sporadic and familial, show TDP-43 proteinopathy with nuclear delocalization of TDP-43 and its accumulation in the cytoplasm. The aim of our project is to identify differentially translated transcripts in the axon of cortical neurons overexpressing wt-TDP-43 or a mutated form (A315T), a cellular model of ALS neuronal damage, relative to control axons. Neurons were cultured in microfluidic chambers, in order to physically separate axons from cell bodies. In this model, we performed next generation sequencing of free and polysome-engaged mRNAs isolated from cell bodies and axons. Cortical neurons overexpressing hTDP-43 (wt or A315T) show disease-related features, including the presence of TDP-43 proteolytic fragments, TDP-43 cytoplasmic accumulation, increased oxidative stress and reduced exocytosis relative to control cells. By RNA-seq analysis we have identified mRNAs involved in oxidative stress response and synaptic vesicle trafficking whose axonal levels and/or translation efficiency are deregulated in ALS axons compared to controls. The results of this study will contribute to the dissection of molecular mechanisms underlying ALS and may uncover novel potential therapeutic targets.

NO05 | BRafV600E mutation in postnatal Sox2-expressing Schwann cell precursors (SCP) drives schwannoma formation

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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 1 and 2 (NF1/NF2). Recent studies suggest that NFs arise as a result of gene mutation at earlier stages in the Schwann cell precursors, SCPs, as well as in closely related cells termed boundary cap cells, that colonize nerves and dermis up to neonatal period. 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 neurocytomas. One hundred percent of Sox2CreErt BrafV600E Ptenflox/flox tamoxifen treated mice also showed a nodular lesion localized ventrally to the hindbraind, near the pons. At higher magnification we found that the lesion contained ganglionic cells entrapped by whorls of hyperproliferating cells indicating a Scarpa ganglion schwannoma. In addition, dissected spinal cord from tamoxifen injected mice also showed dorsal root ganglion (DGR) schwannomas, that stained positive for S100. Our results suggest that a population of Sox2-expressing SCPs is present in adult DRGs that can undergo cell transformation when Ras/Raf/Mapk signaling is overactivated.

NO06 | A diagnostic and monitoring circulating miRNA signature impacts glioma biology

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Gliomas are diffusely growing brain tumors and challenging cancers for diagnosis and treatment. The identification of genetic/epigenetic markers has led to an integrated diagnosis, composed of a histological diagnosis and a molecular profiling of the tumor. Among the key genetic events, the isocitrate dehydrogenase (IDH) mutation is noteworthy. Altered miRNA profiles have been observed not only in tumor tissues but also in biofluids, where they circulate in a very stable form. We recently identified a 10 serum miRNA signature differentially regulated in patients with IDH-wild type (-wt) and IDH-mutant (-mut) gliomas able to stratify gliomas according to survival and IDH mutational status high specificity and sensitivity. Among them, the combination of three out of 10 miRNAs led to an improvement of the diagnostic performance, compared to each single miRNA of the signature and it is significantly correlated both with *Overall Survival* and *Progression Free Survival* of glioma patients. Serum profiling in a validation cohort of glioma patients showed that all the three miRNAs of the signature are significant downregulated in gliomas compared to healthy controls. We also found that alteration of this restricted signature in serum reflects their changes in tumor. Moreover, higher expression of the miRNA signature and release in extracellular fraction (conditioned medium) of IDH-mut *versus* IDH-wt cells was observed, in agreement with that obtained in glioma patients. Of note, this restricted signature acts as tumor suppressor in IDH-wt cells by impacting several biological functions. Finally, we identified common targets of the miRNA signature which are highly associated with important signaling pathways. Our data highlights the potential of a restricted serum miRNA signature, obtainable with minimal invasiveness, as biomarker to aid diagnosis/prognosis, and for the early identification and monitoring of tumor with a noteworthy potential impact on glioma biology.

NO07 | Exosomes derived from CAR-T cells as novel treatment for pediatric brain tumors

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Pediatric Central Nervous System (CNS) malignant tumors are the main cause of cancer-related death in children. Although current treatments have resulted in prolonged free survival rates (60-70%), the effects of surgery, chemo/and radiotherapy in the developing brain of children may cause long-life neurologic irreversible effects, underlying the urgent need to find more specific and less toxic treatments. Chimeric Antigen Receptor T cell (CAR T) have demonstrated potent anticancer efficacy in B-cell malignances but in solid tumors their efficacy is limited. Main hurdles for a successful CAR T cell therapy for pediatric brain tumors are the lack of specific Tumor Associated Antigens (TAAs), the hostile Tumor Microenvironment (TME), the difficulty to trespass the Blood Brain Barrier (BBB) and the need to avoid inflammation and neurotoxicity. NKG2D CAR T cells target up to 8 different NKG2D ligands that are upregulated in CNS tumors and in Myeloid Derived Suppressor Cells (MDSCs) and could overcome tumor heterogeneity and hostile TME. Additionally, they have shown efficacy against pediatric CNS tumor cell lines *in vitro*. Exosomes derived from CAR-T cells (exo-CAR T) maintain the TAA recognition ability and anti-tumor properties of their parental CAR T cells while presenting some advantages: 1) their nanoscale size (30-120 nm), which can potentially facilitate trespassing of the BBB. 2) The lack of expression of inhibitory molecules such as PD1, providing enhanced resistance to the immunosuppressive TME. 3) Inability to release inflammatory cytokines and thus minimizing the risk of Cytokine Released Syndrome (CRS). Thus, our project aims to investigate the potential of exosomes derived from NKG2D CAR T cells (Exo-NKG2D CAR) as an advantageous therapeutic approach to treat pediatric CNS tumors. In this study we aim to isolate, characterize and test the anti-CNS tumor ability of Exo-NKG2D CAR both *in vitro* and in a stereotaxic mouse model.

NO08 | CITK loss leads to DNA damage accumulation impairing homologous recombination by BRCA1 mislocalization in medulloblastoma

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor. The current therapy consists in surgery, followed by irradiation of the entire neuroaxis and high dose multi-agent chemotherapy. Despite the improvement in patient survival, many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed. Citron Kinase (CITK) is validated as target for MB treatment. Its knockdown induces cytokinesis failure and apoptosis in MB cell lines and reduces tumor growth *in vivo*. Moreover, loss of CITK leads to DNA double strand breaks (DBSs) accumulation and it impairs homologous recombination (HR), one of the major pathways used to repair DNA damage. Our aim was to uncover how this occurs assessing which HR proteins are altered after CITK loss. We found that loss of CITK reduces the BRCA1 nuclear levels and BRCA1 colocalization with DNA double strand breaks sites without altering phospho-RPA recruitment in MB cell line. These data indicate that impairment in HR after CITK loss is likely due the reduced levels of the HR protein BRCA1. These results suggest that CITK is a key determinant of DNA damage response in MBs. Our future effort will be to discover how it regulates BRCA1 recruitment at DNA damage site. This will be important to propose CITK inhibition combined with irradiation or cisplatin, treatments used in clinical practice that increases DBSs load in tumor cells.

PNE05 | 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. Western blot and phalloidin staining analyses on reliable cell models show respectively an increase in the activity of Rac1 downstream effectors and F-actin density upon treatment with the peptide. Furthermore, to envision a translation to the human setting for innovative pharmacological screens, we started the generation of human iPSCs isogenic clones harboring in-del inactivating mutations in ARHGEF6 and RAC1.

PNE06 | Human umbilical cord-derived mesenchymal stem cells limit oligodendrocytes precursors cells damage in an in vitro model of encephalopathy of prematurity

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To date, 1 in 10 babies is born premature and, even if the mortality decreased by 50% in the past years, brain damage still occurs leading to motor, cognitive and neuropsychiatric disabilities. The most frequent cause of these lesions is diffuse White Matter Insult, caused by impaired oligodendrocytes maturation and development of the myelin sheath with consequent altered cortical development. Due to their fundamental role in myelination and brain development during the third trimester of gestation, Oligodendrocyte Precursor Cells (OPCs) are considered the key cellular target in preterm brain injury. The aim of this study is to validate, in a simplified in vitro model, human Umbilical Cord-Mesenchymal Stem Cells (hUC-MSCs) secretome as a strategy to limit the OPCs inflammatory damage caused by encephalopathy of prematurity. We investigated the effect of hUC-MSCs on survival, proliferation and differentiation of OPCs, using a non-contact co-culture system. OPCs were obtained from 5-day-old pups mice, selected and cultured in proliferating medium for 72h followed by 48h in differentiating medium with/without hUC-MSCs under control or inflammatory condition (medium conditioned by inflammatory microglia). MSCs benefit from being cultured in hypoxia (1%O_2), compared to normoxia (21%O_2). Therefore, hUC-MSCs were maintained in naïve and hypoxia conditions before being co-cultured with OPCs. At the end of the co-culture, OPCs were exposed to EdU or stained for MBP, OLIG2 and DAPI Ab for ICC analysis. hUC-MSCs promoted OPCs survival and differentiation both in control and under inflammatory insult, with an enhanced effect in the hypoxia-treated condition. These promising results indicate that hUC-MSCs secretome have protective effect on insulted OPCs and further experiments are required to unravel the molecular pathways that drive this rescue.

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PNE07 | Characterization of mitochondrial impairment in a mouse model of CDKL5 deficiency disorder

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CDKL5 deficiency disorder (CDD) is a severe neurodevelopmental disorder, caused by mutations in the X-linked cyclin-dependent kinase like gene (*CDKL5).* It represents one of the most common genetic cause of epilepsy in infants and it is characterized by intellectual disability and autistic features. By using Magnetic Resonance Spectroscopy, our lab revealed the presence of a metabolic dysregulation suggestive of mitochondrial dysfunction in the *Cdkl5* null mouse hippocampus. We mainly used western blots to validate and further characterize the mitochondrial defect and to investigate its onset and progression with the disease. Eventually, we evaluated whether the defect is extended to other brain regions. Based on our results we have started a pre-clinical study assessing the therapeutic potential of a pharmacological approach modulating AMPK activity, a key player of energy metabolism. Brain tissues from *Cdkl5* KO mice and WT controls were used for western blots and ATP measurement. The drug acting on AMPK was administered for 10 days (i.p., n: WT=18; KO=17) and novel object recognition (NOR) test was performed to evaluate short memory. A longitudinal study (P10, P40 and P70) highlighted that in *Cdkl5* KO pAMPK/AMPK ratio increases at P10, it stabilizes at P40, while it decreases at P70 along with ATP levels. We speculated that at P70 *Cdkl5* KO hippocampus suffers from reduced amount of ATP and it is not able to restore ATP homeostasis. Western blots revealed reduced AMPK phosphorylation also in thalamus, whereas no defect was found in cerebral cortex. Preliminary results after pharmacological treatment showed a tendency toward the recovery in the NOR test. Molecular analyses are currently evaluating whether the treatment ameliorates the mitochondrial impairment.

PNE08 | Genotoxic stress and TP53 activation in neural progenitors lead to microcephaly syndrome

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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 in 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 and neural progenitors cells derived from CIT mutated patients iPSCs, we found that CITK inactivation induces DNA damage accumulation and chromosomal instability in human and mouse neural progenitors, similar to CITK loss. Moreover, recruitment of RAD51 to DNA damage foci is compromised by CITK loss or inactivation indicating that CITK is involved in homologous recombination. In both conditions, these alterations result in activation of the TP53 pathway and induction of apoptosis. Exploiting CIT ko and CIT inactivation models, we illustrated how accumulation of DNA damage and activation of the TP53 pathway can lead to apoptosis during the neurodevelopment. This suggests that an alteration in these pathways can represent a common thread between unrelated microcephaly syndromes.

NP15 | Molecular fingerprinting of potentiated synapses via genetically encoded probes

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Memory formation is the result of functional, biochemical and physical changes within neuronal circuits exposed to incoming stimuli. Current neuroscientific research focuses on cellular engrams, defined as those neurons that are necessary and sufficient for the acquisition and recall of a given behavior. However, the richness and plasticity of synapses lead to think that synapses are actually the fundamental memory storage and engram formation unit. In this regard, dendritic spines – which host the majority of excitatory synapses in the brain – undergo extensive structural and molecular rearrangements to serve memory acquisition and maintenance. However, obtaining a complete picture of the molecular repertoire specific of a learning-activated synapse has so far been elusive. In this regard, one of the key elements in the organization and function of the post-synapse is PSD-95, a membrane protein that functions as a hub for proteins at the post-synapse. In this study, we characterized the specific PSD-95 interactome of potentiated synapses by exploiting our new method, named "*SynActive*" (SA), which uses regulatory sequences from the 5'UTR and 3'UTR of the mRNA for the immediate-early gene *Arc* to achieve activity-dependent synthesis of the desired polypeptide at dendritic spines. In this case, we used SA to control the expression of FLAG-tagged PSD-95, acting as a bait for interacting proteins. We used AAVs to deliver this construct to the hippocampus of mice that subsequently underwent contextual fear conditioning. Then, we used immunoprecipitation and mass spectrometry to isolate proteins enriched at synapses activated by learning of a new task. We have obtained a database that is guiding us towards the ultimate outcome of defining the molecular fingerprint of potentiated synapses.

NP16 | Unravelling the inhibitory activity of Botulinum Neurotoxins on the Enteric Nervous System

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Botulism is a rare, mainly foodborne, neuroparalytic syndrome caused by the ingestion of foods contaminated with Botulinum toxin (BoNT), one of the most poisonus biological substances known. The syndrome is characterized by the inhibition of neurotransmitter release, in particular that of cholinergic neurons, causing the characteristic descending flaccid paralysis and, in worst cases, death by respiratory failure. The cellular and molecular mechanism of action of BoNTs on somatic motor neurons has been largely characterized. On the other hand, despite in natural botulism the toxin is adsorbed through the intestinal wall, with constipation as one of the first symptoms, little is known about the possible action of BoNTs on the Enteric Nervous System (ENS). The ENS, also-called “second brain”, is a very complex subdivision of the autonomic nervous system with a central role in the control of enteric motility, secretion, blood flow and response to infections. Therefore, we are investigating the action of BoNTs on the great variety of neurons present in the ENS. By using immunofluorescence, we show for the first time ever the proteolytic activity of BoNTs in enteric cholinergic and non-cholinergic neurons, after gavaging animals with BoNT serotype A and B. Moreover, we identified a dose of BoNTs that leads to a significant slowdown on peristalsis with instead no systemic signs of botulism. This preliminary result opens many legit questions about the effects of BoNTs on gut physiology that are usually underestimated. For this reason, we are now investigating the possible effects on gut microbiota composition and on the enteric neuroimmune crosstalk, both fundamental for host protection against infection. By doing this, we propose to shed some new light on the interaction between the toxin and this very complex nervous network, thus considering the intestine not just a route of entry for the toxin, but also its first important site of action.

NP17 | Physiological Characterization of Layer 5 Pyramidal Neurons of the Anterior Cingulate Cortex in Inflammatory Pain

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The neural network activated by noxious stimuli and that contributes to the suffering involves several brain regions. Of primary interest is the contribution of the anterior cingulate cortex (ACC) in processing the affective component of pain. The ACC is consistently activated by noxious stimuli and it has been shown to play a role in determining the subjective experience of pain by integrating cognitive, emotional, and motivational component. Here we are testing the hypothesis that specific neuronal populations within the ACC undergo different plastic changes in response to inflammatory persistent and stimulus-evoked pain. To dissect the functional pain responsive neurons in the ACC we used a rodent inflammatory pain model, by injecting the Complete Freund's Adjuvant (CFA) in the hind paw of male mice. A group of mice injected with CFA was considered to be in persistent pain given solely by the injection. Another group of mice injected with CFA has additionally received a daily noxious stimulation via pinprick needle for a week as a model of stimulus-evoked pain. To investigate how the peripheral injury changes the general neuronal activity we performed whole cell patch clamp recording in brain slices. All CFA mice developed mechanical and thermal hyperalgesia, as measured with the electronic von Frey, the thermal plate and tail immersion test respectively, as compared to the saline mice. At the cellular level, we observed changes in intrinsic electrical properties between CFA and saline mice in both acute (d1) and persistent phase of inflammation (d7). Changes occur only in a subpopulation of the neurons that has been previously shown to project to subcortical areas (L5SC), whereas neurons projecting contralateral show no differences between groups and/or conditions. Data show that plasticity in the ACC is observed in CFA mice at d1 with a lower excitability of the L5SC pyramidal neurons; after a week of ongoing inflammation, only mice that received a daily pinprick stimulus showed increased excitability of the L5SC neurons. Intrinsic electrical properties differ in input resistance, rheobase threshold, firing rate and action potential features. Persistent and stimulus-evoked pain can influence differently the activity of the ACC. Although, the function of this plastic adaptation of pyramidal neurons in L5 is not known yet. Given that those neurons are the main output projections from the ACC, we are currently working on the identification of the downstream targets that could be affected by these changes in the excitability.

NP18 | Role of D-aspartate oxidase on brain development and in neurodevelopmental disorders

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The *D-aspartate oxidase* (*DDO*) gene encodes for the catabolizing enzyme of D-aspartate (D-Asp), a free D-amino acid that occurs in mammalian brain at high concentrations in the embryonic phase and decreases after birth. D-Asp stimulates glutamatergic NMDA and mGlu5 receptors. Previous works reported the alteration of D-Asp metabolism with neurodevelopmental disorders such as Schizophrenia and Autism Spectrum Disorder (ASD). During the second year of my PhD project, we focused our attention on the still unknown role of precocious D-Asp occurrence on brain morphology and functioning. To clarify this issue, we generated a knock-in mouse model in which *Ddo* is overexpressed starting from the zygotic stage, to remove D-Asp in prenatal and postnatal life. Next, we translated our study from mouse to human. We found a clinical case of severe intellectual disability and ASD-related symptoms associated with duplication of *DDO* gene. Biochemical analysis of the patient's serum revealed that the duplication of *DDO* gene reduced the ratio of D-Asp versus total aspartate as compared with related controls. In conclusion, the patient's neuropsychiatric profile combined with abnormalities observed in the mouse model underline a key role for D-Asp metabolism in the regulation of neurodevelopmental processes associated with early glutamatergic transmission.

NP19 | Unraveling novel players in astrocyte-mediated phagocytosis

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Elimination of unwanted and potentially harmful material is crucial for Central Nervous System development and function. Astrocytes are responsible for the clearance of dead cells, tissue debris, amyloidogenic-toxic proteins and obsolete synapses. However, the molecular machinery involved in the recognition and degradation of such material is poorly characterized. Recent studies revealed that astrocytes contribute to synaptic clearance both in health and disease. The aim of this research is to dissect novel players of synapses clearance mediated by astrocytes following an unbiased approach. To achieve this goal, we optimized an *in vitro* phagocytosis assay to evaluate the phagocytic capacity of astrocytes. In this assay, we used brain-purified neuronal terminals named synaptosomes which manifest several features of live synapses including the ability to be recognized and internalized by glial cells. To follow internalization in astrocytes, we conjugated synaptosomes with a pH-sensitive dye which start to emit bright red fluorescence within acidic organelles. Primary striatal astrocytes plated in multiple well plates where transfected with siRNA mouse library containing around 3000 hits, all potentially druggable. Fluorescence was acquired over time using high-content imaging system. We are now applying bioinformatic tools to prioritize hits for subsequent validation. Overall, this research will highlight novel proteins involved in astrocytes-mediated phagocytosis that can be easily targeted in pathologies.

NP20 | 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. Several PDE 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 from one-month-old mice. Histological analysis of isolated brains revealed a thinning of the hippocampal cortex from *ko* mice, but we did not observed 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.

NP21 | Hyperactivity of Rac1 GTPase pathway affects the development of cortical inhibitory neurons

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GTPases of the Rho family are components of signaling pathways linking extracellular signals to the control of cytoskeleton dynamics. Among these, Rac1 plays key roles during brain development, ranging from neuronal migration to neuritogenesis, synaptogenesis and plasticity. Rac1 activity is positively and negatively controlled by GEFs and GAPs respectively, but the specific role of each regulator in vivo is poorly known. The GTPase-activating protein ArhGAP15 is a specific negative regulator of Rac1, expressed during development in migrating cortical interneurons (CINs) and in a fraction of most subtypes of adult CINs. During development loss of ArhGAP15 causes reduced morphological complexity and altered directionality of the leading edge of tangentially migrating CINs. Likewise, time-lapse imaging of embryonic CINs reveals a poorly coordinated directional control also during radial migration, possibly due to a hyperexploratory behavior. In the adult ArhGAP15$^{-/-}$ cortex, the observed migration defects lead to subtle layering defects of distinct CIN subtypes, hyperexcitability of pyramidal neurons, spontaneous subclinical seizures and increased susceptibility to the proepileptic drug pilocarpine. These results indicate that ArhGAP15 imposes a fine negative regulation on Rac1 that is required for normal control of directionality during CIN migration, with consequences on their laminar distribution and inhibitory function.

NP22 | Human iPSCs-derived oligodendrocytes and astrocytes as the first Autosomal Dominant Leukodystrophy-relevant cellular models

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Autosomal Dominant Leukodystrophy (ADLD) is a rare genetic disease, characterized by autonomic dysfunction and movement disorder, and associated with white matter loss in the central nervous system (CNS). The genetic cause is the presence of three copies, instead of the two normally present, of the gene that contains the instructions to produce the lamin B1 (LMNB1) protein, which belongs to a group of structural proteins forming the nuclear membrane of the cell. Although it is well known that LMNB1 regulates nuclear mechanics and integrity, interacts with chromatin, and regulates gene expression, pathogenic mechanisms in ADLD have only initially been explored. Moreover, a therapy to treat this disease is not available at the moment. Disease-relevant human models are therefore crucial to study disease pathogenesis and to further screen for effective therapies. Based on evidence showing glial pathology in ADLD patients, we set out to generate human glia including both oligodendrocytes and astrocytes from healthy donors (CTRL) and ADLD human induced pluripotent stem cells (hiPSCs). Toward this goal, we established a differentiation protocol based on three stages: the commitment to neural progenitors, the production of gliospheres and a further maturation step into authentic oligodendrocytes and astrocytes. Preliminary observations indicate a lower gliogenic potential in the hiPSC ADLD lineages, as reveled by gliosphere production and the expression of primary pathological readouts. Thus, the developed model appears as a promising "disease-in-a-dish" platform to further reveal so far unknown dysfunctions of the diseased cells and, prospectively, aid the development of effective therapeutic strategies for this rare genetic disease.

NP23 | Virus-like particles: towards a new tool to unmask local RNA pools in neurons

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The regulation of local protein synthesis in neurons is crucial in the molecular control of synaptic plasticity. In this context, translational repression by local micro-RNAs (miRNAs) is essential to refine synaptic strength. Nevertheless, the composition of the local miRNome in living individuals has been poorly characterized so far due to the lack of appropriate tools to selectively separate the pre- and post-synaptic compartments *in vivo*. Our goal is to develop a tool to finely isolate the local pool of miRNA molecules in the dendritic compartment of living species. To this aim, HIV-derived virus-like particles (VLPs) were engineered to be targeted to dendrites and pack local miRNAs. Our results indicate that functionalized VLPs are successfully produced not only by gold-standard virus-producing cells, but also by primary mouse neurons. Confocal microscopy imaging on neurons expressing VLP constructs confirmed targeting effectiveness of particles bearing the dendritic localization signal. Total RNA purified from miRNA- seizing VLPs was significantly enriched in small RNAs as compared to control with an abundance of 20/22 nucleotide species, which is consistent with miRNA length. RT-qPCR analysis of endogenous miRNAs as well as ectopically expressed *D.melanogaster* miR125-3p confirmed a significant enrichment of these molecules in comparison with control VLPs. As our data pinpoint VLPs great potential to characterize local RNA pools, our future plans will be to sequence miRNAs isolated through this tool out of the mouse brain to achieve post-synaptic miRNomes in either physio- and pathological conditions.

NP24 | Role of group I metabotropic receptors in the synaptic alterations of the dorsal striatum in the R451C-NLGN3 mouse model of autism

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Autism Spectrum Disorders (ASD), comprise heterogeneous neurodevelopmental disorders, characterized by the early onset of communication and social interaction difficulties, restricted interests, repetitive and stereotyped behaviors. Gene sequencing studies have identified hundreds of genes potentially implicated in ASD, which converge on two main biological pathways: gene expression regulation and neuronal communication. The projection neurons of the striatum, the input nucleus of 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 ASD patients, and a correlation of the dorso-ventral anatomo-functional subdivisions of the striatum with specific domains of ASD symptoms was proposed. In support of this hypothesis, synaptic alterations in the dorsal and ventral area of the striatum were described in the R451CNlgn3 knock-in (KI) mouse model of ASD. In particular, in a previous work we described the loss of corticostriatal long-term depression (LTD) in the dorsal striatum of KI mice. LTD was partially rescued by enhancing the endocannabinoid tone or activating CB1 receptors. Here, we aimed at identifying more effective strategies to rescue corticostriatal LTD. Activation of group I metabotropic glutamate receptors (mGluIRs: mGlu1R, mGlu5R) activates downstream signaling pathways, involving production of endocannabinoids. We therefore attempted a pharmacological rescue of LTD in KI mice, by targeting mGluIRs. Both a mGluIRs agonist, 3,5-DHPG, and a selective mGluR5 positive allosteric modulator, CDPPB, were able to rescue LTD expression. By means of immunoblotting experiments, we found that mGlu5R protein expression was significantly reduced in the dorsal striatum of KI mice, suggesting a molecular basis of corticostriatal LTD impairment. With the aim of testing the impact of such a pharmacological strategy on ASD relevant behaviors, we then used different tests (3-chamber, reciprocal interaction, marble, rotarod), that revealed significant impairments in KI mice.

NP25 | Effect of 3D synthetic microscaffold Nichoid on the morphology of hippocampal neurons

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The human brain is the most complex organ in biology. This complexity is due to the number and the intricate connections of brain cells and has so far limited the development of in-vitro models for basic and applied brain research. We decided to create a new, reliable, and cost-effective in-vitro system of hippocampal neurons and astrocytes co-cultured based on the Nichoid, a 3D microscaffold microfabricated by two photon laser polymerization technology. After 21 days in culture, we morphologically characterized the 3D spatial organization of the hippocampal astrocytes and neurons within the microscaffold and we compared our observations to those made using the classical 2D co-culture system. We found that the co-cultured cells colonized the entire volume of the 3D devices. Using confocal microscopy, we observed that within this period of time the different cell types had well differentiated. This was further elaborated with the use of Drebrin and PSD-95 antibodies as markers for mature and differentiated dendritic spines. Drebrin and PDS95 labelled the majority of neurons both in the 2D as well as in the 3D co-cultures. Using scanning electron microscopy, we found that neurons in the 3D co-culture displayed a significantly larger amount of dendritic protrusions compared to neurons in the 2D co-culture. This latter observation indicates that neurons growing in a 3D environment may be more prone to connections than those co-cultured in a 2D condition. Our results show that the Nichoid can act as a 3D device that can be used to investigate structure and morphology of neurons and astrocytes in a 3D volume. In the future, this model can be used as a tool to determine the factors at the basis of different human brain diseases, by plating cells derived directly from patients. This system may potentially further be used for drug screening in various brain diseases.

NP26 | Modulation of climbing fibers intrinsic excitability can reshape the architecture of olivo-cerebellar circuit

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The structure and function of neuronal circuits can be modified by experience during the encoding of memories, or under pathological conditions. Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. These fibers are the neuronal projections of the inferior olivary nucleus, localized in the brainstem. It was suggested that CFs may undergo dramatic structural changes either after a general block of neuronal firing in the cerebellar cortex or, to a lesser extent, by increasing neuronal activity in the inferior olive and this may rely on the growth-associated protein 43 (GAP-43) that is highly expressed in these fibers. However, the extent of such plastic events, their mechanisms and their relevance to cerebellar physiology, are still largely unclear. Here we investigate how modifications of the CFs intrinsic excitability affect their own structure, and the physiology of olivo-cerebellar circuit. To do this, we chronically altered CF intrinsic excitability and tonic firing by *in vivo* knocking-down of voltage-gated sodium channels (Nav 1.1/1.2 and Nav 1.6). We analyzed CFs 3D morphology, presynaptic terminals and postsynaptic spines, as well as the functional consequences in their synaptic transmission, showing that activity-dependent structural plasticity can occur at CFs (affecting CF length and density of varicosities) and PCs (affecting spine density at the dendritic territory innervated by CFs), and that these changes can be potentially relevant for cerebellar function. This study provides the first direct evidence, to our knowledge, of an activity-dependent structural plasticity of cerebellar climbing fibers that can potentially contribute to encode cerebellar memories.

NP27 | Diurnal rhythm in chloride homeostasis in pyramidal neurons underpins variation in network excitability

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The main inhibitory synaptic currents, gated by gamma-aminobutyric acid (GABA), are mediated by Cl^--conducting channels, and are therefore sensitive to changes in the chloride electrochemical gradient. GABAergic activity dictates the neuronal firing range and timing, which in turn influences the rhythms of the brain, synaptic plasticity, and flow of information in neuronal networks. The intracellular chloride concentration ($[Cl^-]_i$) is, therefore, ideally placed to be a regulator of neuronal activity. Chloride levels have been thought to be stable in adult cortical networks, except when associated with pathological activation. Here, we used 2-photon LSSm-ClopHensor imaging, in anaesthetized young adult mice, to show that $[Cl^-]$ inside pyramidal cells exhibit a physiological diurnal rhythm, with an approximately 2-fold increase at times when mice are typically awake (midnight), relative to when they are usually asleep (midday). This change of $[Cl-]_i$ alters the stability of cortical networks, as demonstrated by a much stronger epileptogenic effect of 4-aminopyridine at midday, compared to midnight. Importantly, both $[Cl]_i$ and seizure susceptibility, in night-time experiments, were shifted in line with day-time measures, by inhibition of the cation-chloride-co-transporters NKCC1 with bumetanide. This oscillatory changes in $[Cl-]_i$ are strictly dependent on the oscillatory behaviour of the molecular machinery regulating this ion. Indeed, we found diurnal fluctuations in the expression and phosphorylation states of the cation-chloride-co-transporters, KCC2 and NKCC1. Consequently, we observed a greatly reduced chloride extrusion capacity at night during the awake period. Thus, our data demonstrate altogether that functional changes in brain excitability are modulated by diurnal fluctuation in specific proteins that finally regulate changes in $[Cl-]_i$. We speculate that the same mechanism could be at the basis of altered brain states also in pathological conditions.

NP28 | Neural stem cell-derived exosomes counteract insulin resistance-induced impairment of brain plasticity

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Overnutrition and metabolic disorders induce cognitive deficits by affecting both neural stem cell (NSCs) niche and mature neurons. In particular, type 2 diabetes-related cognitive impairment is correlated with decreased adult neurogenesis in the hippocampus due to defective proliferation, differentiation and cell survival. In physiological conditions, adult neural stem cells release extracellular vesicles (exo-NSC) contributing to intercellular communication and potentially regulating brain cell activity. Recently, numerous studies revealed the capability of exo-NSC to ameliorate brain functions and counteract cognitive decline occurring in experimental models of neurological diseases, but the underlying molecular mechanisms are still poorly understood. We investigated the effects of intranasal administration of exo-NSC on brain plasticity in a well-established experimental model of brain insulin resistance (i.e. mice fed with a high fat diet). Our data demonstrated that intranasal administration of exo-NSC was able to deliver the vesicles into the hippocampus of mice and to restore the HFD-dependent proliferation/senescence unbalance of neurogenic niche. Chronic administration of exo-NSC also prevented HFD-induced memory impairment. Interestingly, exo-NSC seem to differently modulate intracellular molecular cascades in mature neurons and NSCs. In particular, exo-NSC prevented the inhibition of BDNF/TrKB/CREB signaling in differentiated neurons, whereas they rescued IRS1/FoxOs-mediated transcription of pro-proliferative genes in NSCs. Collectively, our findings highlight the role of extracellular vesicle cargo in the regulation of brain plasticity and provide evidence of the potential therapeutic effect of these vesicles against metabolic disease-related cognitive deficits.

POSTER SESSION 3

NIM07 | Evaluation of the effect of the sample size in DTI and AMURA measures: a migraine clinical study.

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Neuroimaging group studies, such as those using diffusion MRI, usually suffer from small sample sizes. Hence, it would be desirable to employ imaging features that have a high discriminative power. Our purpose is to evaluate how traditional Diffusion Tensor Imaging (DTI) parameters compare to those obtained using a new approach called Apparent Measures Using Reduced Acquisitions (AMURA) in terms of their behavior when reducing the sample size in a group study. To that end, 50 Healthy Controls (HC), 50 Episodic Migraine (EM) and 50 Chronic Migraine (CM) patients were recruited. Brain diffusion Magnetic Resonance Imaging data were acquired using a single-shell scheme (b=1000 s/mm^2). Three DTI-based and eight AMURA-based measures were calculated. A region-based analysis was performed iteratively, reducing in five the group size for each iteration and considering 201 different subsamples in each, until no regions with statistically significant differences were found. The average value for each region from the JHU white matter atlas was calculated using the 2% and 98% percentiles and compared with an ANOVA test. *Post-hoc* two-by-two comparisons were carried out considering $p < 0.05$ for statistical significance. For two out of three comparisons carried out (EM vs. HC and CM vs. HC), AMURA-based measures showed a remarkable discriminative power even when the sample size was significantly reduced. For instance, using 20 subjects per group, AMURA-based Non-gaussianity and Q-Space Mean Square Displacement showed statistically significant differences in 12 and 6 white matter regions for EM vs. HC, respectively. However, for DTI-based measures, the number of regions with differences drastically decreased for group sizes lower than 45. Moreover, most regions identified by DTI were also recognized by AMURA, but not the other way around. In conclusion, our results indicate AMURA to be able to detect relatively subtle differences between groups with smaller sample sizes than DTI.

NIM08 | Gliomas' functional connectivity and its relevance to patients' survival

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Gliomas have recently been found to be integrated into brain circuitry via active synapses (Venkatamarani 2019). To explore the functional integration between solid tumor and healthy brain tissue in humans, we used resting-state functional magnetic resonance imaging (rs-fMRI). 54 patients with newly diagnosed and recurrent glioma were included. Lesions were manually segmented and rs-fMRI was analyzed via seed-to-voxel analysis using the solid tumor mask as seed region. A regression model between Functional Connectivity (FC) of the solid tumor with respect to the rest of the brain and overall survival (OS) was performed. Solid tumor masks of newly diagnosed gliomas (n=18) displayed significant FC with occipital cortices and bifrontal regions ($p<0.05$), overlapping with the Fronto-Parietal Control Network, Ventral Attention Network (VAN) and the Visual Network. Recurrent gliomas (n=36) showed FC with bilateral fronto-parietal regions ($p<0.05$), overlapping with Dorsal Attention Network (DAN) and VAN. In newly diagnosed high-grade gliomas (HGG) significant solid tumor FC ($p<0.001$) with healthy brain areas were found, strongly correlating with OS: (i) bilateral frontal ($r=0.96$; OS variance explained by FC, $R^2=92\%$) and (ii) right occipito-temporal regions ($r=0.90$; $R^2=82\%$). In HGG patients at recurrence, solid tumor FC with right frontal areas correlates with OS ($r=0.72$; OS variance explained by FC, $R^2=52\%$). FC patterns of multiple control regions did not show any significant relation. A regression model including FC predictors, clinical, genetic and demographic ones (age, gender) highlighted tumor-to-brain FC as the best predictor of survival, outperforming age and genetic profile (IDH-status) by a factor of 2. We found significant FC between solid tumor and healthy brain structures. Moreover, the FC profile of solid tumor predicts OS in both newly diagnosed and recurrent HGG, possibly reflecting the recently postulated integration of gliomas into brain network.

NIM09 | MRI correlates of apathy in Multiple Sclerosis: a clinical-radiological study

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MRI correlates of apathy in multiple sclerosis (MS) have not been investigated yet. We prospectively enrolled 126 MS patients (pts) to identify clinical, neuropsychological and cognitive features and biomarker of brain damage associated with apathy. Pts underwent Expanded Disability Status Scale (EDSS), Apathy Evaluation Scale (AES), Hospital Anxiety and Depression Scale (HADS-A; HADS-D), Symbol Digit Modality Test (SDMT), California Verbal Learning Test (CVLT) and Brief Visuospatial Memory test (BVMT-r) and brain 3T MRI. Total brain and grey matter (GM) volumes were obtained using Freesurfer. Diffusion Tensor Imaging and Spherical Mean Techinque metrics were extracted from GM. Binary logistic regression analysis, adjusted for age, sex, educational level, psychiatric comorbidities, EDSS, HADS-D, HADS-A and BVMT-r, were used to identify predictors of apathy. Of the 126 MS pts (mean age 40.3±11.5; female 61.9%; RRMS 74.6%; psychiatric comorbidities 11.1%) apathetic pts showed lower education level (p=0.012), higher EDSS (p=0.021), HADS-D (p<0.001), HADS-A scores (p=0.007), and worse performance at BVMT-r (p=0.029). BVMT-r score was correlated with lingual gyrus, cuneus and lateroccipital cortex volumes (p=<0.05, R^2 range 0.19-0.22) At univariate analysis lingual gyrus (p=0.002), cuneus (p=0.007), pericalcarine (p=0.009) and lateroccipital cortex (p=0.022) volumes were predictors of apathy (p<0.001, R^2 ranging 0.41-0.45). Apathetic pts showed atrophy in lateral, medial orbitofrontal and rostral middle frontal cortices (p=0.022, p=0.024, p=0.037 respectively). None of them was predictor of apathy. On the contrary, caudal middle frontal extra Mean Diffusivity (MD) (p=0.045; model p<0.000, R^2=0.45) and caudal anterior cingulate extraMD (p=0.036, model p<0.001, R^2=0.42) were predictors of apathy. Our results suggest that atrophy of the posterior areas and microstructural changes in the prefrontal regions of the brain seem to play a relevant role in explaining apathy in MS.

NIM10 | Investigating patterns of white matter disconnections in gliomas: a single-subject tractography-based approach

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Gliomas are infiltrative brain tumors originating from white matter (WM) cells often associated with poor prognosis. Having a clearer depiction of the involvement of WM pathways could be of paramount importance to predict the progression of the disease. To this end, diffusion MRI (dMRI) offers unparalleled potential for the in vivo dissection of the human brain. Tractography techniques allow the reconstruction of WM fiber pathways connecting different brain regions. Although rich in information, full tractograms are difficult to navigate in search for the impairment of WM bundles due to the pathology. To obviate this issue, inspired by past work on defining structural disconnections, we here introduce a method to conveniently visualize the alteration caused by the presence of tumor on brain tissues. 48 glioma patients were scanned at the University Hospital of Padova. Tumor core was manually segmented by an expert neuroradiologist using FLAIR, T1w (pre/post contrast medium) and T2w scans. Based on a rich dMRI protocol, we performed multi-shell multi-tissue spherical deconvolution and reconstructed the tractograms (10M streamlines) using a probabilistic, anatomically constrained algorithm. In each subject, for every streamline in the tractogram, we initially tested whether it crossed the tumoral lesion. We subsequently transformed the subset of streamlines for which this overlap was found (i.e., affected streamlines) into a visitation map, detailing in each voxel how many altered streamlines were passing through it. We label these maps direct Structural Disconnection maps (dSD), in contrast to indirect ones, which compute the visitation maps projecting the lesion mask on selected population tractography atlases. Our study supports the use of dSD maps, which use subject-specific tractograms and are thus less prone to biases due to modified brain morphology (e.g., due to mass effect) potentially altering the predictive ability of the WM disconnection information.

NI15 | Toward a new tolerogenic strategy: could dendritic cells educated through exposure to specialized pro-resolving mediators be possible therapeutic agents in neuroinflammation?

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Dendritic cells (DCs) play a crucial role in the immune system activation and in the regulation of immunological tolerance. DCs are present within the central nervous system (CNS), in the choroidal plexuses and in the perivascular spaces regulating the CNS immune surveillance and tolerance in both mouse and human. In experimental autoimmune encephalomyelitis (EAE), a pathology mediated by encephalitogenic T-cell activation induced by auto-reactive DCs, the loss of immunological tolerance is one of the main autoimmune pathological mechanisms. We propose to generate tolerogenic DCs using a novel approach whereby DCs are exposed to specialized pro-resolving mediators (SPMs) a novel class of bioactive lipids that play a fundamental role in the resolution of inflammation. SPMs act as immunoresolvents by reducing the infiltration and activation of pro-inflammatory leukocytes, such as neutrophils, monocytes/macrophages and T lymphocytes, and by shifting their immune response into an anti-inflammatory phenotype as well as promoting tissue healing and regeneration. Accordingly, we hypothesized that DCs conditioned by exposure to SPMs could acquire a tolerogenic phenotype and could reduce the activation of T cells, thus ameliorating EAE. qPCR analysis showed that differentiation of DCs induced to mature with LPS-INFγ in the presence of SPMs imparts a tolerogenic phenotype, with downregulation of pro-inflammatory markers and concomitant upregulation of tolerogenic markers. Flow cytometry analyses confirmed that SPMs induce the anti-inflammatory phenotype of mature DCs by upregulating the surface markers of tolerance, ILT3 and PD-L1, as well as other anti-inflammatory such as MerTK, CD206, and CTLA4. Moreover, LPS-INFγ-activated DCs differentiated in the presence of SPMs maintained the upregulation of those tolerogenic markers after 24h and 48h and displayed a cytokine profile commensurate with a tolerogenic phenotype. In addition, activated T cells co-cultured with DCs or stimulated with the supernatant of DCs generated in the presence of SPMs, produced low levels of pro-inflammatory cytokines INFγ and IL-17 and displayed a reduced mRNA expression of the relative transcription factors. Our preliminary data point to a novel role of SPMs in the induction of a tolerogenic phenotype for DCs.

NI16 | Modulation of neuroendocrinal and peripheral immunogical biomarkers by rehabilitation in sarcopenic subjects

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Sarcopenia is an aged-related condition characterized by loss of muscle mass and function, whose risk factors include, among others, inflammation and a complex imbalance of the neuroendocrine system. No pharmacological agents have been FDA approved for the treatment of sarcopenia, and the management of this disease is primarily focused on physical therapy for muscle strengthening and gait training. Because the crosstalk between the neuroendocrine and immune system is modulated by rehabilitation, the aim of this study was to verify the efficacy of the rehabilitation in reducing inflammation in sarcopenic patients. Sixty sarcopenic patients undergoing a specifically designed rehabilitation program, were enrolled in the study. At the time of recruitment (T0), and at the end of the end of the rehabilitation program (30-days; T1) patients underwent a comprehensive geriatric multidimensional evaluation that included lower extremity function evaluation with the Short Physical Performance Battery (SPPB), fall risk assessment evaluation (Tinetti score), and the evaluation of performance in activities of daily living (Barthel index), as well as the analysis of the plasmatic concentration of proinflammatory (IL-1β, TNFα, IL-6, IL-18) and antiinflammatory cytokines (IL-10) and the quantification in serum of neurotransmitters noradrenalin, adrenalin, dopamine, and serotonin. Rehabilitation resulted in a significant improvement of physical and cognitive conditions. This was accompanied by significantly increased concentrations of IL-10 and noradrenalin ($p=0.02$ and $p= 0.016$, respectively) that were positively correlated with the improvement in the scores of the Tinetti ($p=0.02$) and of the (SPPB) tests ($p=0.004$). IL-18 concentation was significantly reduced as well at T1 ($p = 0.008$), and this was negatively correlated with Barthel index ($p= 0.0085$) and SPPB ($p= 0.05$) test scores. Results herein show a correlation between the rehabilitation efficacy and the reduction of the inflammation, and identify the peripheral immunological and neuroendocrine biomarkers which are modulated by rehabilitation.

NI17 | Differential regulation of hematopoiesis and regulatory T-cell generation in mouse models of glioma

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Glioblastoma (GBM), the most common and malignant of all primary brain tumors in adults, is notorious for its immunosuppressive features, and for its resistance to immunotherapy. The immunogenicity of glioma is lost during tumor progression. Indeed, low-grade glioma displays abundance of infiltrating lymphocytes, while in high-grade gliomas a shift of the immune infiltrate towards a pro-tumorigenic phenotype has been observed. Tumor strategies to evade the immune system include sequestration of T cells in the bone marrow (BM) and promotion of regulatory T-cell (Treg) activity. Neuro-inflammation is known to elicit the generation of sympathetic nervous system (SNS) signals that control hematopoiesis and lymphopoiesis in BM and thymus. We propose that the progressive reduction of neuro-inflammation during brain tumor progression may alter SNS transmission to BM and thymus, impacting on generation and release of lymphocytes, finally contributing to the process of tumor immunoediting. To assess our hypothesis, we have exploited a platelet-derived growth factor-B (PDGF-B) overexpressing mouse model of glioma. These mice can either develop low-grade (LG) or high-grade (HG) tumors. We have performed a single-cell gene expression analysis of LG and HG gliomas, to define changes in the inflammatory signature of these tumors. We used flow cytometric analysis to monitor the differentiation of hematopoietic stem cells (HSC) in BM, of T-cell maturation and Treg generation in thymus and of T and B lymphocytes in BM and blood. We observed an impairment of Treg generation in thymus of HG gliomas bearing mice, associated with a reduction of T and B lymphocytes in BM and blood, and with promotion of HSC differentiation in BM. Our results clearly indicate that the maturation of immune cells in BM and thymus differs between LG and HG gliomas bearing mice. We are now assessing the possible signals which drive the regulation of immune cells that lead to promotion of LG vs HG tumors.

NI18 | Effects of MAGL inhibitor on striatal synaptic dysfunction in a mouse model of 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 rodent 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 surprising 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 to induce a less severe disease course accompanied by an improvement in motor activity evaluated by the grip strength test at the onset of the disease. Electrophysiological recordings revealed a selective 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 explored the inflammatory status of the striatum and by immunohistochemistry we observed a significant reduction of the microglia activation. 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.

NI19 | Air pollution and neuroinflammation

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Air pollution consists of a complex mixture of chemicals, particular matter (PM), organic compounds, metals, ions and elemental carbon which can harm living organisms, including humans. The several PM adverse effects may relate to its physicochemical characteristics, including mass, size, number, surface area, concentration, source and composition. Since the last decade, the central nervous system (CNS) has also been proposed to be a target organ for the detrimental effects of airborne pollutants. Emerging evidence from epidemiological, clinical, and experimental studies suggest that certain neurological diseases, such as Alzheimer's disease, may be strongly associated with ambient air pollution. Although the precise mechanisms underlying neurodegenerative diseases still remain elusive and are not fully understood, environmental pollution is believed to exert its neurotoxic function through oxidative stress, glial activation and cerebrovascular damage. The aim of our study is to explore the effects of airborne pollution on CNS elements and brain homeostasis focusing on microglia, brain cells of nonneural origin, as predominant regulators of neuroinflammation. To explore the effects of airborne pollution on microglia we treated, at different time-points, an immortalized line of murine microglia (i.e. BV2) with different concentration of a standard reference material of diesel exhaust particles (DEP), one of the main component of airborne pollutants at urban area. After the treatment, we evaluated cell viability, cell morphology and intracellular calcium waves by means of calcium imaging technique. Our preliminary data suggest that BV2 cells actively interact with DEP after 24 hours treatment. DEP seems to be also chemoattractant factor for BV2 but the exact mechanisms are still unknown. We are going to deeply investigate how DEP is able to interact with BV2 functions thus triggering neuroinflammation and neurodegeneration.

NI20 | Microglia-derived extracellular vesicles in perinatal stroke-induced neuroinflammation

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Microglia are key orchestrators of the inflammatory response to ischemic stroke (IS). However, their role is still debated, as they contribute to both detrimental and regenerative processes following IS, depending on their activation phenotypes. Perivascular microglia have been suggested to exert protective functions, by contributing to the modulation of angiogenesis, a fundamental process for stroke recovery. However, mechanisms of microglia-endothelium crosstalk following IS remain mostly uncharacterized. In this respect, extracellular vesicles (EVs) have emerged as crucial means of communication between cells of the central nervous system (CNS), and microglial EVs as key players in brain homeostasis and pathology. Here, we hypothesized that microglia-derived EVs might be involved in modulating vascular remodeling post-stroke. To reproduce microglia-activating stimuli present in the stroke-induced CNS microenvironment, we stimulated HMC-3 cells with an oxygen-glucose-deprivation and reperfusion (OGD/R) protocol or with a pro-inflammatory cytokine mix. After characterization of EVs released by activated microglia, we evaluated their effects on the migratory and tubulogenic potential of human brain microvascular cells (HBEC-5i). We showed that, depending on the priming stimulus, microglial EVs exert different biological effects, when taken up by HBECs. Indeed OGD/R activated microglia secreted EVs with potential pro-angiogenic activity, as supported by additional in vitro (matrix digestion) and in vivo assays (zebrafish), and by analysis of differentially expressed angiogenesis-related EV cargo proteins. On the other hand, cytokine-primed microglia released EVs with a potential role in HBEC activation, through up-regulation of vascular adhesion molecules, pointing to an implication in inflammation propagation. Further validation of these findings and investigation of putative activated pathways, will shed light on potential targets for IS therapy.

NI21 | Translocator Protein modulation of human microglia activity: the involvement of neurosteroids production

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Dysregulation of microglial activity is related to the development of chronic neuroinflammation and neurodegenerative diseases. The mitochondrial translocator protein (TSPO, 18 kDa) has emerged as a promising target against neuroinflammation due to its overexpression in activated glia. Several cellular processes have been proposed to underlie TSPO immunomodulatory activity, including neurosteroidogenesis induction. However, the precise role of TSPO in the modulation of reactive microglia is still unclear. We investigated the potential role of TSPO during the inflammatory response in a model of immortalized human inflamed microglia. In addition, we aimed to elucidate the mechanism underlying its activity. Our results demonstrated that TSPO stimulation by the use of a selective ligand attenuated microglia activation promoting the shift towards the restorative phenotype. Moreover, this phenomenon was abolished in the presence of an inhibitor of the neurosteroidogenesis cascade, suggesting a possible role for neurosteroids in the modulation of microglial phenotype. Therefore, we investigated the putative neurosteroidogenic activity of microglia, and our results showed that microglia express all the key members for neurosteroid production, including TSPO, StAR, and CYP11A1, which convert cholesterol into pregnenolone as the first limiting step of neurosteroids synthesis. In line with these results, pregnenolone was accumulated by human microglia cells in a time-dependent manner, and the pharmacological stimulation of TSPO drastically increased pregnenolone release. In conclusion, the results obtained in our experimental setting suggested that TSPO contributes to the preservation of microglia well-being, exerting a negative regulation on neuroinflammatory mechanisms and this activity could be attributed to the induction of neurosteroidogenesis.

ND35 | The modulation of mitochondria function as a possible mechanism for GM1 oligosaccharide-derived neuroprotection

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic (DA) neurons in the brain *substrantia nigra*. Although its etiopathogenesis is still poorly understood, the mitochondrial (mit) dysfunction was described to have a crucial role in the exacerbation of neuronal degeneration. Hence, mit targeted protective compounds capable to minimize mit dysfunction constitute hopeful therapeutic strategies for PD. Here, we describe the properties of GM1 oligosaccharide (OligoGM1), the bioactive portion of GM1 ganglioside, that by interacting with and activating the NGF TrkA receptor at cell surface triggers crucial cellular pathways responsible for mit neuroprotection. Using proteomic and biochemical approaches, we demonstrated that OligoGM1 is able to induce the mitochondriogenesis and to enhance the mit function. Wild-type Neuro2a cells treated with OligoGM1 showed an increased number of mitochondria and, at functional level, an increased expression of mit complexes, boosted ATP levels and mit respiration. Importantly, OligoGM1 treatment determined the rescue of mit activity and respiration in a Neuro2a model of mit dysfunction. On the other hand, OligoGM1 proved to efficiently counteract both *in vitro* and *in vivo* the neurotoxicity associated to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a PD-linked neurotoxin that acts by inhibiting the mit complex I. Specifically, OligoGM1 pre-treatment strongly reduced the mit ROS overproduction and P38 MAPK hyper-phosphorylation due to MPTP exposure leading to increased cell viability and neurite network in Neuro2a cells and DA neurons together with enhanced ATP levels and mit complexes expression. Collectively our data indicate that OligoGM1 is able to protect neurons possibly via mit function restoration and oxidative stress reduction, opening a new perspective for the use of OligoGM1 in diseases where these organelles are compromised, including PD.

ND36 | Phenotypic and molecular characterization of mutations in Plekhg5 gene associated with Amyotrophic Lateral Sclerosis

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Pleckstrin homology domain-containing family G member 5 (PLEKHG5) gene encodes for a Rho GEF protein mainly expressed in the nervous system and vascular endothelium. Several pathogenic variants have been reported in association with a wide spectrum of neurological disorders ranging from Motor Neurons Disease (MND), including Amyotrophic Lateral Sclerosis (ALS), to peripheral neuropathies with motor and sensory symptoms. Exploiting CRISPR/Cas technology, we generated a new point-mutant mouse model to investigate the pathological mechanisms associated with a PLEKHG5 variant identified in ALS patients. Detailed histological and behavioural analysis did not reveal the salient pathological features of MND in 1-year-old transgenic mice. However, the peripheral nerves of mutant mice displayed aberrant myelin infoldings along with increased macrophages infiltration indicative of focal axonal pathology and neuroinflammation. In parallel, we established in vitro assays based on expression of GFP-tagged PLEKHG5 variants to study the effects of several pathogenic mutations on cellular processes regulated by PLEKHG5. Consistent with recent findings implicating PLEKHG5 in the control of autophagy in motor neurons, we found that pathogenic variants affect autophagosome biogenesis. In conclusion, our novel MND mouse model based on PLEKHG5 mutation points to peripheral nerve pathology as an early disease event. Furthermore, the ongoing in vitro characterization of PLEKHG5 variants might unveil pathological mechanisms masked in complex animal models.

ND37 | Stem cells differentiation in a 3D environment for the study of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects both upper and lower motor neurons in cortex, brainstem and spinal cord, causing weakness, muscle atrophy and spasticity. Unfortunately, there are only symptomatic treatments available. An important innovation of recent years is 3D Bioprinting, which allows the creation of a 3D model for the study of interaction between cells and between cells and environment. Moreover, an important newness is the use of induced pluripotent stem cells (iPSCs). They are pluripotent stem cells derived from adult somatic cells, which can allow the creation of a more realistic pathological model advancing the field of personalized medicine. Aim of this work was the development of a protocol of 3D stem cells differentiation and their characterization. We first obtained iPSCs from peripheral blood mononuclear cells (PBMCs) of ALS patients and healthy subjects and differentiated them in neural stem cells (NSCs). NSCs were then included in Cellink Bioink and printed in 3D structures. Cells were differentiated in 3D in motor neuron progenitors (MNPs), immature MNs and at the end mature MNs. Every step was tested for cells viability and characterized by confocal microscopy and RT-qPCR. At the end we tested the electrophysiological characteristics of included Mouse Motor Neuron-Like Hybrid Cells (NSC34). We found that included NSCs maintain a good proliferation rate during the differentiation in 3D. Moreover, we confirmed the good differentiation process both by confocal microscopy and RT-qPCR, using specific markers of the different steps. Finally, we confirmed that NSC34 cells maintain their electrophysiological characteristics when printed in a 3D structure. In conclusion, we confirmed that 3D Bioprinting can be considered a good model for the study of the pathogenesis of ALS allowing the growth and proliferation of cells in a more physiological environment.

ND38 | Pathological hallmarks in brain organoids derived from sALS patients

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Amyotrophic lateral sclerosis (ALS) is considered a non-cell autonomous disorder and 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 early and late pathogenic mechanisms. Cerebral organoids are pluripotent stem cell-derived self-organizing structures that recapitulate brain development and allow in vitro generation of 3D tissues. We developed a new method for the generation of motor neuron organoids 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 sALS patients. We differentiated iPSCs into neural stem cells (NSCs). NSCs were dissociated using StemPro Accutase and a cell strainer. Then, NSCs were plated on low-attachment 6-well plate and were cultured in floating conditions using an orbital shaker. NSCs were differentiated in these conditions into motor neuron progenitors (MNPs), immature motor neurons (MNs) and finally mature MNs. We then characterized cells by phase-contrast and confocal microscopy. We found that brain organoids 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. In conclusion, brain organoids represent a promising tool for the investigation of pathogenic mechanisms of ALS. Indeed, we found typical pathological hallmarks of the pathology, such as the presence of gliosis, the smaller length of neurites and the decreased level of mature MNs.

ND39 | Effect of long-term transplantation of hMSN progenitors and enriched environment on striatal circuits reconstruction in a rat model of Huntington's Disease

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Huntington's Disease (HD) is a neurodegenerative disorder characterized by the prominent loss of striatal medium spiny neurons (MSNs). To replace the affected cells, we grafted MSN progenitors derived from H9 human embryonic stem cells (hESCs) into the striatum a preclinical model of HD [i.e adult immunodeficient rats in which the striatum was lesioned by monolateral injection of quinolinic acid (QA)]. We assessed the survival, maturation and integration of the graft as well as its impact on lesion-dependent motor alterations up to 6 months post-graft. Moreover, by exposing a cohort of QA-lesioned animals to environmental enrichment (EE), we tested whether this protocol could improve graft integration and function. We found that the transplanted progenitors survived up to 6 months post-graft, underwent maturation as striatal neurons (Ctip2, Darpp32, Enkephalin) or interneurons (CB, CR, TH), and were integrated into the host circuits. Viral vector-based tracing experiments revealed that grafted cells received connection from both cells of the graft and of the host (in striatal and extrastriatal areas). Of note, EE increased cell differentiation into the MSN phenotype and connectivity, compared to standard housing condition. Further, behavioral analyses showed that the graft improved motor performances impaired by QA lesion: transplanted animals maintained in EE show the best improvement in motor recovery. These support the therapeutic potential of human MSN progenitor grafts for the replacement of degenerated striatal neurons and suggest that generalised training protocols (such as those provided by EE) can effectively stimulate the maturation and integration of transplanted human progenitors.

ND40 | Characterization of the neuromuscular junction in iPSC-derived 2D and 3D FUS-ALS model systems

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neurons (MNs) death in the spinal cord and brain, leading the loss of skeletal muscle mass (muscle atrophy). So far it has been difficult to investigate the molecular pathways of ALS because of the lack of suitable cell model system. The use of induced pluripotent stem cells (iPSC) carrying ALS mutations introduced by gene editing represents a valuable opportunity for the study of this pathology. Such cell model system has indeed provided important improvements on the understanding of the molecular processes involved in ALS pathology. Mutations in the FUS protein have been reported in several ALS patients. It has been shown that a hallmark of ALS disease is the abnormal cytoplasmatic accumulation of FUS mutated protein. Preliminary data collected in my lab highlight an aberrant increase in axon branching and growth in MNs derived from FUS-mutated iPSCs. Moreover, an interesting aberrant crosstalk between FUS protein and the RNA-binding protein HuD, leading to an upregulation of HuD levels, has been identified. This interaction results in the consequent upregulation of some HuD targets such as the axonal proteins GAP43 and NRN1. The principal aim of my PhD project is to analyze the importance of GAP43 involvement in aberrant axon phenotypes and confirm whether this increase in neurite branching can lead to neuromuscular disruption. Notably, neuromuscular junction (NMJ) degeneration has been observed as an early pathogenic event in ALS. To recapitulate the NMJ circuit formation *in vitro* I am taking advantage from iPSCs to obtain neural-muscle systems by 2D co-cultures and 3D neuromuscular organoids. The study of NMJ impairment will be conducted by morphological and functional assays to assess the contribution of FUS mutation in ALS progression. Finally, understanding of these cellular processes represents a crucial step for to the development of more personalized therapies.

ND41 | Extracellular vesicles showed different immune phenotypes in patients with Amyotrophic Lateral Sclerosis.

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Background. Extracellular vesicles (EVs) play central role in inflammatory processes and they could be plausible targets in ALS related to immunological reaction to motor neuron loss. We have previously demonstrated that leukocyte-derived EVs are upregulated in ALS patients and they can be considered markers of disease progression. Objectives. The aim of this study was to investigate specific immunological surface markers on Large and Small EVs (LEVs and SEVs) from plasma of sporadic ALS (sALS) patients and healthy donors (CTRLs).
Methods. EVs were isolated from plasma of 50 sALS and 30 CTRLs by differential centrifugation and characterized by Nanoparticle Tracking Analysis (NTA), Atomic Force Microscopy (AFM), and COlorimetric NANoplasmonic method (CONAN). For a simultaneous identification of 37 surface protein markers in each sample, we used a multiplex bead-based flow cytometric assay (MACSPlex Exosome Kit).
Results. Endosome-specific tetraspanins (CD63 and CD9), leucocyte, platelet and endothelial cell adhesion molecules (leukocyte common antigen (CD45), platelet endothelial cell adhesion molecule (PECAM-1, CD31), human Integrin alpha 5 (CD49e), E-selectin (CD69), B-lymphocyte antigen (CD20) were more expressed in SEVs derived from CTRLs than in sALS patients ($P<0,05$). LEVs derived from CTRLs were enriched in platelet surface expression of CD62P (P-selectin) ($P<0,05$) and CD63 ($P<0.001$) compared to sALS patients.
Discussion. Endosome-specific tetraspanins decrease in both LEVs and SEVs in accordance with the autophagy-endolysosomal system dysregulation described in ALS. LEVs from sALS patients also had fewer platelet activation markers in line with the literature as suggested by the platelet variation in blood of ALS patients. These data suggest that LEVs and SEVs carry different surface markers which might discriminate their role in ALS pathogenesis.

ND42 | Presenilins γ-secretase independent functions: role of PS2 in cellular bioenergetic and lipid metabolism maintenance

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Presenilins (PSs: PS1 and PS2), widely known as the catalytic subunits of the γ-secretase complex, are responsible for the cleavage of the Amyloid Precursor Protein (APP), generating the amyloid beta peptide (Aβ), the major constituent of the senile plaques found in the brain of Alzheimer's disease (AD) patients. A growing number of cellular functions have been recently attributed to the two homologs, such as cell fate decision and AD progression. Dissecting the molecular mechanisms related to PSs-dependent functions, it is important to understand the physiological and pathological implications in the nervous system and in peripheral tissues. We used Mouse Embryonic Fibroblasts (MEFs) from wild type (PS+/+), PS1/2 double knock-out (PS-dKO), PS1KO and PS2KO embryos, and primary neurons and astrocytes obtained by crossing PS2+/- and PS1flox/flox animals; while genetic downregulation of PS1 was by lentiviral expression of the Cre recombinase. Interestingly, we found that PS1 and PS2 show differential subcellular distributions and functions. PSs exhibit distinctive specificity in APP and Notch cleveage, in the maintenance of mitochondrial function and cellular bioenergetics. Since, lipid homeostasis and extracellular vesicles (EVs) formation are essential for various physiological processes and have been associated to AD, we studed the precise contribution of PSs. We found that while the increase in EVs release is similar, the lipidomic profile is different in PS1KO vs PS2KO MEFs. Moreover, only the absence of PS2 perturbed the levels of both membrane and intracellular cholesterol and by a transcriptome analysis, we observed that the expression of the lipoprotein lipase was drastically increased. Taken together these data indicate that PS2 has a crucial role in the maintenance of lipid homeostasis, on the cholesterol machinery, on membrane fluidity and intracellular lipid droplets formation. Investigation of these events may contribute to understanding and treating AD.

ND43 | An agonist of the CXCR4 receptor accelerates the recovery from the peripheral neuroparalysis induced by Taipan snake envenomation

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Envenomation by snakes is a major neglected human disease. Hospitalization and use of animal-derived antivenom are the primary therapeutic supports currently available. There is consensus that additional, not expensive, treatments that can be delivered even long after the snakebite are needed. We recently showed that the drug dubbed NUCC-390 shortens the time of recovery from the neuroparalysis caused by traumatic or toxic degeneration of peripheral motor neurons. These syndromes are characterized by the activation of a proregenerative molecular axis, consisting of the CXCR4 receptor expressed at the damaged site in neuronal axons and by the release of its ligand CXCL12α, produced by surrounding Schwann cells. This intercellular signaling axis promotes axonal growth and functional recovery from paralysis. NUCC-390 is an agonist of CXCR4 acting similarly to CXCL12α. Here, we have tested its efficacy in a murine model of neuroparalytic envenoming by a Papuan Taipan (*Oxyuranus scutellatus*) where a degeneration of the motor axon terminals caused by the presynaptic PLA2 toxin Taipoxin, contained in the venom, occurs. Using imaging of the neuromuscular junction and electrophysiological analysis, we found that NUCC-390 administration after injection of either the purified neuroparalytic Taipoxin or the whole Taipan venom, significantly accelerates the recovery from paralysis. These results indicate that NUCC-390, which is non-toxic in mice, should be considered for trials in humans to test its efficacy in accelerating the recovery from the peripheral neuroparalysis induced by Taipans. NUCC-390 should be tested as well in the envenomation by other snakes that cause neuroparalytic syndromes in humans. NUCC-390 could become an additional treatment, common to many snake envenomings that can be delivered after the bite to reduce death by respiratory deficits and to shorten and improve functional recovery.

ND44 | Characterization of intracellular signaling leading to synaptopathy in 5xFAD mouse model across different ages.

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Alzheimer disease (AD) is the first leading cause of dementia worldwide and no treatments are available to cure or slow down the pathology. One of the key features of AD is the synaptic dysfunction, the first event in the neurodegenerative process. Identify modulators of this process is of particular significance in the treatment of AD. Several evidence in literature suggest that the c-Jun N-terminal Kinases (JNKs) have a pivotal role in AD pathogenesis. To study JNKs function in the AD synaptopathy we analyzed 4, 6, and 10 months old male and female 5xFAD mice, an established mouse model of AD, evaluating the progression of cognitive deficits with the 6-Arms-Water Maze and the synaptic dysfunction correlated to the biochemical changes at the post-synaptic element (TIF fraction). Our findings showed a clear progression of the memory deficit in transgenic (tg) mice, in the three time-points considered, coupled with increased activation of JNK signaling pathway in the total homogenate of cortex and hippocampus, the two main areas impaired in AD. In addition, the biochemical evaluation of the main biochemical markers of the excitatory synapses, *i.e*. AMPA and NMDA glutamate receptors, and scaffold proteins like PSD95, Shank3 and Drebrin in the PSD-region (TIF) reveals a general disorganization of the dendritic spines in tg vs wt mice. These alterations correlate with JNKs activation, in this subcellular compartment, that increased during time and strongly correlate with cognitive deficit. Lastly, at six and ten months of age, when mice displayed the stronger memory deficit, the JNK3 isoform levels, the most responsive isoform to stress, in the TIF fraction were sharply increased in tg vs wt animals. These results confirm JNKs as a crucial actor in the AD neurodegenerative process in 5xFAD mice and propose JNK3 as a potential target to tackle AD synaptopathy.

ND45 | Exceptionally potent human monoclonal antibodies are effective for prophylaxis and therapy of tetanus in mice

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Tetanus neurotoxin (TeNT) is the causative agent of tetanus, a life-threatening disease of vertebrates, including humans, characterized by neurogenic muscle rigidity and spasticity. Although tetanus can be prevented by a very effective vaccine, worldwide clinical practice in the emergency rooms is the administration of anti-TeNT immunoglobulins (TIG), which are used both for prophylaxis to avoid tetanus development in wounded patients or for therapy to treat patients already carrying tetanus symptoms. TIG is produced from the blood of hyperimmune individuals, either humans or horses (in developing countries). As such, it exposes patients to several possible side-effects, including infections by still-unknown pathogens as well as dangerous anaphylactic reactions. Human monoclonal antibodies (humAbs), which are emerging as superior therapeutics against several diseases, could overcome the drawbacks of TIG. Here, we screened the immortalized memory B cells pooled from the blood of immunized human donors and isolated two TeNT-specific humAbs, dubbed TT104 and TT110, that displays an unprecedented neutralization ability against TeNT neurotoxicity. We determined the epitopes recognized by TT104 and TT110 via cryo-EM and defined how they interfere with the mechanism of neuron intoxication. These analyses pinpointed two novel mechanistic aspects of TeNT activity in neurons unraveling at the same time the molecular bases of the exceptional neutralization ability of TT104 and TT110. Crucially, the combination of TT104 and TT110 displayed a prophylactic activity in mice when injected long before TeNT. Moreover, the two Fab derivatives neutralized TeNT in post-exposure experiments. In both these two paradigms of experimental tetanus, TT104 and TT110 humAbs and their Fabs derivates display an activity fully comparable to TIG. In conclusion, they meet all requirements for being considered for prophylaxis and therapy of human tetanus and are ready for clinical trials.

ND46 | Identification of the transition frequency from theta to alpha band in patients with neurodegenerative disease

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It is well known that brain activity is modulated by oscillations within different frequency bands, each of them related to specific brain states. Common practice in electroencephalography (EEG) signal processing consists in analysing the data at specific frequency bands chosen according to the interest and target of the study. It is straightforward that an optimal identification of the frequency bands is the starting point to reach good results. Many studies still rely on a standard frequency band subdivision, however a subject-specific frequency band subdivision is gaining more interest. This is of utmost importance, for instance, when dealing with data from Alzheimer's patients, since it has been demonstrated a progressive shift towards the low frequencies of the power spectrum profile with the progression of the disease.
The key point in a good subject-specific frequency band subdivision is the identification of the individual alpha peak (IAP) and the transition frequency (TF) from theta to alpha band. While the literature presents robust methods for the identification of IAP, there still is a lack of methods for the identification of the TF. The main method reported in literature requires both resting state and task EEG recordings, however this is limiting as it is not rare to only have resting state data at disposal for the analysis.
We propose a novel method for the identification of the TF, which only requires resting state EEG recordings. The key idea of the presented method is to identify two EEG channel groups, characterized by a strong presence of alpha activity in the first group and theta activity in the second. The two groups play the role of the resting state and task EEG recordings in the classical method. We validated the method on a EEG open source dataset from both Parkinson's patients and healthy subjects. The novel method that we propose is easy to implement and yet very robust, thus it can be of great interest for the neuroscientific community.

ND47 | A bidirectional relationship between Alzheimer's disease and sleep fragmentation in 5xFAD mice

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Alzheimer's disease (AD) is the most common age-related disorder characterized by loss of memory and cognitive functions. It is due to both genetic and environmental factors and it is correlated to aging. Recent studies have demonstrated that sleep disorders can be a risk factor for developing AD. Indeed, between AD and sleep disorders a bidirectional relationship exists. Actually, the acute effect of sleep disorders results in an increase in Ab concentration, besides the sleep quality in AD patients is impaired, leading to a possible further accumulation of Ab. Literature data demonstrate a correlation between AD and acute sleep deprivation. In this study, we performed and validated a mouse model of AD and sleep fragmentation, a sleep disorder that mimic more correctly a real condition of intermittent and continuous awakening. The fragmentation method lasts 30 days all day long and includes a tilting time-controlled, which it swings every 3 min for about 10 sec epochs. We analyzed the effect of sleep fragmentation in 5xFAD and wild type (wt) mice of 2 months of age, since 5xFAD mice at this age already develop senile plaques. In this study, we observed in fragmented 5xFAD and wt mice respect to their control (no-fragmented) an increase in the amount of Ab1-42 production, tau phosphorylation, and GSH/GSSG ratio. Moreover, we observed a modulation in the expression of the kinases involved both in the oxidative stress pathway and in tau phosphorylation, such as pJNK, pERK in fragmented wt and 5xFAD mice. For these reasons, we would examine in depth the modulation of tau protein in mice expressing human tau. In conclusion, since the first signs of AD appear 15-20 years after its pathogenesis begins, it's possible to say that a bad sleep quality could be a risk factor for AD, but also an aggravating of the pathogenesis and probably speeding it up. A more in-depth study could lead to the development of new preventive therapies in patients with chronic sleep disorders.

ND48 | The effect of Exosomes isolated from murine Adipose-derived Stem Cells on two Motor Neurons disorders: ALS and SMA

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The use of stem cells represents a possible treatment for neurodegenerative disorders, like Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA). In particular, their beneficial action seems to be due to the paracrine release of exosomes, main mediators of intercellular communication. Indeed, through the release of their content (proteins, miRNA and nucleic acids) they are able to promote neurogenesis, inhibit apoptosis, enhance immunomodulation in different pathophysiological contexts, recapitulating the effect of origin cells.
Despite ALS and SMA are two distinct neurodegenerative diseases caused by different pathogenic mechanisms, our aim is to investigate the protective influence of exosomes in two different *in vivo* models. We therefore isolated and characterized exosomes from murine adipose-derived stem cells (ASC-exosomes) and delivered them via intranasal administration in the SOD1(G93A) mice, the murine model of ALS, and with intracerebroventricular injections in the SMNΔ7 murine model, the most widely used one for SMA.
The results showed that ASC-exosomes could improve the motor performance of animals, both in treated SOD1(G93A) and SMNΔ7 mice. They could also protect lumbar spinal cord motor neurons from neurodegeneration and modulate the neuroinflammation in the central nervous system. Moreover, in the peripheral tissues we could observe a higher number of innervated neuromuscular junctions and an attenuated skeletal muscle atrophy in the treated SMNΔ7 group.
These outcomes could allow to better understand the effects of ASC-exosomes and to target them as a possible approach in the treatment of neurodegenerative disorders.

ND49 | A CXCR4 receptor agonist strongly stimulates the recovery of axonal connections after neuronal damage

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The activation of the G-protein coupled receptor CXCR4 by its ligand CXCL12α is involved in a variety of physiological and pathological processes, including the growth of B cell precursors, autoimmune diseases, stem cell migration, inflammation, and several neurodegenerative conditions. Recently, we have demonstrated that CXCL12α potently stimulates the functional recovery of damaged neuromuscular junctions via interaction with CXCR4. This result has prompted us to test the pro-neuroregeneration activity of small molecules acting as CXCR4 agonists, endowed with better pharmacokinetics if compared to the natural ligand. We have focused on NUCC-390. This latter has recently been shown to activate CXCR4 in in vitro models. We have designed a novel and convenient chemical synthesis method of NUCC-390, hence we have tested the molecule for 1) its ability to induce regeneration of motor axon terminals, upon administration of presynaptic neurotoxin α-Latrotoxin; or 2) boosting sciatic nerve regrowth after mechanical damage. By using imaging and electrophysiological techniques, we have provided novel and compelling evidence that CXCR4 is expressed at the injury site within the axonal compartment, whilst its ligand CXCL12α is expressed in Schwann cells, demonstrating that this molecular axis is involved in the recovery of neurotransmission of injured nerve. More importantly, the small molecule NUCC-390 is a strong promoter of the functional and anatomical recovery of the nerve, by acting very similarly to CXCL12 α. This makes NUCC-390 a potential candidate molecule to stimulate nerve repair by promoting axonal elongation. We aim at testing this molecule in other models of neuronal damage, to define a solid ground to deploy in the design of clinical trials.

ND50 | Effects of traffic-related air pollution on the development of Alzheimer's disease: the role of the olfactory pathway.

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Abstract: Ambient air pollution, a heterogenous mixture of gases and fine and ultrafine particles, has been shown to potentially cause adverse health effects, including respiratory and cardiovascular diseases. More recent investigations have also shown that ultrafine particles can deposit in the brain and increase the risk of developing neurological disorders such as Alzheimer's disease (AD). In previous investigations, we already showed that diesel engine exhaust can aggravate amyloid plaque formation and motor function impairment in a mouse AD model. However, not only the molecular and cellular mechanisms behind it are still unknown, but also if there are any biomarkers indicating air pollution's effects on the brain. In order to address these questions, we exposed female 5X Familial AD (5XFAD) mice and their female wildtype (WT) littermates for 5 h/days, 5 days/week during 2 or 4 consecutive weeks at a traffic-dominated location to concentrated ambient particles (CAPs), particle filtered air (FA) or clean air (CA). Our preliminary results revealed that traffic-related air pollution accelerates AD like pathologies in the 5xFAD mice, albeit to different extent by CAPs and FA exposures. Analysis of the olfactory bulbs of 5xFAD vs. WT mice from the respective exposure groups by cytokine array indicated that the olfactory route might be involved in these adverse effects. Potential individual biomarkers were thus chosen for further evaluation by immunohistochemical analysis, ELISA or Western Blot.

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NO09 | A nanoformulated 11-microRNA pool synergistically modulates tumor cell invasion and growth in orthotopic glioblastoma murine models

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Although small interfering RNAs are increasingly used in the clinic, the therapeutic potential of oncogene co-regulation via multiple microRNAs is still underexplored. Here, functional synergism of a pool of 11 miRNAs involved in neural stem cell differentiation and down-regulated in human glioblastoma (GBM) was investigated in patient-derived GBM cultures and in a mouse model of human GBM. Re-administration of the 11-miRNA pool affected multiple cell adhesion and stemness pathways, impairing GBM cell invasiveness in monolayer and spheroid cultures. Moreover, the 11 miRNAs downregulated a subset of proteins, involved in Collagen pathways and correlated with GBM grade/subtype, supporting the ability of the pool to modulate tumor aggressiveness. The 11-miRNA pool, encapsulated in Apolipoprotein E-coated lipid nanoparticles, was intratumorally delivered in orthotopic xenograft models of human GBM: a single administration significantly impaired tumor growth, extending mice survival by >50%. This study highlights the role of miRNAs' functional synergism in anti-cancer therapies, proposing a clinically relevant nanomedicine approach for miRNA delivery.

NO10 | Oncolytic HSV reestablish an immune antitumoral environment resulting in high grade gliomas eradication and memory against tumor cells

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Oncolytic viruses are a safe, remarkable and dynamic alternative to common chemotherapies. We evaluated the efficacy of an oncolytic HSV (oHSV), R-115, retargeted against Her2 and expressing murine IL12 in our model of high-grade murine glioma. We estimated the effect of this oHSV on gliomas with high expression (>90%) or low expression (<30%) of Her2 on tumor cells. Mice inoculated with high Her2 expressing cells showed a stronger tumor eradication if compared to mice injected with low Her2 expressing cells, underlying the deep antitumoral activity mediated by the virus capable to target, infect and lyse Her2 expressing cells. Despite dissimilarities between differently composed gliomas, R-115 was capable to prolong survival and eradicate tumors even in low expressing Her2 tumors. We also compared different schedule treatments, single or double R-115 administration, which resulted in an improved frequency of tumor eradication and prolonged survival. Moreover, virus therapy allows to establish a solid immune response in surviving mice which were capable to eradicate secondary transplanted tumors (even not expressing Her2). Finally, we evaluated the status of the immune system characterizing the rescued population. Surviving mice showed and enhanced production of IFN-γ, augmented CD8 cell division and capability to restore MHC I expression on tumor cells.
Our deeply established murine model gave us the opportunity to analyze the effect of virus therapy and immune response to high grade gliomas in immunocompetent mice. Indeed, our results underline the fundamental role of the immune system in tumor fight and eradication and the synergistic effect mediated by the oncolysis and stimulation induced by the viral infection.

NO11 | Functional and anatomical modifications of peritumoral neurons that occur during glioma progression

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In recent years, the interaction between cancer cells and tumor microenvironment has emerged as one important regulator of tumor progression. In particular, it has been proved that neuronal and glioma cells interact each other, establishing a bidirectional crosstalk in which glioma cells induce peritumoral dysfunctions that alter the normal neuronal physiology. At the same time, it has also been demonstrated that the activity of pyramidal and parvalbumin neurons have an opposite effect on glioma proliferation. Using the GL261 mouse model, we performed *in vivo* electrophysiological and two photons analyses to longitudinal assess modifications of peritumoral neurons that take place along with glioma progression. For electrophysiological studies GL261 cells were implanted in the visual cortex, while for two photons analysis they were inoculated in the somatosensory cortex of Thy1GFP mice. To measure the responsiveness and the connectivity throughout the cortical thickness of the visual cortex, we took advantage of recordings made with a laminar 16-channel electrode placed within 300 µm from the tumor burden. Glioma-bearing mice were recorded awake and both VEP (Visual Evoked Potential) and multi-units responses at different contrasts and brightness of gratings stimuli were analyzed. With respect to controls, glioma-bearing animals showed a dampening of visual responses which started from day 14 after cell transplant; interestingly, in almost 50% of recorded glioma-bearing mice was found interictal activity around 18 days after tumor inoculation. With two-photon microscopy we also identified an early deterioration of dendritic spines in glioma-bearing animals with respect to control. Elucidating the mechanisms underlying the decay of the sensory response is of paramount importance in order to shed light not only on biomarkers, that might be used for an early diagnosis of the disease, but also on novel molecules, that might be targeted with innovative approaches.

PNE09 | A strange case of fetal hydrops: the mystery of the vein of Galen.

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We are presenting a case report of a preterm neonate born at 31 weeks and 6 days of gestational age (birth weight 2300 g) by emergency c-section due to fetal hydrops, mainly characterized by abundant pleural fluid with lungs compression and mild pericardial effusion. Echocardiography showed a good biventricular function. At birth, the baby was asphyxiated (Apgar 3 and 4), then intubated and underwent external cardiac massage, treated with a dose of endotracheal surfactant in the birth room. She was then transferred to the Neonatal Intensive Care Unit in extremely severe conditions. The post-natal echocardiography showed iso-systemic lung hypertension without structural cardiopathy. The newborn was treated with invasive ventilation for 13 days and for 11 with non-invasive ventilation. The karyotype, the hemoglobin, the metabolism and the infection parameters were all normal. The brain sonography was normal up to the first month of life, when it showed multiple micro-calcifications in the posterior right frontal parenchyma. The following magnetic resonance examination showed a small ectasia of the straight sinus with an apparent aneurismatic dilation of the vein of Galen. It will be re-evaluated with angiography sequences.
The following brain sonographies evidenced with some difficulties the aneurismatic dilation of the vein of Galen but they showed several doppler velocity grams inside the same vein. Two were arterial characterized by an IR of 0.5 and one was venous. The pericallosal artery showed a systolic velocity that ascends in a steep way, and one diastolic that showed a notch and an IR of 0.9.
The echocardiography is normal.
Now the newborn is in discreet general conditions and is gaining weight.
At 9 months the Magnetic Resonance and the angio-magnetic resonance examination showed a minimal dimensional increment of the venous ampulla of Galen and the ventricular system. Nevertheless, the psycho-behavioral and motor profile of the baby was normal.

PNE10 | Functional assessment of KCNB1 variants: loss- versus gain-of-function, and related electro-clinical phenotypes

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Purpose: Pathogenic variants in the *KCNB1* gene, encoding the voltage-gated K^+ channel (Kv) a-subunit, are associated with a spectrum of phenotypes ranging from severe developmental and epileptic encephalopathies (DEE) to mild intellectual disability without epilepsy. Kv2.1 exerts an electrical role in neurons and mutations may be associated with loss of the channel voltage-dependence or loss of the ion currents conductance. We evaluated the functional effect of different pathogenic variants in *KCNB1* and correlated the results with the electro-clinical phenotype of patients.

Method: *KCNB1* pathogenic variants were identified through Next Generation Sequencing (NGS). Kv2.1 mutants were expressed in HEK293 cells and membrane currents were evaluated by patch-clamp technique. Cells were stimulated with constant pulse-potentials ranging from -80 to +120 mV, D=20 mV (n≥4 experiments for each mutation). Patients were deep-phenotyped through clinical charts collected from referring clinicians.

Results: We identified 5 *KCNB1* pathogenic variants: p.T210M; p.T804A; p.R293C; p.A406V; p.F416L (4 *de novo*, p.T804A inherited from the affected mother). In p.T804A mutant, K_V2.1 showed to stay open at >+50 mV potentials, whereas the K_V2.1 p.R293C mutant showed a different voltage-dependence and a modified reversal potential as compared to the wild type channel (p=9.35e-6). Both variants were associated with a milder phenotype (focal epilepsy with mild intellectual disability) in two and eight patients, respectively. The remaining K_V2.1 mutants showed loss of the ion current conduction. These variants were found in 48 patients with severe phenotypes (DEE).

Conclusions: *KCNB1* mutations may impact patients' phenotypes depending on the functional effect on the channel: milder phenotypes are more often associated with gain-of-function mutations whereas severe phenotypes are associated with loss-of-function mutations.

PNE11 | Development of a new drug screening system for Rett Syndrome Therapy

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Rett Syndrome (RTT) is a devastating neurodevelopmental disorder, caused by mutations in the X-linked *MeCP2* gene, primarily acting as transcriptional repressor. Although RTT proved to be reversible in mice, no cure is yet available. Animal-based drug screenings are used to evaluate drug efficacy, requiring a large number of animals and elevated costs. New drug screening approaches *in vitro* have emerged, based on neuronal morphological analysis. However, our lab demonstrated that the transcriptional rescue ensures a better neuronal functional restoration than morphological analysis. For this reason, we aim at developing a new cell-based system for *in vitro* drug screening on *Mecp2* KO neurons, based on a customized high-throughput 96x96 qRT-PCR Array. A longitudinal bulk RNASeq analysis was performed on *Mecp2* KO neurons differentiated from neuronal precursor cells (NPCs) at three different time along maturation (DIV7-14-18). Time-specific bioinformatics analysis identified the greatest number of DEGs at DIV14, related to pathways involved in RTT, suggesting to set DIV14 as the timepoint to perform the screening. Since we aim at identifying consistent RTT-neuronal transcriptional defects, we selected a first group of 96 neuronal DEGs to be validated using 96x96 Microfluidic Array Cards (Fluidigm), using either primary neurons or NPC-derived cells. Primary neurons proved to be the most stable cell culture for the screening, confirming the reproducibility of a greater number of DEGs. We then tested the effects of CX546, whose efficacy was confirmed *in vivo* in our lab, as a proof of concept for DEGs validation. The analysis corroborated that CX546 rescues the expression of half of the reproducible DEGs. All in all, our preliminary validation suggest that the transcriptional platform could be a valid drug screening system to pre-select drugs with a higher chance of success in pre-clinical studies.

PNE12 | 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, $GABA_A$, 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 aim to map 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.

PNE13 | Developmental path of local/global preference in vision and touch.

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Several studies have investigated local/global perception in vision, but less attention has been given to touch. Here we explore its developmental path and compare performance for tactile and visual stimuli. Forty-four typically developing individuals between 6 to 26 years completed a similarity judgment task on 3D printed stimuli made of local elements (squares or triangles) arranged to form a global shape (square or triangle). Stimuli were presented in groups of three: a target and two probes. One of the probes had the same global shape as the target stimulus, the other had the same local shape, and participants reported which one was more similar to the target. Responses were categorized as “global” when the chosen stimulus had the same global configuration as the target and “local” otherwise. Across trials, we varied the size and density of the local stimuli and the modality with which they were presented: tactile (presented to blindfolded participants) or visual (participants could not touch the stimuli). The results suggest a three-way interaction between age, size and modality. For stimuli made of small local elements, the proportion of global responses increased with age, in both vision and touch. The same happened for stimuli made of large local elements presented visually; however, when these were presented haptically, there was no global preference, irrespectively of age. As a consequence, local-global preferences in vision and touch became systematically more different in adults than in children. At the same time, children were far more likely than adults to display idiosyncratic differences between vision and touch (variable in sign and amplitude across individuals and conditions, yet reliable across repetitions). These results confirm that local/global preferences develop with age and so does the coordination between vision and touch, although the development of the two modalities may follow partially distinct trajectories.

NP29 | Somatosensory processing deficits and altered cortico-hippocampal connectivity in Shank3b−/− mice

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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. Patients with Phelan-McDermid syndrome (PMS), caused by mutations in the SHANK3 gene, show ASD-like symptoms associated with aberrant tactile responses and hypo-responsiveness to sensory stimuli. However, the neural underpinnings of these somatosensory abnormalities are still poorly understood. Here we investigated, in Shank3b−/− adult mice, the neural substrates of whisker-guided behaviors, a key component of rodents' interaction with the surrounding environment. To this aim, we assessed whisker-dependent behaviors in Shank3b−/− adult mice and age-matched controls, using the textured novel object recognition (tNORT), a texture-based novel object recognition test and whisker nuisance (WN) test. Shank3b−/− mice showed deficits in whisker-dependent texture discrimination in tNORT and behavioral hypo-responsiveness to repetitive whisker stimulation in WN. Notably, sensory hypo-responsiveness was accompanied by a lack of activation of the primary somatosensory cortex (S1) and hippocampus, as measured by c-fos mRNA in situ hybridization, a proxy of neuronal activity following whisker stimulation. Moreover, resting-state fMRI showed a significantly reduced S1-hippocampal connectivity in Shank3b mutant mice. Together, these findings suggest that impaired crosstalk between hippocampus and S1 might underlie Shank3b−/− hypo-reactivity to whisker-dependent cues, highlighting a potentially generalizable form of dysfunctional somatosensory processing in ASD.

NP30 | Strong regulation of vesicular release probability by tomosyn

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Presynaptic terminals release neurotransmitters in response to an actional potential (AP) through fusion of transmitter-filled vesicles with the plasma membrane. The release rate of synaptic vesicles is a key determinant for successful and reliable communication between neurons. The presynaptic protein tomosyn is known to bind to proteins that mediate vesicle fusion and, therefore, has the potential to regulate neuronal communication. To study the role of tomosyn in synaptic vesicle release, conditional double knockout (KO) neurons, lacking both isoforms of tomosyn, were generated using the Cre-lox system. Single neurons were grown on glial islands and whole-cell patch clamp recordings were performed. Depleting neurons of tomosyn leads to a strong effect on synaptic release: Neurons lacking tomosyn have more spontaneous miniature release, stronger evoked release and altered presynaptic plasticity, pointing to a higher release probability of synaptic vesicles. To test this hypothesis, neurons were subjected to hyperosmotic sucrose solutions and the responses were analyzed using a computational model for the release rate of vesicles. This approach reveals a strong increase in the vesicular fusion rate and a reduction in the energy barrier for fusion in tomosyn KO neurons, confirming that tomosyn reduces the vesicular release probability. Together, this shows that tomosyn negatively regulates synaptic vesicle fusion, which directly impacts the strength of neuronal communication.

NP31 | Diurnal chloride oscillations in pyramidal neurons affect cortical dynamics

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Intracellular chloride concentration ($[Cl]_i$) influences the action of gamma-aminobutyric acid (GABA) on neurons. Usually, this neurotransmitter elicits inhibition binding to $GABA_A$ receptors allowing chloride ions to flow inside the postsynaptic cell. However, GABA was reported to exert an excitatory action early during development and in pathology. In these cases, GABA acts as a depolarizing neurotransmitter because of a higher $[Cl]_i$ allowing an outflow of chloride ions outside the cell. We reported for the first time diurnal oscillations in intracellular chloride levels in vivo in pyramidal neurons of the primary visual cortex of rodents; $[Cl]_i$ was low at midday and increased at midnight, and brain excitability was affected accordingly with a reduced threshold towards hyperexcitability. Can these oscillations underpin the depolarizing action of GABA when $[Cl]_i$ increases and affect brain processing? We checked whether diurnal chloride oscillations drive physiological changes in the way how the nervous system responds and elaborates external stimuli. To do that, we made use of the visual cortex as a paradigmatic model. Electrophysiological recordings were acquired to evaluate the visually-driven response at day and night, the time at which $[Cl]_i$ differs the most, resulting in a different response at different daytime. To further analyse the cause of this difference, parvalbumin interneurons were optogenetically activated at different daytime; we found that a diverse inhibitory strength is exerted at day and at night, with the minimal inhibitory activity reported at night. These results matched with our previous ones reporting a change in brain excitability during daytime and shed light on a new scenario in which GABA changes its effect according to $[Cl]_i$ also in the cortex of adult mammals following diurnal oscillations. As GABA exerts plenty of physiological action, further analysis will be carried out to assess new biological questions.

NP32 | Septal cholinergic input to CA2 hippocampal region controls social memory via nicotinic receptor-mediated disinhibition

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In spite, its well-established neuromodulatory effect on attention, memory and learning, the role of acetylcholine (ACh) in social memory, meaning the capacity of an animal to discriminate between a stranger and a familiar one is virtually unknown. Medial Septum/Diagonal Band of Broca (MSDB) nuclei contain cholinergic neurons projecting to different brain areas including the hippocampal CA2 region, that is known to be involved in social memory encoding. Here, different strategies have been used to control the activity of cholinergic neurons in the MSDB to evaluate its effect on social memory and on CA2 circuit functionality. Hence, AAVs carrying floxed tetanus toxin (TeNT), inhibitory and excitatory DREADD hM4 and hM3 respectively, or channelrhodopsin (ChR2) were stereotactically delivered in the MSDB of ChAT-Cre mice. After four weeks behavioral (three-chamber test) and electrophysiological experiments were performed. ChAT-Cre mice expressing Tetanus toxin (TeNT) showed an impairment in the novelty discrimination task, an indicator of social memory. This effect was mimicked by both i.p. and hippocampal administration of CNO, activating the inhibitory hM4, and by application of selective antagonist of nicotinic AChRs. *Ex vivo* recordings from hippocampal slices, provided insight into the underlying mechanism, as activation of nAChRs by nicotine or CNO binding to hM3 increased the excitatory drive to CA2 principal cells *via* disinhibition. In line with this observation, optogenetic activation of cholinergic neurons in MSDB enhanced the firing of CA2 principal cells *in vivo*. In conclusion, our results point to nAChRs as essential players in social discrimination by controlling inhibition in the CA2 region.

NP33 | The neural substrate of serial dependence: an EEG classification study

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Serial dependence is a perceptual phenomenon that consists in observers' responses being attracted towards recent sensory history. Although the bias was described in behavior in a variety of domains and paradigms, its neurophysiological underpinnings are still unclear. Here, we recorded electroencephalographic (EEG) signal from 17 participants during a visual target discrimination task. By classifying averaged trials with support vector machines, we demonstrate that EEG activity maps contain traces of prior stimulation. Both task-relevant and task-irrelevant features of the previous stimulus are reactivated, but only after a new visual presentation, while the signal remained silent during the inter-trial interval. We illustrate that this memory trace is akin to the late perceptual representation of a visual stimulus, implying that observers might reignite a high-level engram to compare it with a new item. Remarkably, we show that serial dependence in individual observers robustly correlates with the strength of this memory trace. These findings show that the neurological substrate of serial dependence may consist in an activity-silent memory trace that is reactivated with new stimulation. Looking at evidence from our results and from previous studies on the phenomenon, we maintain that the function of this reactivation may be the construction of a perceptual continuum, known as "continuity-field" hypothesis, whereby successive stimuli are considered to belong to the same perceptual unit, which would be an ideal assumption in natural perception. In this framework, serial dependence stabilizes small drifts in the visual world by grouping series of stimuli in time, making sense of the redundancies of the evolving scene.

NP34 | Bile acids directly act on the dopamine transporter mediated currents

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Dopamine (DA) is a neurotransmitter implicated in different physiological functions and the dopamine transporter (DAT) plays a key role in regulating DA homeostasis in the brain. Bile acids (BAs) are molecules derived from cholesterol participating in dietary fat absorption. Several studies report the presence of BAs in the brain where act as regulators of neuronal function. Recently, in mice, it has been discovered that a bile diversion surgery that increases circulating BAs affects DA dynamics in the nucleus accumbens and reduces reward-related behavior induced by cocaine. Feeding obeticholic acid (OCA), an FDA-approved semi-synthetic bile acid, to mice induced the same effects of bariatric surgery. This study investigates the interactions of the OCA with DAT and mechanistically defines the regulation of DAT activity via two-electrode voltage clamp approach on *Xenopus laevis* oocytes heterologously expressing murine DAT and through molecular modeling. Electrophysiological data show that the OCA acts directly on mDAT inducing a small fast-inward Na^+ dependent current. OCA also inhibits the DAT-mediated Li^+ leak current, supporting the hypothesis of direct binding. Dose-response experiments in the presence of OCA resulted in unaltered DAT I_{max} and $K_{0.5}$. Perfusing a different bile acid, lithocholic acid (LCA), induces similar effects on mDAT. Docking simulations were performed to identify the potential binding sites of OCA to DAT. The residue D421, which is physiologically involved in coordinating the binding of the Na^+ ion, may contribute to the action of OCA on DAT. Moreover, the binding to the residues R445 and D436 may stabilize the inward-facing open state by preventing the reformation of the salt bridges required for substrate transport. These preliminary results indicate a novel and undocumented interaction between DAT (and potentially other SLC6) and BAs, raising new questions about the role of these molecules in brain function and in associated behaviors.

NP35 | Chitosan micro-grooved membranes with increased asymmetry for improving Schwann cell response in nerve regeneration

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Peripheral nerve injuries are a common condition in which a nerve is damaged, affecting more than one million people every year. Nowadays, there are still no efficient therapeutic treatments for these injuries. Artificial scaffolds can offer new opportunities for nerve regeneration applications, and in this framework, chitosan is emerging as a promising biomaterial. Here, we set up a simple and effective method for the production of micro-structured chitosan films by solvent casting, with high fidelity in micro-pattern reproducibility. Three types of chitosan directional micro-grooved patterns, presenting different levels of symmetricity, were developed for application in nerve regenerative medicine: gratings (GR), isosceles triangles (ISO), and scalene triangles (SCA). The directional patterns were tested in vitro with the RT4-D6P2T-GFP glial Schwann cell line. The most asymmetric topography (SCA), although polarized less efficiently cell shaping, promoted higher cell proliferation and a faster cell migration, both individually and collectively, with higher directional persistence of motion. Overall, the use of micro-structured asymmetrical directional topographies may be exploited to enhance the nerve regeneration process mediated by chitosan scaffolds. Moreover, these results provide important information on the use of specific topographical features for other neural tissue engineering applications and for the fabrication of novel, bio-compatible neural scaffolds.

NP36 | Endocrine disruptors and neurodevelopment: study of their effect on the glutamatergic system in primary hippocampal neurons

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Endocrine disruptors (ED) are a heterogeneous group of chemicals found in everyday products which is in the spotlight because of toxicity due to the interaction with the endocrine system. Within the nervous system (NS), hippocampus is a brain area which expresses functional hormone receptors and which is sensitive to their action: sex steroids and thyroid hormones influence dendritic outgrowth and synaptogenesis, playing a role in hippocampal development. ED exposure starts *in utero* and emerging evidence suggest that selected ED could alter synaptic plasticity inducing a remodeling of glutamatergic spines. In the hippocampus, the GluN2B/GluN2A switch of the N-methyl-D-aspartate (NMDA) receptor subunits is one of the mechanisms involved in spine formation. Aim of our study has been to evaluate ED effect on the expression and synaptic distribution of different subunits of the glutamatergic ionotropic receptors, during the development of hippocampal primary neurons. Neurons were exposed to NS active ED: Atrazine (ATZ), Ethinylestradiol (EE) and Cypermethrin (CYP). Different experimental schemes were adopted, ranging from day in vitro (DIV) 1 to 18, or from DIV 7 to 18, to depict their action in a time window relevant for the GluN2B/GluN2A switch. Selected concentrations were 0.02 to 200 nM, close to exposure conditions, and that did not alter cell viability (decreasing at 10 µM for ATZ and CYP). Neurons were analyzed by western blot for the main subunits of NMDA (GluN2A, GluN2B, GluN1) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (GluA1, GluA2) receptors in the homogenate and post-synaptic site. ATZ treatment from DIV 1 to 18 at both 0.02 and 2 nM concentrations increased the GluN2B/GluN2A ratio in total homogenate, while EE and CYP revealed no effects. These preliminary results suggest that specific ED could finely act on NMDA receptors subunits along development in the hippocampus at concentrations in a range compatible to environmental exposure.

NP37 | Dendritic processing implements spike-timing dependent plasticity (STDP) in cerebellar Golgi cells

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The existence of a precisely organized neuronal circuitry in the cerebellum is crucial for information processing and long-term synaptic plasticity induction. This latter determines persistent modifications in neuronal activity and synaptic transmission providing the basis for learning and memory. Timing in the cerebellar circuit is tightly controlled by inhibitory interneurons. Among these, Golgi cells located in the granular layer play a primary role in modulating the input to Purkinje cells. Golgi cell receive inputs on apical and basal dendrites by parallel and mossy fibers, respectively. However, the mechanisms through which these neurons integrate input patterns is still unclear. In fact, little is known about how Golgi cells process the input signals, generate plasticity and modulate the gain and timing of signals conveyed to Purkinje cells. Recently, theoretical models predicted that spike-timing dependent plasticity (STDP) at mossy fiber-Golgi cells synapse would be a pivotal mechanism of Golgi cells inhibition. Furthermore, a computational model suggested the mechanisms through which Golgi cells dendrites might integrate and process the inputs transmitted by parallel and mossy fibers, implying that dendritic processing and plasticity in Golgi cells would depend on the spike time intervals between these two inputs. This project investigated Golgi cell STDP using whole-cell patch-clamp recordings in acute mice cerebellar slices. Mossy fibers spikes either anticipated or followed those on parallel fibers with a phase difference of ±75 ms. Mechanistically, NMDA channel unblock at mossy fiber synapses on basal dendrites are the potential coincidence detectors of mossy and parallel fiber activity. This hypothesis is now under experimental testing. In summary, this work shows that dendritic processing is instrumental to STDP and opens new perspectives on the role of Golgi cells in determining learning and plasticity in the cerebellar circuit.

NP38 | Vulnerability and resilience to stress-induced anhedonic phenotype are associated to a different modulation of oxidative balance

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Stress is an important environmental risk factor for the development or exacerbation of major depression, which affect around 10% of the world population. However, the impact of stress is highly variable, with the majority of the subjects exposed to adverse situations being resilient, able to positively cope with it, and a smaller percentage being susceptible, developing psychopathologies and needing drug treatment. Although the molecular mechanisms underlying resilience and vulnerability are still elusive, their characterization is crucial to identify systems that might be the target of more efficient therapeutic strategies, able to correct the alterations observed in a vulnerable subject but also to favour the resilient response. To this aim we evaluated, at preclinical level, the involvement of oxidative balance mediators -known to be altered in psychiatric disorders- in the differential response to the chronic mild stress (CMS) model of depression. Adult male rats were exposed to 2 weeks of CMS paradigm, and the sucrose intake test was used to assess the insurgence of an anhedonic phenotype. The animals have been then divided in vulnerable, that develop the anhedonic phenotype, and resilient, that did not. 24 hours after the test, we performed the molecular analyses to evaluate the gene and protein expression of oxidative balance mediators. Our study shows that chronic stress has a significant impact on the balance between pro- and antioxidant factors in different cerebral areas implicated in psychopathologies. Interestingly, resilient animals showed a marked antioxidant response characterized by the Nrf2 pathway activation in the prefrontal cortex. Conversely, in vulnerable animals we observed a region-specific increase in oxidative stress. Our results suggest the Nrf2 antioxidant pathway as a possible target to consider in developing new pharmacological therapies for stress-related disorders, as its activation could favour a positive behavioural and molecular response to stress.

NP39 | Novel Synaptotagmin-1 mutation causes neurodevelopmental disorder and dominant gain-of-function spontaneous transmission

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Pathogenic mutations in the presynaptic fusion machinery are increasingly found to underlie neurodevelopmental disorders. In this study, we describe the functional consequences of a novel pathogenic mutation in the calcium sensor Synaptotagmin 1 ($Syt1^{P401L}$), found in an individual with neurodevelopmental disorder. Expression of the disease variant ($Syt1^{PL}$) in Syt1-*null* and WT murine neuronal cultures reveals that neurons expressing $Syt1^{PL}$ have shorter dendrites, resulting in a reduced number of synapses. Patch clamp recordings show that in addition to slowing down synaptic transmission, $Syt1^{PL}$ causes a >3-fold increase in spontaneous release rates when expressed in either Syt1-*null* or WT neurons. Further measurements show that $Syt1^{PL}$-expressing neurons have a 2.5 fold increased release rate compared to Syt1 KO neurons. Hence, the P401L mutation causes a partially dominant negative phenotype regarding dendritic growth and release synchronization, and a fully dominant gain-of-function phenotype regarding spontaneous transmission. These data expand the scope of NDD-associated Syt1-mutations, previously only found in calcium-binding regions, and provide new insights into synaptic alterations caused by pathogenic mutations in the core fusion machinery.

Keywords: Neurodevelopmental disorder, Synaptotagmin-1, Synaptic Transmission, Clamping of spontaneous release.

NP40 | Glycogen quantification 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 for glycogen breakdown and its further conversion to lactate, which is consequently 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 among fresh, paraformaldehyde-perfused and microwaved-fixed tissues. Our results reveal that microwave-fixation better preserves glycogen stores, by halting enzymatic activities and fixing the brain metabolites in vivo while maintaining anatomic integrity. Finally, we decided to compare different working protocols in microwave-fixation system to determine the most 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 need to be finely tuned.

NP41 | Assessment of behavioural outcomes after stroke in a mouse model

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Cerebral stroke is one of the major causes of disability worldwide. Spontaneous recovery of neurological function often occurs due to plastic rearrangements of perilesional spared tissue. However, recovery is highly variable in both individual patients and experimental animals, and appears to depend on many factors (lesion location and volume, topology of structural and functional connectivity damage, etc.). Great is therefore the need for a deeper understanding of the cellular plasticity mechanisms underlying neurological recovery, as well as the identification of novel biomarkers that could represent predictive signals of recovery. In this scenario, a combination of multiple biomarkers, able to document the processes of post-stroke plasticity and predict the extent of spontaneous recovery, is needed to unravel the factors important to the recovery process and tailor specific treatments for individual patients. The aim of my project is to study the longitudinal evolution of both electrophysiological and behavioural parameters after a stroke in a mouse model. I use a Middle Cerebral Artery occlusion (MCAo) model to better represent human stroke characteristics and variability. Functional measures are taken from a peri-infarct zone, i.e. the forelimb primary motor cortex (caudal forelimb area, CFA). Electrophysiological measures are carried out in Thy1-ChR2 transgenic mice that express the light-gated cationic channel ChR2 mainly in layer V corticospinal neurons. I started with the creation of a behavioural protocol comprising Rotarod rotating test, Gridwalk and Grip strength test to assess the degree of forelimb motor impairment. For the electrophysiological measures of neuronal plasticity recordings of local field potentials (LFP) are carried out from the perilesional CFA following optogenetic stimulation delivered to either the contralateral CFA or the ipsilateral RFA (rostral forelimb area, equivalent to the premotor cortex).

NP42 | Conformational anti-Arc intrabodies to decipher the role of Arc capsid formation.

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Activity-regulated cytoskeleton-associated protein (Arc) has emerged as a key regulator of neuronal plasticity and memory. Arc is induced as an immediate early gene and acts as an effector downstream of multiple neuronal signaling pathways. Loss of function studies support a role for Arc in long-term potentiation and long-term depression of synaptic transmission and homeostatic synaptic scaling. These diverse responses are mediated by distinct Arc protein-protein interaction complexes in the postsynaptic dendritic compartment and the neuronal nucleus. However, experiments with more spatial resolution are still lacking. Interestingly, recent advances highlight a structural and functional relationship between Arc and Gag polyprotein. Recombinant Arc was shown to self-assemble into spheroid particles resembling HIV capsid. The significance of a Gag domain in a plasticity molecule is uncertain and raises a plethora of biological questions. We exploited our Intracellular Antibodies Capture Technology (IACT) to select working intrabodies (idAbs) against Arc as ideal nanoscale tools to achieve Arc interference, imaging and even degradation in different subcellular compartments. Specifically, a conformer sensitive selection was designed against the NT-region of Arc (1-140), in which a 7 amino acids motif (113-119) has been demonstrated to be critical for oligomerization and capsids formation. Out of 80 initial candidates, that were all re-screened against the NT-region in which the critical motif was mutated to 7 alanins (s113-119a), we successfully identified 4 idAbs. Moreover, two of them turned to be conformational sensitive for the mutant against the wt. In conclusion, these idAbs form part of a robust toolbox for targeting Arc protein with highly spatially-restricted subcellular localization (even in spines), allowing for modulation of function at those sites and interference with specific stages in the process from oligomerization to capsids.

NP43 | Substrate's stiffness changes the behaviour of immortalized mesencephalic neuron-derived cells

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Cells undergo an enormous amount of mechanical stimuli from extracellular matrix and surrounding cells and tissues. These stimuli have been underestimated for years, but recently, several studies highlighted their importance. Indeed, many pathological conditions contribute to create peculiar environments that lead to biomechanical abnormalities, as occurs in tumors or in neurodegenerative diseases. However, how these mechanical stimuli are converted into biological signals is still largely unknown. Piezo1 is a mechanosensitive channel that acts as calcium-permeable ion channel. Its expression could be involved in the maintenance of cerebral homeostasis, making it a relevant potential pharmacological target. This project aims to outline the role of Piezo1 channel in mouse immortalized mesencephalic neuron-derived cell line (A1) by its overexpression (A1-OV) and pharmacological modulation, in different stiffness culture settings. *In vivo,* cell environment displays lower elasticity (0.2-64 kPa) as compared to the common *in vitro* culture conditions ($1x10^7$kPa). We evaluated how cells change and adapt to a different stiffness substrate, analysing viability, shape, and functional activity. A1 cell viability is stiffness-dependent, paralleling substrate stiffness. A1-OV and A1-WT cells plated in softer substrates change their shape modifying the expression of cytoskeleton components, more evident in Piezo1 overexpressing cells. Using single-cell calcium imaging, we directly evaluated Piezo1 activity. Data show that A1-OV cells and WT cells plated in a softer substrate have a higher concentration of basal calcium. Despite that, they also displayed an increased response to Yoda1, a Piezo1 agonist. These data show that neuron-like cells adapt in different stiffness settings changing their characteristics based on the features of the substrate in which they are plated. These changes could, at least partially, involve Piezo1, making it an interesting pharmacological target.