BOOK OF ABSTRACTS

Brayn

25th 26th NOVEMBER 2020

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3rd BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

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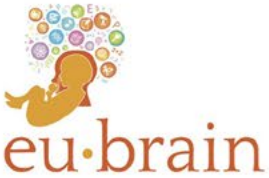

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Dear Young Neuroscientists,

We are delighted to introduce you to the 3rd Brainstorming Research Assembly for Young Neuroscientists, the BraYn conference. Inspired and organized by researchers under the age of 40 from different backgrounds and with different scientific approaches, our meeting aims establish connections between the future protagonists of neuroscience. Every day, young Neuroscientists face the difficulties of carrying out their research at several levels; our conference is intended to be a useful meeting point to maximize our scientific investigation to its full potential.

The philosophy of the conference is simple: to meet, connect, collaborate and share. Indeed we need to encourage cooperation among different research groups in order to broaden our horizons, and to contribute to the improvement in quality of research. By hosting and involving neuroscientists from abroad, our goal is to make the BraYn conference a flagship event for young European researchers. Thanks to the past editions of BraYn, the number of connections between the laboratories in Italy and Europe has been increased, thus improving the chances of potential collaborations.

Almost 600 delegates have registered to the BraYn conference 2020. They include experienced senior leaders, attending as mentors and discussants, and five invited speakers. We have scientists attending from different disciplines of neuroscience including neurodegeneration, paediatric neuroscience & perinatal neurology, neuroimmunology, neurophysiology & neural plasticity and neuro-oncology who will show the most recent advances in these fields.

We are looking forward to welcoming you to the 3rd BraYn conference!

Due to COVID-19, this year has been an extraordinarily difficult time, and we hope that all the BraYn members and their families stay as safe and healthy as possible.

The BraYn Staff

NOVEMBER 25th

15:20 Opening ceremony | **Giovanni Ferrara**

15:45 **Marta Boccazzi** (Starting Grant 2019 Winner) (*Chairmen*: C. Calì)
The immune-inflammatory response of oligodendrocytes in a murine model of preterm white matter injury: the role of TLR3 activation.

16:00 Lecture | **LUCAS SCHIRMER** (*Chairwoman*: E. Volpe)
Multilineage vulnerability and reactivity in chronic neuroinflammation.

SESSION 1 • NEUROINFLAMMATION • ORAL COMMUNICATIONS

Chairpersons: S. Angiari, M. Campolo

16:20 **Simona Schiavi**
Non-invasive quantification of inflammation, axonal and myelin injury in multiple sclerosis.

16:35 **Giulia Cisbani**
Peripheral cytokine and fatty acid associations with neuroinflammation in AD and aMCI patients: An exploratory study.

BraYn Educational Symposia

Chairpersons: P. Illiano, G. D'Arrigo

16:50 **BraYn Educational Symposium 1 • Merck Serono**
Andrea Paolillo, *Merck heritage and commitment in the new MS era: past, present and future.*

17:10 **BraYn Educational Symposium 2 • Sanofi**
Francesca Sangalli (IRCCS Ospedale San Raffaele, Milano), *Innovation through patient needs: Bruton Tyrosine Kinase Inhibitor (BTKi) development journey.*

17:30 **Adriano Lama**
Converging mechanisms of palmitoylethanolamide on metainflammation and neuroinflammation: relief of anxiety-like behavior in obese mice.

17:45 **Carolina Nunes**
Cardiac drug amiodarone accumulation and neurotoxicity in the iPSC-derived human 3D model BrainSpheres.

SESSION 2 • NEURO-ONCOLOGY • ORAL COMMUNICATIONS

Chairpersons: G. D'Alessandro, M. Tamborini

18:00 **Giulia Pericoli**
Exosome-mediated inter-clonal interactions in pediatric GBM and DIPG.

BraYn Educational Symposia

Chairwomen: M. Velasco, E. Stanzani

18:15 **BraYn Educational Symposium 3 • Beckman Coulter**
Claudia Maria Radu (Università di Padova), *Extracellular vesicles as emerging biomarkers for diagnosis and therapy.*

18:35 **BraYn Educational Symposium 4 • Fujifilm VisualSonics**
Philippe Trochet, *See the Whole Mouse Brain in vivo and in Real-time.*

18:55 **Elena Tantillo**
Characterization of glioma microenvironment: effects of glioma growth on cortical activity.

19:10 **Francesca Bufalieri**
The RNA-Binding Ubiquitin Ligase MEX3A Affects Glioblastoma Tumorigenesis by Inducing Ubiquitylation and Degradation of RIG-I.

19:25 Lecture | **MANUEL VALIENTE** (*Chairwoman*: G. D'Alessandro)
Strategies to challenge an unmet clinical need.

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NOVEMBER 26th

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Chairwomen: E. Boda, R.C. Paolicelli, G. Calabrese

9:30 **Claudia Alia**
Novel cell-based strategies to promote brain repair and motor function after stroke in mice.

9:45 **Ajesh Jacob**
"SynActive"- a genetic toolbox to study the proteome and connectome of learning and memory associated synapses.

BraYn Educational Symposia

Chairpersons: P. Lippiello, S. Paglia

10:00 **BraYn Educational Symposium 5 • Miltenyi Biotec**
Chloè Dominici (AMU, IBDM, Marseille), *3D analysis of neural network remodeling in pancreatic cancer.*

10:20 **BraYn Educational Symposium 6 • Becton Dickinson**
Daniela Carnevale (IRCCS Neuromed, Università Sapienza, Roma), *Neural regulation of immunity in cardiovascular diseases.*

10:40 **Gabriele Nardi**
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10:55 **Giulia Nato**
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11:10 Lecture | **ALESSANDRA PIERANI** (*Chairwomen*: E. Boda, R.C. Paolicelli, G. Calabrese)
Life and death of transient neurons in the maturation of functional and dysfunctional cortical circuits.

11:30 **BraYn Educational Workshop** • Vincenzo Di Ruocco (Beckman Coulter) (*Chairman*: G. Sansevero)
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15:20 **Alessandro Falconieri**
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BraYn Educational Symposia

Chairpersons: M. Di Paolo, S. Schiavi

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16:45 **Rebecca Piccarducci**
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17:00 **Samuele Negro**
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17:15 **Serenella Anzilotti**
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17:30 Lecture | **CESARE MONTECUCCO** (*Chairmen*: S. Angiari, S. Negro)
Neurodegeneration and Neuroregeneration in the Peripheral Nervous System.

18:00 Closing Remarks • BraYn Awards (Best Oral and Poster Presentation and BraYn Starting Grant)
(*Chairpersons*: E. Vannini, I. Gallo, V. Chiurchiù, N. Iraci, C. Calì.)

Neuroinflammation

Neuro-oncology

Neurophysiology & Neural Plasticity

Paediatric Neuroscience & Perinatal Neurology

Neurodegeneration

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NI17 | Maria Cristina Mariani
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NI18 | Martina Nazzaro
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NI19 | Marta Graziano
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NI20 | Giulia Magni
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NI21 | Luca Pangrazzi
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NI23 | Margherita Prosperi
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NI24 | Carolina Ricci
Tools for large specimen clearing: applying SOCRAT to the auditory system of small and large mammals.

NI25 | Riccardo Rossetti
What does the microanatomy of the choroid plexus tell us on its function?.

NI26 | Giulia Santamaria
Role of microglia in synaptic dysfunction in the Fragile X Syndrome mouse model.

NI27 | Magdalena Chustecka
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NI28 | Sarah Adriana Scuderi
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NI29 | Giulia Borgonovo
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NO07 | Gianmarco Pallavicini
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NO08 | Lucia Lisa Petrilli
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NO09 | Vinoshene Pillai
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NO10 | Valentino Ribecco
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NO11 | Giovanna Casili
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NNP26 | Sara Pepe
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NNP27 | Irene Petrizzo
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NNP28 | Livia Vignozzi
Longitudinal multifactorial evolution after stroke in mice.

NNP29 | Giulia Palla
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NNP30 | Lidia Pollara
In vitro modelling of Joubert syndrome.

NNP31 | Chiara Olmeo
Cell proliferation and amount of doublecortin-positive neurons in the dentate gyrus of different mammalian species.

NNP32 | Nelly Redolfi
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NNP33 | Danilo Bondi
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NNP34 | Gabriele Sansevero
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NNP35 | Teresa Tommasini
Can the cellular interference hypothesis find an evidence through single unit analysis?.

NNP36 | Francesca Montarolo
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NNP37 | Carlotta Baroni
Stress-induced neurobehavioral dysfunctions in obese mice: a translational model for neuroprotective nutraceutical strategies.

NNP38 | Francesca Tozzi
Associative learning and synaptic plasticity in the Lateral Entorhinal Cortex.

NNP39 | Alan Consorti
Active training promotes visual functions recovery in adult amblyopic rats.

NNP40 | Aurelia Viglione
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NNP41 | Francesca Mottarlini
Neuroadaptive changes in the nucleus accumbens following induction of activity-based anorexia in adolescent female rats.

NNP42 | Marialuisa Tognolina
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NNP43 | Enrico Pracucci
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ND27 | Sara Bosticardo
Look over the correlation between serum neurofilament light polypeptide and global structural connectivity in multiple sclerosis patients.

ND28 | Andrea Stoccoro
Decreased mitochondrial DNA methylation levels in sporadic amyotrophic lateral sclerosis patients.

ND29 | Valeria Vasciaveo
Estrogen effects on miRNA-218 and Tau alterations in Alzheimer's disease.

ND30 | Keagan Dunville
Establishment of a neurogenic niche with dentate gyrus identity from human iPSCs.

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Zerbo Effects of exosomes derived from IFNγ-primed mesenchymal stem cells on the phenotype of astrocytes cultured from late symptomatic SOD1G93A mice.

ORAL COMMUNICATIONS

The immune-inflammatory response of oligodendrocytes in a murine model of preterm white matter injury: the role of TLR3 activation

Marta Boccazzi* [1] - Juliette Van Steenwinckel [2] - Anne-Laure Schang [3] - Valérie Faivre [2] - Zsolt Csaba [2] - Claudia Verderio [4] - Marta Fumagalli [1] - Shyamala Mani [5] - Pierre Gressens [2]

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A leading cause of preterm birth is the exposure to systemic inflammation (maternal/fetal infection) which leads to neuroinflammation and permanent white matter injury (WMI). A wide range of cytokines and chemokines are expressed and upregulated in oligodendrocytes (OLs) in response to inflammation and numerous reports show that OLs express receptors for immune related molecules, which enable them to sense inflammation and to react. Here, we focus our study on toll-like receptor 3 (TLR3) that is activated by dsRNA promoting neuroinflammation and whose expression and role in OLs remain unclear.

We used an *in vivo* mouse model, which mimics inflammation-mediated WMI of preterm born infants, consisting of intraperitoneal injection of IL-1β from P1 to P5. In the IL-1β treated animals, we observed the upregulation of Tlr3, IL-1β, IFN-β, Ccl2 and Cxcl10 in both PDGFRα+ and O4+ sorted cells. This upregulation was higher in O4+ immature OLs (immOLs) as compared to PDGFRα+ OLs precursor cells (OPCs), suggesting a different sensitivity to neuroinflammation. These observations were confirmed in OL primary cultures: cells treated with TLR3 agonist Poly(I:C) during differentiation showed a stronger upregulation of Ccl2 and Cxcl10 compared to cells treated during proliferation and led to decreased expression of myelin genes. Finally, OLs were able to modulate the activation of microglia depending on their maturation state: proliferative OLs increased the expression of immunomodulatory markers whereas the differentiated cells tended to increase the expression of pro-inflammatory molecules in primary microglial cultures. These results show that during inflammation the response of OLs can play an autonomous role in blocking their own differentiation. In addition, this study suggests that the immune activation of OLs during inflammation may play an important role in shaping the response of microglia during this process.

*** Starting Grant 2019 Winner**

Non-invasive quantification of inflammation, axonal and myelin injury in multiple sclerosis

Simona Schiavi [1] - Maria Petracca [2] - Peng Sun [3] - Lazar Fleysher [4] - Sirio Cocozza [5] - Mohamed Mounir El Mendili [2] - Alessio Signori [6] - Kornelius Podranski [2] - James Babb [7] - Sheng-Kwei Song [3] - Matilde Inglese [8]

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Diffusion basis spectrum imaging (DBSI) has successfully distinguished co-existing pathological processes in central nervous system diseases. Here, we determine the feasibility of DBSI in multiple sclerosis (MS) patients and investigate the pathological substrates of tissue damage in lesions and normal appearing white matter (NAWM). 24 relapsing-remitting R- and 19 progressive P-MS patients, and 21 healthy controls (HC) were enrolled. WM lesions were classified in T1-isointense, T1-hypointense and black-holes. DBSI specific maps of fiber fraction (reflecting total content of fibers), non-restricted fraction (reflecting tissue destruction), restricted fraction (reflecting cellularity), axial diffusivity (AD, reflecting fiber injury), radial diffusivity (RD, reflecting myelination), fractional anisotropy (FA, reflecting fiber integrity) were measured from whole brain WM lesions and from both lesions and NAWM of the corpus callosum (CC). Mixed model analysis of covariance was used to compare the lesion types and patient groups in terms of the DBSI metrics with adjustment for cofactors at the subject-level (age, gender, disease duration) and lesion-level (lesion volume). An exact paired-sample Wilcoxon signed rank test was then used to compare the NAWM and lesions of patients' CC in terms of each subject-level imaging measure. Finally, the 3 subject groups were compared in terms of the DBSI metric in the CC NAWM. Significant differences were found between T1-isointense and black-holes and between CC T2-hyperintense lesions and CC NAWM for all DBSI metrics. Comparing the 3 groups in terms of DBSI metrics derived from CC NAWM, a significant difference was found between HC and R-MS for all metrics except restricted fraction and FA; between HC and P-MS for all metrics except restricted fraction and between R-MS and P-MS for FA and RD. Our findings suggest that DBSI is a promising tool to investigate MS pathophysiology, monitor disease progression and treatment response.

Peripheral cytokine and fatty acid associations with neuroinflammation in AD and aMCI patients: An exploratory study

Giulia Cisbani [1] - Alex Koppel [2] - Dunja Knezevic [3] - Ivonne Suridjan [4] - Richard Bazinet [5] - Romina Mizrahi [4]

University of Toronto, Nutritional Sciences, Toronto, Canada (1) - Unviersity of Toronto, Centre for Addiction and Mental Health, Toronto, Canada (2) - University of Toronto, Centre for Addiction and Mental Health, TOronto, Canada (3) - University of Toronto, Centre for Addiction and Mental Health, Toronto, Canada (4) - University of Toronto, Department of Nutritional Sciences, Toronto, Canada (5)

Neuroinflammation is thought to be important in the progression of Alzheimer's disease (AD). To evaluate cerebral inflammation radioligands that target TSPO, a translocator protein strongly expressed in microglia and macrophages during inflammation, can be used in conjunction with positron emission tomography (PET) imaging. In AD patients, neuroinflammation is up-regulated compared to both healthy volunteers as well as to subjects with amnestic Mild Cognitive Impairment. Peripheral biomarkers, such as serum cytokines and total fatty acids (FAs), can also be indicative of the inflammatory state of subjects with neurodegenerative disorders. To understand whether peripheral biomarkers are predictive of neuroinflammation we conducted a secondary exploratory analysis of two TSPO imaging studies conducted in subjects with AD, aMCI and aged matched healthy volunteers. We examined the association between candidate peripheral biomarkers (including amyloid beta, cytokines and serum total fatty acids) with brain TSPO levels. Our results showed that serum IL-6 and IL-10 are higher in AD compared to the aMCI and healthy volunteers while levels of some fatty acids are modulated during the disease. A limited number of associations were observed between region-specific inflammation and fatty acids in aMCI patients, and between amyloid beta 42 and brain inflammation in AD, however no associations were present with systemic cytokines. Our study suggests that while TSPO binding and systemic IL-6 and IL-10 were elevated in AD, serum amyloid beta, cytokines and fatty acids were generally not predictive of the disease nor correlated with neuroinflammation.

Converging mechanisms of palmitoylethanolamide on metainflammation and neuroinflammation: relief of anxiety-like behavior in obese mice

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Metainflammation and neuroinflammation are strictly connected as a common part of the pathogenic mechanisms of obesity and related neuropsychiatric disorders. The role of palmitoylethanolamide (PEA) in both metabolic and central activity has been recently discovered. In this study, we elucidated the effects of PEA in limiting meta- and neuroinflammation, and in blunting anxiety-like behavior and blood-brain barrier (BBB) disruption induced by a high-fat diet (HFD). Male C57Bl/6J mice were divided into 3 groups: control receiving standard diet (STD), HFD and HFD orally treated with ultramicronized PEA (um-PEA, 30 mg/kg/die). The treatment started after 12 weeks of HFD feeding and lasted 7 weeks, along with HFD. To evaluate anxiety-like behavior, we performed the open field test. Serum, hypothalamus and hippocampus were collected for biochemical evaluation of neuroinflammation, immunity and BBB integrity. Furthermore, we evaluated microgliosis, astrogliosis and albumin extravasation in different brain areas by immunohistochemical and immunofluorescence analysis. Um-PEA limited HFD-induced anxiety-like behavior, reducing the thigmotaxis of obese mice. The behavioral effects of um-PEA were associated with a rebalance of monoamines in amygdala. Moreover, um-PEA reduced inflammatory markers in both serum and brain areas (hypothalamus, hippocampus and prefrontal cortex). Furthermore, um-PEA reduced innate immune response, limiting the hippocampal expression of TLR-4, its downstream gene MyD88, and TLR-2. Indeed, in dentate gyrus and stratum radiatum of hippocampus, we confirmed um-PEA effects in limiting microgliosis and astrogliosis. These um-PEA effects were accompanied by a reduction of mast cell proteases. Notably, um-PEA improved hippocampal BBB integrity, increasing tight junction transcription and limiting albumin extravasation. The capability of um-PEA in blunting meta- and neuroinflammation confirms its therapeutic potential for obesity-related CNS disorders.

Cardiac drug amiodarone accumulation and neurotoxicity in the iPSC-derived human 3D model BrainSpheres

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Amiodarone, a Class III antiarrhythmic drug, was linked to side effects such as headache, dizziness, tremor and ataxia, suggesting neurotoxicity. Clinical effects generally require serum concentration >0.5 µM, whereas increased risk of toxicity is associated with a serum concentration >3µM.

The neurotoxicity and distribution kinetics of amiodarone were evaluated, using the iPSC-derived human 3D model BrainSpheres (BS), which comprises neurons, astrocytes and oligodendrocytes and shows spontaneous electrophysiological activity. BS were exposed to amiodarone for 48h or repeatedly between week 6 and 7 *in vitro*. Samples were collected immediately at the end of each exposure scenario and after one week of washout period. Cytotoxicity was evaluated by MTT assay in BS exposed to concentrations ranging from 0.625 to 20µM of amiodarone. IC50 was found at 2.4µM immediately at the end of the repeated exposure, and 2.3µM, after the washout period. At concentrations under IC50, qPCR analysis showed effect on neuronal and astrocytic markers. Effects on the expression of markers for *de novo* lipogenesis and formation of lipid droplets were detected. TempOSeq® analysis also revealed effects of amiodarone in several Gene Ontology biological processes linked to lipid metabolism.

For further *in vitro* to *in vivo* extrapolation of our neurotoxicity data, the *in vitro* distribution kinetics of amiodarone was evaluated. BS were exposed to 1, 2 and 3µM of amiodarone. Chemical extracts from medium, cell and well plate plastic were collected after 1, 3, 6, 24, 48h of exposure, 1 week of repeated exposure and after one week of washout period. Amiodarone levels were quantified by HPLC-UV/fluorescence. Results showing a dose- and time-dependent intracellular accumulation of amiodarone will be used for *in silico* modeling. Taken together, these results confirm the neurotoxic potential of amiodarone.

Exosome-mediated inter-clonal interactions in pediatric GBM and DIPG

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Pediatric Glioblastoma (pGBM) and Diffuse Intrinsic Pontine Glioma (DIPG) are highly heterogeneous brain tumours, characerised by distinct sub-clones interacting in a functional network. Exosomes mediate the crosstalk between tumour and its microenvironment. Therefore, we aimed to investigate the role of exosomes in mediating pGBM and DIPG inter-clonal communication.
By using *Multifluorescence Marking Technology* for single cell-tracking, we first generated two bulk multicolour patient derived-cell lines (DIPG H3.3K27M and pGBM histone WT) from which we obtained two and five single cell-derived clones, respectively. The sub-clones demonstrated significantly phenotypic differences in terms of morphology, growth, adhesion, migration, and invasion properties. When in co-culture, the single cell-derived clones displayed higher speed and greater covered distance, compared to clones cultured individually, suggesting that the cell-cell interaction is key in driving their more aggressive phenotype. Furthermore, we successfully isolated and characterised exosomes from pGBM and DIPG sub-clones and demonstrated that exosomes are actively and differentially internalised by individual clones.
Analysis of circulating microRNAs showed that despite an overall similar profile, interestingly a pool of exclusive exosomal-miRNAs were identified in distinct single cell-derived clones. Three microRNAs from the clone 2B4 (DIPG cell-line) were strongly associated to cell cycle regulation and Wnt pathway, while two microRNAs from clone 5E2 (pGBM cell-line) were associated with the regulation of migration, invasion, and cell cycle.
Our study provides novel insights into the active inter-clonal communication in pGBM and DIPG and identify exosomes as potential key mediators of such cross-talk.

Characterization of glioma microenvironment: effects of glioma growth on cortical activity

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Gliomas grow in a neuronal environment, but the interactions between glioma cells and peritumoral neurons remain poorly understood. Many evidences have shown that glioma cells cause changes in neural peritumoral tissue inducing, in some cases, the insurgence of comorbidity phenomena such as epileptic seizures. Although many studies are now focusing on the effects of glioma on neural networks, little is known about the molecular alterations caused by tumor progression that occur in peritumoral neurons.
Here, we have monitored longitudinal changes in network activity, recording visual evoked potentials (VEP) and local field potentials (LFP) after transplant of GL261 glioma cells (or PBS) in mouse visual cortex. Thanks to this analysis, we detected a progressive deterioration of VEP amplitudes along with tumor progression together with changes in the LFP power spectra typical of focal epilepsy, with a specific increase of the power of delta band and the deterioration of alpha rhythm. To understand the molecular alterations that underlie these perturbed patterns of neuronal activity, we analysed the gene expression profile of microdissected peritumoral pyramidal neurons in cortical superficial layers (i.e., II-III). The data were clear in indicating that glioma induces alterations in both pre- and post-synaptic markers, demonstrating that its progression shapes the network activity of peritumoral areas towards hyperexcitability. Indeed, we recorded the occurrence of seizures in a subset of glioma-bearing animals, finding alterations in the LFP power spectra just before the onset of ictal events. Understanding this complex relationship would add useful information to develop more effective therapeutic approaches for the treatment of this terrible disease.

The RNA-Binding Ubiquitin Ligase MEX3A Affects Glioblastoma Tumorigenesis by Inducing Ubiquitylation and Degradation of RIG-I

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Glioblastoma multiforme (GB) is the most malignant primary brain tumor in humans, with an overall survival of approximatively 15 months. The molecular heterogeneity of GB, as well as its rapid progression, invasiveness and the occurrence of drug-resistant cancer stem cells, limits the efficacy of the current treatments. In order to develop an innovative therapeutic strategy, it is mandatory to identify and characterize new molecular players responsible for the GB malignant phenotype. In this study, the RNA-binding ubiquitin ligase MEX3A was selected from a gene expression analysis performed on publicly available datasets, to assess its biological and still-unknown activity in GB tumorigenesis. We find that MEX3A is strongly up-regulated in GB specimens, and this is associated with very low protein levels of the Retinoic acid-inducible gene I (RIG-I), a tumor suppressor involved in the activation of innate immune response and in the induction of cell growth arrest via apoptosis. We demonstrate that MEX3A binds RIG-I and promotes its ubiquitylation and proteasome-dependent degradation. Further, the genetic depletion of MEX3A leads to an increase of RIG-I protein levels and results in the suppression of GB cell growth both *in vitro* and *in vivo*. Our findings unveil a novel molecular mechanism involved in GB tumorigenesis and suggest MEX3A and RIG-I as promising therapeutic targets in GB.

Novel cell-based strategies to promote brain repair and motor function after stroke in mice

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Brain injuries causing chronic sensory or motor deficit, such as stroke, are among the leading causes of disability worldwide. Cell-based approaches have emerged as an intriguing and promising strategy to promote brain repair.

Recently, we developed protocols to obtain cortical or hippocampal neurons derived from embryonic stem cells (ESCs). These two types of cells showed different degrees of axonal outgrowth and targeted different regions when co-transplanted *in vivo*. In hippocampus, only precursor cells with hippocampal molecular identity were able to extend projections towards CA3 neurons. Conversely, cortical-like cells were capable of extending long-range axonal projections only when transplanted in motor cortex. A cortical stroke greatly enhanced the capability of cortical-like cells to extend far-reaching projections. Our results indicate that neural precursors generated by ESCs carry intrinsic signals specifying axonal extension in different environments.

As second approach to promote neural repair, we exploited direct reprogramming of endogenous reactive astrocytes into neurons, with the major advantages of obtaining neurons with the correct positional identity and immunogenic profile. To this aim, we forced the expression of pro-neural transcription factors through flexed AAVs injection in GFAP-Cre transgenic mice. Two months later, we observed successful reprogramming in the perilesional tissue. Moreover, motor tests were used to evaluate the therapeutic effect of this approach in promoting motor function after stroke, alone and in combination with motor rehabilitation. Currently, electrophysiological experiments are used to shed light on the effective integration of newborn cells in the host damaged circuitry, assessing their role in achieving post-stroke motor recovery.

Our results are important to move the field forward and to bridge the gap between pre-clinical studies and clinical developing of new combined therapeutic strategies for stroke patients.

"SynActive"- a genetic toolbox to study the proteome and connectome of learning and memory associated synapses

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Brain circuits store and encode memories by experience-dependent recruitment of neuronal ensembles, termed cellular "engrams". The formation of engrams relies on long-lasting modifications in synaptic strength, such as the insertion of new neurotransmitter receptor proteins and local, activity-dependent translation of mRNAs. However, methods to identify and manipulate individual synapses undergoing structural plasticity are still imperfect. Recently, we developed "SynActive" (SA), a strategy based on regulatory sequences taken from Arc mRNA, to label and map hippocampal potentiated synapses. Here, we have extended the SA toolbox for two main purposes. First, to identify potentiation-specific molecular changes, we expressed FLAG-tagged PSD95 under SA control. AAVs were delivered to the mouse hippocampus and the PSD95-interactome was immunoprecipitated from potentiated synapses after contextual fear conditioning. Constitutively expressed PSD95-FLAG was used as a control. Mass spectrometry and comparative bioinformatics analyses allowed us to pinpoint the molecular fingerprint of a potentiated spine. Second, to map the topography of potentiated synapses in a circuit-specific fashion, we combined SA with GFP Reconstitution Across Synaptic Partners (SA-GRASP). In SA-GRASP, one split-GFP was expressed constitutively from the pre-synaptic neuron and the other from the postsynaptic potentiated spines, under activity-dependent SA control. Potentiated synapses between these two separate neuronal populations were labeled by GFP reconstitution. In primary hippocampal neurons, SA-GRASP was expressed in response to chemical LTP induction and correlated with higher AMPAR immunoreactivity at single dendritic spines. Our data provide the proof of concept of a new approach to characterize and map experience-dependent plasticity, allowing the shift of engram study from the whole cell to the single synapse scale in both physiological and pathological, (e.g. neurodegeneration) contexts.

Modelling focal cortical dysplasia by driving PTEN gene deletion with a novel Cre-amplifying reporter

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Somatic de novo mutations in neuronal cells are emerging to be associated with neurodevelopmental diseases, autism spectrum disorders and epilepsy, but modelling mosaics of healthy and knock-out (KO) cells *in vivo* is still a challenging task. Recently we described Beatrix (Trovato et al, NatComm 2020), a general purpose Cre-amplifier embedded in a double-fluorophore Cre-switch-based reporter. Beatrix can amplify and preserve weak or transient Cre-events with a tenfold increase in sensitivity compared to canonical reporting strategies. Starting from a population of cells with a floxed gene, it allows to generate highly reliable and finely tuneable mosaics of wild type and KO cells that can be easily discriminated by conventional fluorescence microscopy. After tool validation, we exploited Beatrix to investigate the physiological role of PTEN, a constitutive inhibitor of the mTOR pathway, during brain development. PTEN loss-of-function characterizes focal cortical dysplasia, a neurodevelopmental disorder of the mTor-pathies family, which causes abnormal neuronal growth and cortical delamination. We created mosaics of deletion of PTEN by transfecting a $PTEN^{flox/flox}$ mouse line with Beatrix during development, either via *in utero* electroporation in the cerebral cortex or via postnatal electroporation in the subventricular zone. With 1- and 2-photon imaging we confirmed that PTEN KO neurons show: increased soma and axon size, increased spine density and impaired migration in cortical layer 2/3. Local field potential recordings from the mosaic area of anesthetized mice often showed epileptiform activity. Moreover, we demonstrate for the first time that the loss of PTEN alters slow wave oscillations both in the mosaic area and in the contralateral untreated hemisphere. Eventually, Beatrix gives the green light to investigate the activity of healthy and mutated cells at the same time, *in vivo*, at single-neuron resolution with functional imaging and electrophysiology.

Astrocyte-generated neurons functionally integrate into the lesioned striatum

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After excitotoxic lesion, subsets of striatal astrocytes undergo a spontaneous neurogenic activation leading to the local generation of a large amount of neuroblasts for at least six months post-lesion. Yet, the identity of the lesion induced neurons and their functional integration remain unclear. Fate mapping and 3D reconstruction analyses show that striatal neuroblasts undergo a maturation process in which initially they organize in clusters, subsequently disperse as individual cells, and gradually attain complex morphologies often showing dendritic spines. These neurons fail to express typical markers of striatal neurons and live transiently, similar to other models of physiological and pathological striatal neurogenesis. Surprisingly, rabies virus-based monosynaptic tracing indicated that despite their transient life, striatal neuroblasts receive local inputs from striatal projection neurons and interneurons as well as long-range connections from different cortical and thalamic areas. Electrophysiological recordings in acute brain slices showed that many of these local-generated cells acquired membrane properties similar to immature neurons, displaying transient inward currents and generating single action potentials in response to depolarizing current steps. Further, some individual neuroblasts received spontaneous excitatory synaptic inputs while others, likely consistent with a more mature status, generated action potentials repetitively and exhibited inhibitory postsynaptic currents. These results indicate that striatal neuroblasts functionally interact with pre-existing circuits, thus potentially taking part in post-lesion network plasticity supporting functional recovery after damage.

Sensory abnormalities in Cntnap2-deficient mice, a genetic model of autism spectrum disorders.

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Sensory abnormalities are nowadays recognized as diagnostic criteria in autism spectrum disorders (ASD). About 90% of ASD individuals have atypical sensory experiences, described as both hyper- and hypo-reactivity, with abnormal responses to tactile stimulation representing a very frequent finding. Similarly, altered sensitivity to somatosensory stimuli has been described in mice lacking ASD-associated genes. We recently reported that mice lacking the Engrailed-2 gene (En2-/- mice) have a significantly reduced synchronization in the somatosensory cortex, suggesting the presence of aberrant somatosensory processing in these mutants. Accordingly, when tested in the whisker nuisance test, En2-/- but not wild-type (WT) mice of both sexes showed fear behavior in response to repeated whisker stimulation, accompanied by decreased c-Fos-positive neurons in layer IV of the primary somatosensory cortex and increased immunoreactive cells in the basolateral amygdala as compared to WT littermates. Conversely, mice lacking the ASD-associated gene Cntnap2 (Cntnap2-/- mice), when compared to their WT controls, display a decreased behavioral reactivity in response to repeated whisker stimulation. Following the whisker nuisance test, Cntnap2-/- mice exhibited a decreased c-Fos staining in the somatosensory thalamus (ventral posterior medial nucleus, VPM) but not in the primary somatosensory cortex as compared to WT littermates, thus suggesting a significant suppression of central somatosensory processing in these mutants. Taken together, our findings reinforce the need of studying sensory features of ASD in mouse models and suggest that tactile impairment in mice, akin to human ASD tactile abnormalities, could be explained through somatosensory processing defects in the nervous system.

A novel role of CDKL5 at inhibitory synapses and a possible therapeutic strategy for CDKL5-related defects

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Mutations in the cyclin-dependent kinase like 5 gene (*CDKL5*) have been found in individuals with a rare X-linked neurodevelopmental disorder characterised by early-onset epileptic encephalopathy, severe intellectual disability, intractable seizures and infantile spasms. The disorder affects mostly females who are heterozygous for CDKL5 deficiency and mosaic for the mutated allele. At present, no cure exists for patients with CDKL5 deficiency disorder.

Although the role of CDKL5 at excitatory synapses is widely accepted, its possible role in regulating inhibitory neurotransmission is still unknown. The investigation of its possible function at the inhibitory synapses would allow characterising, in more details, the molecular aspects underlying the epileptic, cognitive and autistic phenotypes. Our data suggest that CDKL5 activities converge at both excitatory and inhibitory synapses and the loss of CDKL5 impacts the main molecular actors of postsynaptic inhibitory compartment, among which $GABA_A$ receptors ($GABA_ARs$) and gephyrin, the main inhibitory scaffolding protein. We found that CDKL5 loss leads not only to a reduced surface expression of $GABA_A$ receptor γ_2 subunit ($GABA_AR\ \gamma_2$) but also to a reduction in the number of gephyrin-positive puncta suggesting that CDKL5 might directly control the stabilisation of synaptic $GABA_ARs$ through gephyrin. These defects are accompanied by a reduction in the frequencies of miniature inhibitory postsynaptic currents. Therefore, we speculate that CDKL5 controls $GABA_AR$ expression and functioning in part through its direct interaction with proteins at the postsynaptic sites. Interestingly, we found a restoration of surface expressed $GABA_AR\ \gamma2$ and gephyrin clusters in *Cdkl5*-KO neurons treated with a synthetic pregnenolone derivative, pregnenolone methyl ether. We therefore hypothesize that CDKL5 may regulate $GABA_AR$ expression also through its control of microtubule dynamics.

Axonal regeneration and magnetic nanoparticles: a new scenario in regenerative medicine?

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Neuronal regeneration is a topic of great interest to the scientific community, as well as the search for new targets to increase the speed of axonal elongation. The growth cone is the motor that govern axonal elongation, influencing its movement, orientation and development in response to chemical signalling. However, recent discoveries have shown that mechanical forces could significantly influence phenomena related to axonal growth. We developed a method to generate extremely low forces, very closed to endogenous ones, by labelling primary neurons with magnetic nanoparticles (MNPs) and by stretching them via exposure to a permanent magnetic field. We found that the picoNewton force, generated by the action of MNPs under the effect of the magnetic field, it's able to stimulate axonal elongation in mouse hippocampal neurons, as well as sprouting and maturation of the stretched axons. MNP-mediated stretching also induces changes in axonal ultrastructure such as an increase in microtubules linear density and an accumulation of cisternae from the endoplasmic reticulum. As the MNPs and static magnetic fields are biologically compatible and their use has already been approved for human therapies, the perspective of exploiting MNPs in regenerative medicine seems particularly intriguing and fascinating.

Brain-targeted polymeric nanoparticles: A breach through the blood-brain barrier for enzyme replacement therapy in Krabbe disease.

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Lysosomal storage disorders (LSDs) are a large group of metabolic diseases, individually rare but collectively common (1:5,000 live births). Usually, they result from an enzyme deficiency within lysosomes, which ultimately causes accumulation of undegraded substrates. The most clinically applied method to treat LSDs is the systemic administration of the missing enzyme. This approach, however, is not effective in the case of LSDs that involve the central nervous system (CNS); the presence of the blood brain barrier (BBB), in fact, forbids translocation of big molecules into the brain. Here, a new enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into poly-(lactide-co-glycolide) (PLGA) nanoparticles (NPs) functionalized with brain targeting peptides (Ang2, g7 or Tf2) is demonstrated for Krabbe disease (KD), an inherited neurodegenerative LSD caused by the genetic deficiency of the enzyme galactosylceramidase (GALC). We firstly synthesize and characterize Ang2, g7 and Tf2-targeted GALC CLEA NPs. Then, we study NP cell uptake and trafficking, assessing their capability to reinstate enzymatic activity *in vitro*. Finally, we successfully test our NP formulations in the Twitcher mouse, the spontaneous murine model of KD. We report enzymatic activity measurements in the nervous system and in typical accumulation districts upon NP intraperitoneal injections, demonstrating GALC activity recovery in the brain up to the level of unaffected control mice. These results open new therapeutic perspectives for KD, and for all LSDs with major CNS-involvement.

Melatonin promotes regeneration of injured motor axons via MT1 receptors

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Melatonin is a hormone produced by the pineal gland in a photoperiod controlled mode, and released in the blood flow and the cerebrospinal fluid, thus reaching many organs and tissues in the body. It is a major regulator of the sleep/wake cycle via its binding to plasma membrane G-protein coupled receptors dubbed MT1 and MT2. Once in the body fluids, melatonin displays a broad range of actions: circadian rhythm regulator, free radical scavenger, anti-oxidant, anti-inflammatory and immunoregulating molecule, and oncostatic agent. A strong neuroprotective activity of melatonin has been described in a variety of neuronal models, but comparatively less attention has been dedicated to its possible contribution to nerve regrowth and neuroregeneration. To dissect the role of melatonin in peripheral nerve regeneration we first exploited an innovative experimental model recently set up, based on the neurotoxic action of the spider toxin α-latrotoxin. This presynaptic neurotoxin causes the rapid and selective degeneration of motor axon terminals without inflammation, with complete recovery within a week in mice, thus providing an ideal model to investigate the molecular determinants of nerve regeneration. Indeed, using this model system we recently identified the signaling axis CXCR4-CXCL12α as important contributors of the rescue of function of the injured neuromuscular junction. We have also tested the activity of melatonin in established forms of prolonged damage (compression and transection of the sciatic nerve). We found that in both cases melatonin administration accelerates the process of nerve repair. This pro-regenerative action is MT1-mediated, and at least in part due to a sustained activation of the ERK1/2 pathway. These findings reveal a receptor-mediated, pro-regenerative action of melatonin in vivo that holds important clinical implications, as it posits melatonin as a safe candidate molecule for the treatment of a number of peripheral neurodegenerative conditions.

Red blood cells α-synuclein heteroaggregates in Alzheimer's Disease and Lewy Body Dementia patients.

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Alzheimer disease (AD), and Lewy bodies dementias (LBD), including Dementia with Lewy body (DLB) and Parkinson disease dementia (PDD), are the most frequent dementia-related neurodegenerative disorders (NDs). These pathologies share common pathological mechanisms, involving the accumulation in cerebrospinal fluid (CSF) of multiple misfolded proteins. A contemporary evaluation of the accumulation of these proteins is needed for every biomarker-based study aiming to classify patients within the AD and LBDs groups.

The research attention is focusing on peripheral biomarkers, moving from CSF to blood, due to accessibility and cheap costs. Red blood cells (RBCs) are involved in the accumulation and clearance of misfolded proteins, representing an interesting model to study the peripheral pathological alterations proved in neurodegeneration.

The current study aimed to investigate the diagnostic value of total α-syn, b-amyloid ($A\beta_{1-42}$), tau, and their heteroaggregates (α-syn/$A\beta_{1-42}$ and α-syn/tau) in RBCs of LBDs and AD patients compared to healthy controls (HC).

Employing enzyme-linked immunosorbent assays, RBCs concentrations of the aforementioned were measured in 27 subjects with LBDs (Parkinson dementia disease, PDD, n = 17; Dementia with Lewy body, DLB, n = 10), 51 subjects with AD (AD dementia, n = 37, prodromal AD, n = 14), and HC (n = 60).

Total α-syn and tau concentrations as well as α-syn/tau heterodimers were significantly lower in the LBDs group and AD group respectively, compared with HC. The concentration of a-syn/$A\beta_{1-42}$ was significantly lower in the AD dementia group only. RBC α-syn/tau heterodimers had a higher diagnostic accuracy for differentiating patients with LBD vs controls (AUROC = 0.80).

Overall, these data demonstrated that RBC α-syn heteromers may be useful for differentiating between neurodegenerative dementias (LBD and AD) and HC. In particular, RBC α-syn/tau heterodimers have demonstrated good diagnostic accuracy for differentiating LBDs from HC.

Hydrogen peroxide: a key signal in nerve regeneration?

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Peripheral nerve injuries represent a global health issue with insufficient therapeutic solutions. Despite the peripheral nervous system (PNS) has retained through evolution an intrinsic capability for repair and regeneration, the molecular mechanisms underlying these processes are only partially known. We recently identified hydrogen peroxide (H_2O_2), produced by stressed mitochondria of injured motor axon terminals, as one of the key mediators for the Schwann cell (SC)-dependent functional recovery of the neuromuscolar junction (NMJ) upon an acute damage induced by α-latrotoxin, a presynaptic neurotoxin. Given that, I test if H_2O_2 triggers motor axon regeneration which occurs upon sciatic nerve crush, one of the most employed experimental models to study PNS regeneration.

In collaboration with the laboratory headed by Prof. G. Schiavo at UCL-Institute of Neurology in London, I monitored H_2O_2 levels in the sciatic nerve of a living anesthetized mouse before and at different time points after sciatic nerve injury by using H_2O_2-specific probes. Moreover I performed *in vivo* imaging of the axonal transport of single endosomes in motor neurons after sciatic nerve crush or H_2O_2 incubations, in live anaesthetised mice. Labelling of endosomes is achieved using a fluorescently tagged probe: the atoxic binding fragment of tetanus neurotoxin (HCT). I then test if H_2O_2 released by the degenerating neuron, affects the time course of recovery upon injury, and impairs the activation of myelinating axonal SC, tested by activation of the MAPK pathway and cJun transcription factor.

With the described experimental systems, I detected H_2O_2 production in the sciatic nerve after crush, and I found that H_2O_2 promotes axonal transport of endosomes and acts as a paracrine signal on myelinating SC by inducing the activation of MAPK and c-Jun.

I believe that the present study will help to define H_2O_2 as an important signal molecule that activate and support the regenerative capability of PNS.

Maintenance of NCX1 and NCX2 activation in a mouse model of familiar ALS prevents misfolded SOD1 accumulation, reduces neuroinflammation, ameliorates motor behavior and prolongs survival rate

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Background: Imbalance in cellular ionic homeostasis is a hallmark of several neurodegenerative diseases including ALS. Sodium-calcium exchanger (NCX) is a membrane antiporter that, operating in a bidirectional way, couples the exchange of Ca2+ and Na+ ions in neurons and glial cells, thus controlling the intracellular homeostasis of these ions. Accordingly, the identification of new compounds capable of increasing NCX activity may be a suitable strategy to limit this devastating disorder. The aim of the present study is to evaluate the possible neuroprotective effects of NCX activator, Neurounina, on motor neurons degeneration in a mice model of familiar ALS.
Methods: We performed real time PCR, western blotting, microfluorimetry and confocal microscopy experiments on spinal cord of SOD1 G93A and wild type mice
Results: ALS mice showed a reduction in the expression and activity of NCX1 and NCX2 consistent with disease progression, therefore we aimed to investigate their role in ALS pathophysiology. Notably, we demonstrated that, in SOD1G93A mice, the prolonged NCX1 and NCX2 pharmacological activation:(1)prevented the reduction in NCX activity observed in spinal cord;(2) preserved motor neurons survival in G93A mice;(3)prevented the spinal cord accumulation of misfolded SOD1;(4)reduced astroglia and microglia activation in the spinal cord;(5)improved the lifespan and mitigated motor symptoms of ALS mice.
Conclusions: The present study highlights the significant role of NCX in the pathogenesis of this neurodegenerative disorder and it might represent an important step in defining a new pharmacological approach for ALS.

POSTER SESSION 1

NI01 | Longitudinally detection of JCPyV miR-J1-5p in urine samples of Natalizumab-treated Multiple Sclerosis patients

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The use of Natalizumab drug in Multiple Sclerosis (MS) can cause polyomavirus JC (JCPyV) reactivation, leading in rarely cases on the development of the fatal disease called progressive multifocal leukoencephalopathy (PML). For this reason the identification of JCPyV infection and reactivation in human is crucial, as some JCPyV infected subjects result seronegative for antibodies searching. Here, we investigated whether JCPyV miR-J1-5p – a miRNA that downregulates the early phase protein T-antigen, promoting the viral latency - is longitudinally detected and quantified by digital droplet PCR (ddPCR) in urine of 25 MS patients under 24 months of Natalizumab therapy (at baseline, before the first dose of Natalizumab (T0), and after 1 (T1), 12 (T12) and 24 months (T24)). The miR-J1-5p was detected in urine of 7/25 MS patients (28 %) during Natalizumab therapy: for three subjects it was detected at T24, for two subjects at T12, whereas for the remaining two the miRNA was detected in two different time-point: in one case at T1 and T12, and in the other at T0 and T1. Importantly, two of these patients were seronegative for JCPyV Ab, and the viral DNA had been never found at any time point in biological samples (urine and blood). To note, only in one case the miR-J1-5p was detected before the first dose of Natalizumab. Overall, this study suggests a potential use of the miR-J1-5p detection in urine, obtained by a very easy and non-invasive methodology, as a biomarker to monitor the JCPyV infection and reactivation, to promptly identify the risk of developing PML among Natalizumab-treated MS patients.

NI03 | Combined inhibition of histone deacetylases and BET family proteins as epigenetic therapy for nerve injury-induced neuropathic pain

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Accumulating evidence suggests that histone acetylation plays essential roles in chronic pain. Also, the analgesic activity of histone deacetylases (HDACs) inhibitors is documented. Bromodomain and extra-terminal domain (BET) proteins are epigenetic readers that interact with acetylated lysine residues on histones. In this study, we aimed at investigating the possible potentiation of the effect of HDAC and BET inhibitors, administered in combination, on neuropathic pain. The spared nerve injury (SNI) mouse model of neuropathic pain was used. Thermal and mechanical nociception was assessed, and the effect of HDAC/BET inhibitor combination was examined. Expression and localization of microglia and neuroinflammation markers were investigated by western blotting and immunofluorescence assay. i-BET762 (BET inhibitor) or SAHA (HDAC inhibitor) administration attenuated pain hypersensitivity and this effect was improved by the co-administration of both drugs. SNI mice showed increased spinal expression of HDAC1 and Brd4 proteins, robustly reduced by the combination therapy. SAHA and i-BET762 co-administration counteracted the SNI-induced microglia activation by inhibiting the expression of markers of neuroinflammation. This effect was much more evident than the one observed with the administration of the single drugs. Our results indicate a key role of acetylated histones and their recruitment by BET proteins in microglia-mediated spinal neuroinflammation, which is involved in the development of neuropathic pain symptoms.

NI04 | Exposure to different early-life stress experiences results in differentially altered DNA methylation in the brain and immune system

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The existence of a proportional relationship between the number of early-life stress (ELS) events experienced and the impoverishment of child mental health has been hypothesized. However, different types of ELS experiences may be associated with different neuro-psycho-biological impacts, due to differences in the intrinsic nature of the stress. DNA methylation is one of the molecular mechanisms that have been implicated in the "translation" of ELS exposure into neurobiological and behavioral abnormalities during adulthood. Here, we investigated whether different ELS experiences resulted in differential impacts on global DNA methylation levels in the brain and blood samples from mice and humans. ELS exposure in mice resulted in observable changes in adulthood, with exposure to social isolation inducing more dramatic alterations in global DNA methylation levels in several brain structures compared with exposure to a social threatening environment. Moreover, these two types of stress resulted in differential impacts on the epigenetic programming of different brain regions and cellular populations, namely microglia. In a pilot clinical study, blood global DNA methylation levels and exposure to childhood neglect or abuse were investigated in patients presenting with major depressive disorder or substance use disorder. A significant effect of the mental health diagnosis on global methylation levels was observed, but no effect of either childhood abuse or neglect was detected. These findings demonstrate that different types of ELS have differential impacts on epigenetic programming, through DNA methylation in specific brain regions, and that these differential impacts are associated with the different behavioral outcomes observed after ELS experiences.

NI05 | Different susceptibility of T and B cells to cladribine depends on deoxycytidine kinase activity as a result of activation state

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Deoxycytidine kinase (dCK) and 5' deoxynucleotidase (NT5C2) are involved in metabolism of the immunomodulatory drug for multiple sclerosis, cladribine (2CdA), and therefore important in its mode of action leading to cell death. We aim at determining if their expression and/or activity differ in particular progenitor and mature immune cells and are influenced by activation and/or exposure to 2CdA. dCK and NT5C2 protein expression was measured by flow cytometry and Western blotting. T and B cells isolated by negative selection were activated with anti-CD3/CD28 antibodies or IL15/CpG, respectively. Cell viability was measured by flow cytometry. dCK and NT5C2 mRNA expression was quantified by qPCR. The activity of dCK protein enriched by anion exchange chromatography was measured as luminescence corresponding to residual ATP using KinaseGlow kit. Flow cytometry analysis showed no difference in dCK/NT5C2 ratio in progenitor and mature immune cells. 2CdA induced apoptosis in stimulated T and B cells and unstimulated B cells. dCK expression was enhanced by 2CdA at mRNA and protein levels in activated T cells and mRNA level in activated B cells. dCK activity was higher in activated T and B cells, but such an increase was abrogated in activated B cells, but not T cells, upon exposure to 2CdA.
These results highlight the importance of measuring dCK activity to better dissect the impact of 2CdA on B and T cells according to their activation status; they will be crucial to further understand how 2CdA treatment might affect lymphocyte subsets in treated MS patients.

NI06 | Daily changes in MAPK signaling pathway activity in chicken pinealocytes in vitro.

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In birds, the pineal gland is one of three equivalent central biological clocks. The pineal gland synthetizes melatonin (MEL), a hormone regulating the rhythms of various physiological processes, at night. In the innate immune response, MEL is part of a dynamic network that acts throughout the inflammatory response, integrating signaling pathways and regulatory processes at the molecular, cellular and organism levels. One of the signaling pathways engaged in immune response is mitogen-activated protein kinases (MAPK). On the other hands MAPK signaling pathway regulates function of biological clock. Thus, we hypothesized that MAPK signaling pathway is under clock control and shows daily changes in activity under different light conditions. The aim of our study was to examine the MAPK daily activity under light:dark (LD, 12:12), constant darkness (DD) and light (LL) conditions in chicken pinealocytes. The second aim was to investigate if exogenous administration of MEL and noradrenaline (NA) may influence this activity. The pinealocytes taken from pineal glands of 16-d-old chickens were cultured 48 h and then transfected with the pNiFty3-A-Lucia plasmid, which allows to assess MAPK activity by measuring the luminescence of the reporter gene in the culture medium. 22 h after transfection, starting from ZT0, the culture medium was replaced every 2 h into clear medium or supplemented with MEL or NA. The removed medium was collected to measure luminescence. We found that the activity of the MAPK signaling pathway changes over the course of the day, and these changes are rhythmic under LD conditions regardless of cell's treatment. Additionally, the rhythm of the MAPK signaling pathway activity is sustained under LL conditions, but only in control pinealocytes whereas constant darkness disrupts this rhythm. Supplementation with MEL, but not with NA restores rhythm in DD pinealocytes. The study was supported from grant UMO-2016/21/B/NZ3/00364.

NI07 | Bone channels and inflammation routes in the rat auditory system

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Hearing loss is a major risk for dementia. One of the proposed reasons is the presence of a pro-degenerative process affecting the auditory system with particularly high efficiency, and subsequently affecting other brain structures. In the brain, skull bone marrow connects to the dura and CSF spaces through bone channels and releases immune cells into the brain parenchyma. Similarly, the temporal bone contains marrow patches in association with the cochlea, vestibular labyrinth and endolymphatic sac, but given the inner ear complex morphology, they have been little characterized. We are exploring the hypothesis that dysregulation of temporal bone marrow-derived cells may be an early sign for age-related neurodegeneration. As a first approach, we performed iDISCO clearing on rat temporal bones in control conditions and after LPS-induced systemic inflammation.
The unique regulation of cochlear fluid composition and blood flow poses strong constraints on its neuroinflammatory responses, making the ear more susceptible than the brain to neuroimmune dysregulation. Cochlear inflammation is involved in most non-genetic forms of hearing loss, and affects a large population of resident macrophages, nerve-associated glial cells and fibrocytes from the cochlear lateral wall, a mesenchymal-derived structure which surrounds cochlear neurosensory components similarly to the brain meningeal lining. All these cell populations are at least partially renewed from bone marrow.
The inner ear labyrinth includes an immune-related membranous structure (the endolymphatic sac) which associates with periosteal dura and is surrounded by tortuous bone channels. The latter change upon inflammation, and the connection of at least a subset of them with bone marrow patches suggests a role in the local regulation of neuroimmune responses. Channel remodeling impairment would likely affect inflow of immune cells to the cochlea upon chemotactic factor production.

NI08 | Hydroxychloroquine modulation of immune response in lymphoblasts derived from patients with Aicardi-Goutières syndrome

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Aicardi-Goutières syndrome (AGS) is a rare genetic disorder. Mutations in the 7 AGS genes lead to an accumulation of endogenous nucleic acids (NAs), such as RNA:DNA hybrids, which are recognized as foreign NAs by the organism and may trigger a type I interferon response through the cGAS-STING or Toll-like receptors (TLRs) pathways. Hydroxychloroquine (HCQ), hampers the NAs binding to cGAS and TLRs and accumulates within lysosomes inhibiting their action and blocking the autophagic flux. Aim of this study was to assess the effectiveness of HCQ treatments in modulating IFN-α response in lymphoblastoid cell lines (LCLs) derived from *RNASEH2A* and *RNASEH2B* mutated patients and its action on the RNA:DNA hybrids discard. An abnormal RNA:DNA hybrids accumulation and colocalization with endolysosomes was found in *RNASEH2B* LCLs compared to control. We evaluated cGAS protein level and *IRF3* expression level, observing an slight increase of both in *RNASEH2A* LCLs, whereas, the expression levels of *MYD88* and *IRF7* genes, which are activated downstream by TLR-7/9, seem to be higher in *RNASEH2B* patients. An overexpression of two ISGs (*IFIT1*, *IFI44*) was found only in *RNASEH2B* LCLs. After a 24h treatment with 25µM of HCQ, we found a reduction in the RNA:DNA hybrids content and lysosomal signal, suggesting an alkalization of lysosomes with a consequent loss of their functions. We no longer identify a colocalization between endolysosomes and RNA:DNA hybrids after treatment and increasing levels of LC3 and p62 have been identified in both control and AGS LCLs. Colocalization of hybrids with LC3 and lysosomes was lost after treatment. In *RNASEH2B* LCLs, HCQ also causes the decrease of *MYD88* and *IRF7* transcripts levels. as well as *IFIT1* and *IFI44* expression levels. Therefore, HCQ seems able to stop IFN-α activation and release in *RNASEH2B* LCLs, determining improvements in patients' condition, possibly representing an effective method to decrease RNA:DNA hybrids.

NI09 | DNA methylation profiling in Aicardi-Goutières Syndrome patients mutated in RNASEH2B

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Aicardi-Goutières syndrome (AGS) is a pediatric rare genetic disorder that mainly affects the brain, the immune system and the skin. Mutations in the 7 AGS genes lead to an abnormal activation of the innate immune system. The most frequent mutation found in AGS is the p.A177T in *RNASEH2B* gene and it is associated with both a severe (with or without extraneurological involvement) or mild phenotype (characterized by a normal IQ in almost all patients). Aim of this study was to identify changes in DNA methylation between classic and mild phenotype AGS patients carrying p.A177T mutation in *RNASEH2B*. The Infinium MethylationEPIC BeadChip was used to interrogate DNA methylation in peripheral blood samples. All p.A177T RNASEH2B patients presented an increased DNA methylation when compared to healthy controls. Severe patients showed an hypomethylation of genes involved in cell adhesion, leukocyte differentiation, hemopoiesis and T-cell activation, and a hypermethylation in genes involved in regulation of viral life cycle, NF-kB activity, T-helper differentiation and IL-5, IL-13, IL-10 release. Mild patients showed an hypermethylation of genes involved in neutrophil degranulation, activation and mediated immunity, whereas a mild-specific hypomethylation was found in genes involved in T-cell activation, lymphocyte differentiation, regulation of antigen receptor-mediated and B cell receptor signalling pathways. Among the most hypomethylated genes we found two interferon regulated genes, *IFI44L* and *MX1*, in accordance with the immune system activation found in both subgroups of patients. At last, *LIG4* gene was more hypomethylated in mild patients, suggesting a higher expression of this ligase involved in DNA non-homologous end joining. Taken together our results showed that severity-specific alterations in methylation can contribute to the pathogenesis and variation in the clinical presentation of AGS between mild and severe patients.

NI10 | Neurosteroidogenic pathway activation reduces microglial reactive phenotype in a human in vitro model of neuroinflammation

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Abnormal activation of microglia, the resident macrophages of the central nervous system (CNS), is considered to promote neuroinflammation and neurodegeneration. Therefore, particular attention has recently been paid to the understanding of the molecular mechanisms underlying microglial activation, to identify new possible therapeutic targets. Our previous study demonstrated that activation of the protein involved in the first phase of neurosteroidogenesis Translocator Protein (TSPO, 18 kDa), by selective ligands, was able to shift the reactive phenotype of human microglial cells stimulated with IL-1β into a restorative one. Moreover, this phenomenon was abolished by the inhibition of the first enzyme of neurosteroidogenesis, CYP11A1. These results prompted us to investigate the characterization of the neurosteroidogenic pathway in microglia, above all the questioned capacity of this type of cells to produce neurosteroids ex-novo. Early evidence suggested that human microglia constitutively express high levels of both TSPO and StAR, an indication of its ability to successfully deliver cholesterol to the mitochondria. Microglia also expresses, albeit at low levels, the enzyme CYP11A1, involved in the first rate-limiting biosynthetic step, consisting in the conversion of cholesterol into pregnenolone. In line with this result, human microglia is capable of producing pregnenolone and furthermore, the neurosteroidogenic activity of microglia is effectively stimulated by synthetic ligands to TSPO. Notably, the inflammatory stimuli increased the expression of these neurosteroidogenic involved proteins. Although a direct correlation between protein expression and neurosteroid production must be still evaluated, the activation of the neurosteroidogenic pathway seems to be an important autocrine/paracrine way to regulate microglia activities and could represent a crucial mechanism to exploit for the exogenous control of neuroinflammation.

NI12 | Exposure to fine particulate matter (PM2.5) hampers myelin repair in a mouse model of white matter demyelination.

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Epidemiological studies show a strong association between exposure to air pollution – and particularly to particulate matter (PM) - increased prevalence of Multiple Sclerosis (MS) and higher rates of hospital admissions for MS and MS relapses. Beyond having immunomodulatory effects and sustaining a systemic oxidative-inflammatory response, PM may participate in MS pathogenesis by targeting also Central Nervous System (CNS)- specific processes, such as myelin repair. Here we show that, in a mouse model of lysolecithin-induced demyelination of the subcortical white matter, post-injury exposure to fine PM hampers remyelination, disturbs oligodendroglia differentiation dynamics and promotes astroglia and microglia reactivity. These findings support the view that exposure to fine PM can contribute to demyelinating pathologies by targeting the endogenous regenerative capability of the CNS tissue.

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NI13 | SCFA's alleviates the nitroglicerin (NTG)-induce migraine in mice.

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Based on global burden of headache reports, migraine is a prevalent disorder that affect approximately 15% of the adult population. Generally migraine attacks are sporadic, however, some individuals develop a chronic disease form. In this study, we aimed to evaluate the role of the short-chain fatty acids (SCFAs), such as sodium propionate (SP) and sodium butyrate (SB) in regulating the pathophysiology of migraine in a mouse model induced by nitroglycerine (NTG).
Mice were orally administered with SB and SP at the dose of 10, 30 and 100 mg/kg, 5 min after NTG intraperitoneal injections. Histological and molecular analysis were performed on the whole brain to detect the expression of inflammatory and oxidative markers. Behavioral tests (Tail flick, hot plate, orofacial formalin and photophobia) were performed 4 h from migraine induction, to evaluate migraine-like pain and migraine-related light sensitivity. SP and SB treatment notably reduced histological damage in whole brain in NTG-injected mice. Treatments with both SCFAs decreased the markers of inflammation and increased the protective antioxidant enzymes, suggesting an important role of SCFAs to exercise neuromodulatory action.
These results provided the evidence that SCFAs exerts a protective effect, suggesting a new insight into the potential application of SCFAs as novel supportive therapies for migraine.

NI14 | Ocrelizumab treatment in patients with relapsing-remitting multiple sclerosis: a single-center real-world experience

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Background: Real word data on efficacy and safety of ocrelizumab (OCR) treatment in patients with Relapsing Remitting Multiple Sclerosis (RRMS) are still scarce.
Objectives: To provide first experience on pts with RRMS treated with OCR in a real-world setting.
Methods: We collected safety and efficacy data from RRMS pts treated with OCR at the MS Center of the University of Genoa. The probability of disability worsening-free, relapse-free and MRI-activity free-survival and NEDA-3 status was calculated with the Kaplan-Meier estimator and Cox proportional hazards regression analysis.
Results: 96 RRMS pts [60 females (62.5%), mean (SD) age 37.3 (10.2) years] with a mean disease duration (DD) of 9.6 (9.3) years, a median (IQR) baseline EDSS of 2.5 (2-4) and a mean ARR of 0.79 (0.73). Reasons for previous DMTs discontinuation were (i) lack of efficacy for 45 (67%), (ii) occurrence of adverse events (AE) for 7 (10%) and (iii) high JCV titer during natalizumab treatment for 5 (7.5%) pts. 28 pts (29.5%) had not received any DMT prior to OCR. Naïve pts had significantly shorter disease duration (2.6 vs 12.5 years; p<0.0001), higher ARR (1.1 vs 0.7; p=0.002) and inflammatory activity on baseline MRI scan (96.3% vs 74.6%; p=0.019). At 1-year FU, MRI-inflammatory activity free survival was 75.9%, relapse free survival was 95.9%, progression free survival was 98.7%. 2-years NEDA-3 status was achieved in 73.6% of pts. At multivariate analyses, 2-years NEDA-3 status was significantly higher in naïve compared with treated pts [90.7% versus 60.8% at the end of the observation period; HR (CI 95%) 0.14 (0.03-0.65); p=0.012]. We recorded 55 AE in 39 pts. No serious infusion associated reactions were reported.
Discussion: OCR treatment allows complete disease control in a high proportion of real-world RRMS pts, with a manageable safety profile. Although ocrelizumab can control disease activity after failure of highly efficacy DMTs, its efficacy seems to be higher in naïve patients.

NI15 | A humanized model of blood brain barrier to investigate immune cell infiltration: toward a personalized medicine approach

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The neurovascular unit (NVU) is the most important biological barrier between vascular districts and central nervous system (CNS) parenchyma, which maintains brain homeostasis, protects the CNS from pathogens, and mediates neuroimmune communication. T lymphocytes migration across the blood–brain barrier (BBB) is affected in different brain diseases, representing a target for novel drug development. *In vitro* NVU models represent a tool to investigate the molecular events occurring at this interface.
Primary human astrocytes (hA) purified from the peripheral area of surgical samples of patients undergoing cerebral tumor resection were characterized to confirm their astrocytic identity. Endothelial cells (hE) and hA were integrated in a contact co-culture NVU model. Abluminal GFAP and Kir4.1 positive hA processes protruding through filter pores and terminating in close proximity of luminal hE were observed, with a morphology reminiscent of end-feet, a key feature of the NVU. hE responded positively to hA integration, improving barrier properties, forming a less permissive model in terms of trans-endothelial electrical resistance (TEER) and permeability. When challenged with the pro-inflammatory stimuli IL-6 or TNFα, the barrier resistance was reduced and the permissiveness increased. $CD4^+$ T cells were isolated from healthy subjects (HS) or Multiple Sclerosis (MS) patients, and transmigration was analyzed. TNF-α increased T cells transmigration. IL-17 producing $CD4^+$ T cells (Th17) and IFN-g producing $CD4^+$ T cells (Th1) subsets were compared for their ability to cross the NVU model. Greater transmigration of Th1 and Th17 cells was observed in MS patients in respect to HS.
To move toward the establishment of personalized therapies, a patient-related NVU *in vitro* model was set, incorporating primary hA integrated into a microfluidic platform. The model represents an advantageous tool to investigate lymphocytes transmigration and the efficacy of drugs affecting this process.

NO01 | Deciphering a serum miRNA signature associated to IDH1 status as non-invasive diagnostic/prognostic biomarkers for glioma patients

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Gliomas are diffusely growing brain tumors and challenging cancers for diagnosis and treatment. The identification of genetic and epigenetic markers has led to an integrated diagnosis, composed of a histological diagnosis, and a molecular profile of the tumor. Among the key genetic events, the isocitrate dedydrogenase 1 (IDH1) mutation is noteworthy, whose detection requires invasive tissue sampling. Biofluids are important sources of information in several diseases, analyzable by liquid biopsies, minimally invasive tools for diagnosis and prognosis. Altered microRNA (miRNA) profiles have been observed not only in tumor tissues but also in biofluids, where they circulate in a very stable form. Several circulating miRNAs have been evaluated in gliomas as single biomarkers. However, it is widely recognized that single miRNA profiles may provide a low accuracy as cancer biomarkers, mostly due to the multifactorial nature of tumor and to the large number of targets for a single miRNA. Preliminary results, obtained by small RNA-seq in the hosting laboratory, showed 10 serum miRNAs as promising diagnostic and prognostic tools, able to stratify gliomas, according to prognosis and IDH1 mutation status, with high specificity and sensitivity. Interestingly, combination of miR-X/miR-Y/miR-J and miR-XX/miR-Y/miR-J (under patent), from the identified 10 miRNA signature, showed a significantly improved accuracy compared to each single miRNA. All these miRNAs have been described as involved in glioma pathogenesis and in potentially druggable molecular pathways. Our data suggest that the detection of circulating miRNAs holds great promises to aid diagnosis and prognostication by monitoring tumor progression, and thus can be considered a pathway towards personalized medicine.

NO02 | Candidate early biomarkers of Glioblastoma Multiforme revealed by longitudinal bottom-up proteomics study on multiple biofluids in a murine model

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Glioblastoma Multiforme (GBM) is an aggressive form of brain tumor, associated with poor prognosis and low survival rates. GBM is diagnosed at an advanced state and few improvements have been made for earlier diagnosis. Here, a GBM murine model was used to investigate longitudinal changes in the protein profile of serum, serum small extracellular vesicles (sEVs) and cerebrospinal fluid (CSF) during GBM progression. Motor tests were used to define a baseline, a pre-symptomatic and an advanced stage of the disease; serum, serum sEVs, and CSF samples were obtained from each animal at each stage. The samples were then analyzed using proteomics workflows specifically developed for the analysis of the small sample volumes collected during such longitudinal investigations. Data were then analyzed using a linear mixed effects model to identify longitudinal changes in expression. Forty-four proteins were dysregulated during GBM progression, several of which have previously been reported as candidate diagnostic (Vtn, Flna, C1qa and Gsn) or prognostic (Ahsg) biomarkers. Most of the proteins exhibited a difference from the baseline already at the pre-symptomatic stage, suggesting that some candidate GBM biomarkers can be dysregulated before symptoms onset.

NO03 | BRAFV600E mutation in combination with loss of tumor suppressor Pten in adult Neural Stem/Progenitor Cells induces glioma formation.

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Gliomas are a heterogeneous group of malignancies of the central nervous system (CNS) that account for about 2% of all cancers.
The landscape of glioma mutations is wide and mostly involves EGFR, PI3K, CDKN2A, P53 and PTEN mutations. PTEN loss of function strongly contributes to brain tumor formation and it is always associated with increased tumor malignancy and poorer prognosis. BRAF mutations account for a restricted number in gliomas and can occur as KIAA1549-BRAF and BRAFV600E.
Since BRAF is a master regulatory gene for oligodendrocyte differentiation , to gain insights into the function of BRaf overactivation in NSCs, we developed a mouse model in which BRAFV600E mutation and *Pten* deletion are driven by the Tamoxifen-inducible Sox2-CreERT2, a deleter specifically active in telencephalic NSCs. Sixty days after Sox2-CreERT2 induction in adult mice, autopsy revealed the presence of tumors originating from the ventricular cavities, that resembled oligodendrogliomas, with a penetrance of 100%, supporting also the hypothesis that NSCs represent the cells of origin of glioma.
To investigate the molecular events involved in tumor progression, we performed in vitro differentiation on transformed NSCs from BRAF/PTEN mutant mice, observing that they were able to segregate toward the oligodendrocyte lineage recapitulating the oligodendroglioma phenotype observed *in vivo.* Moreover, transformed NSCs showed a more proliferative behavior when compared to the control.
Altogether these results suggest that deregulated BRAF, in a *Pten* null background, can drive NSPC differentiation towards the oligodendrocyte tumor lineage.
These results were confirmed by RNAseq analysis on transformed NSCs isolated from our experimental mice.
Our model can represent an important tool to understand the molecular mechanisms that occur in NSPs during malignant transformation and to test valid therapies for oligodendroglioma treatment.

NO04 | CITK loss leads to DNA damage accumulation by altering microtubule stability

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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, these therapies are only partially effective. 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. 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 (DSBs) accumulation, nuclear reduction of the DNA-repair protein RAD51 and it impairs homologous recombination (HR). A class of drugs commonly used against brain tumors are microtubule-targeting agents, which are classified as microtubule 'stabilizers' or 'destabilizers'. Since sensitivity to CITK depletion it has been linked to microtubule dynamics in cytokinesis, we investigated the relationship between CITK and microtubule dynamics for DNA integrity. CITK knockdown combined with the microtubule-stabilizing agent Paclitaxel was able to recover the nuclear levels of RAD51 protein and the DNA damage induced by CITK knockdown. These data indicate CITK loss induces DNA damage accumulation through an alteration of microtubules stability. Indeed, Paclitaxel rescued DNA damage induced by CITK loss while destabilizing agents, as Vincristine, worsen it. These results suggest that CITK molecular function is strictly connected to microtubule dynamics and that combination of CITK inhibition with microtubule-destabilizing agents could synergize for MB's treatment.

NO05 | CTX-CNF1: an innovative therapeutic strategy to treat Gliomas

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The most aggressive form of gliomas is Glioblastoma Multiforme (GB). Unfortunately, the currently used standard-of-care is only partly effective and the overall survival of GB patients is of almost 15 months after diagnosis. Thus, there is a compelling need to find novel approaches to counteract GB. Recent studies highlighted the effectiveness of the bacterial protein Cytotoxic Necrotizing Factor 1 (CNF1) in reducing tumoral mass, increasing survival of glioma-bearing mice and protecting peritumoral neural tissue from dysfunction. However, native CNF1 needs to be delivered into the brain, because it is not able of crossing the blood brain barrier (BBB) per se, hampering its clinical translation. To allow a non-invasive administration of CNF1, we developed a chimeric protein conjugating CNF1 with Chlorotoxin (CTX), a peptide already employed in clinics because capable of passing the BBB and of selectively binding glioma cells. From *in vitro* studies we found that CTX-CNF1 is effective in leading glioma cells to death through the activation of a senescence process. At the same time, CTX-CNF systemic administration was effective in selectively targeting glioma cells with high specificity in vivo. As a consequence, glioma-bearing animals that were treated with CTX-CNF1 showed a significant increase in their survival rate. This result is of paramount importance because the administration of the recombinant molecule was made at a symptomatic stage of the disease. Altogether, our data strongly demonstrate that CTX-CNF1 represents a very innovative and valuable approach for GB treatment.

NO06 | Proteomics analysis of serum small extracellular vesicles for the longitudinal study of a glioblastoma multiforme mouse model

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Small extracellular vesicles (sEV, diameter <150nm) released from cancer cells contain tumor derived material, and thus many studies have focused on their role as potential source of biomarkers. Xenograft mouse models are among the most frequently studied cancer models, but longitudinal blood-sEV analysis during tumor development has been undermined by the lack of purification methods compatible with the low sample volumes that may be obtained from a single mouse at each time point (maximum serum volume for such longitudinal studies is about 75 µl every 14 days). Here we report a method for the proteomics analysis of sEV from 50 µl of serum. Two sEV isolation procedures were first compared; precipitation-based purification (PPT) and size exclusion chromatography (SEC) from 100 µl of mouse serum. Vesicle proteome was then analyzed using a modified SP3 protocol. The methodological comparison revealed that SEC led to purer sEV both in terms of sEV size distribution and sEV proteins. The SEC-EV procedure was then scaled down to 50 ml and the proteolytic digestion further optimized to increase the number of identified proteins and reproducibility. This led to the identification of 277±2 protein groups and greatly improved the reproducibility of protein quantitation (Pearson correlation coefficient >0.9). The SEC-EV optimized method was then applied to a longitudinal study of serum-sEV proteome changes in a glioblastoma multiforme (GBM) mouse model. Serum was collected at three time points: baseline, T1 (pre-symptomatic) and T2 (post-symptomatic) stages. The protocol enabled 274 protein groups to be identified and quantified. Several sEV common proteins were identified: integrins (α6, α-IIb, β1, β3), Anxa2, Gapdh, Adam10, Hspa8, and Tgfb1. The longitudinal analysis revealed 25 deregulated proteins in GBM serum-sEV including proteins previously shown to be associated with GBM induced metastasis and tumor progression (Myh9, Tln-1, Angpt1, Thbs1).

NNP01 | Physiological biomarkers to predict motor recovery after stroke in mice

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Stroke is the second leading cause of death and the third leading cause of disability worldwide. Notwithstanding, many patients survive and display a spontaneous recovery, possible due to a reorganization of spared areas and connections. Currently, there are no ways to determine either the extent or time-course of recovery. Great is therefore the need for biomarkers predictive of spontaneous recovery to allow a better patient stratification in clinical trials and to personalize therapies. In our study we took advantage of a mouse model of middle cerebral artery occlusion (MCAO) to investigate novel prognostic and therapeutic tools in preclinical models. To determine the amount of spontaneous recovery, we conducted, at different time points, a battery of behavioral tests and a retraction task in the M-platform, a robot device that quantitatively evaluates kinetic parameters related to forelimb movement. Moreover, to mimic clinical scales used in stroke patients, we implemented a novel “Motor Score (MS)” comprehensive of the motor performance assessed in all single motor tests. MS was able to detect a global motor deficit after MCAO and, showed the spontaneous recovery variability over time. Mice were also implanted with chronic electrodes in the caudal forelimb area (CFA) to record local field potentials from both hemispheres during the retraction task in the M-platform and in freely moving condition. Novel quantitative methods were also implemented to evaluate lesion size, location and shrinkage in addition to integrity of the corticospinal tract.
Finally, electrophysiological and anatomical changes will be then correlated with the extent of motor outcome to highlight physiological predictive biomarkers of spontaneous recovery.
The results deriving from this study could shed light on the mechanisms responsible for the reorganization of the spared areas following a stroke, and could find reliable biomarkers with high translational potential to human stroke patients.

NNP02 | Prolonged aerobic exercise maintains juvenile synaptic plasticity in hippocampus

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Aerobic exercise (AE) produces beneficial effects on brain health by improving plasticity, connectivity, memory and cognitive functions and reducing the incidence of age related neurodegenerative disorders. In this study, we explored the effects of two continuous AE protocols, that differ in duration and intensity, on hippocampal synaptic plasticity. Young mice (P30) were trained with short term (6 weeks), variable intensity exercise (CV) or long term (12 weeks), progressive increasing intensity exercise (CP), using Rotarod. At the end of training, hippocampus was isolated and synaptic plasticity was measured by electrophysiological recording of long-term potentiation (LTP) on hippocampal slices. We report that LTP from CP mice showed an enhancement on population spikes (PS) respect the corresponding sedentary animals, while CV training did not affect LTP, suggesting that only CP exercise was able to induce an increase of hippocampal synaptic plasticity. Furthermore, to investigate the physiological relevance of our finding, we hypothesized a possible rejuvenating effect of CP exercise. Thus, we compared hippocampal LTP of CP trained mice with LTP detected at different ages. We show that LTP was significantly higher in young mice (P30) with respect to adult (P120) and middle-aged (P12M) animals, indicating that an age-related decrease of synaptic plasticity occurred in hippocampus. Interestingly, we found a strong overlapping between the LTP values recorded from CP trained and young (P30) animals, indicating that CP training maintained a juvenile synaptic plasticity at adult age in hippocampus. In conclusion our results indicate that enhancement of hippocampal plasticity is dependent on intensity and duration of training protocol.

NNP04 | Lactate metabolism in the control of microglial function

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Microglia are the tissue-resident macrophages of the brain. They are implicated in a variety of fundamental processes, including clearance of pathogens and cell debris, as well as synapse pruning and refinement. Not surprisingly, dysregulation in microglial function is linked with the onset of neuropathology. Recently, accumulating evidences are pointing 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, as well as lysosomal acidification in other cell types. The physiological role for lactate in modulating microglial function, however, is still unexplored. In order to address this question, we have generated a microglia-specific conditional *knock out* (cKO) for one lactate transporter, the monocarboxylate transporter 4 (MCT4), which we describe to be specifically upregulated in microglia upon lactate exposure. We analyzed key microglia features during postnatal development, and we found alterations in microglial density and in CD68+ phagocytic vesicles in the hippocampus of cKO mice at two weeks of age. This was associated with alterations in excitatory post-synaptic currents, indicating that microglia-specific depletion of MCT4 is sufficient to induce neuronal dysfunction. Additionally, cKO mice present an anxiety-like phenotype, which becomes more evident in older individuals. In summary, this study will lead to a better understanding of the crosstalk between metabolism and microglial function, highlighting novel mechanisms by which these cells sense environmental and neuronal activity changes. Given the established correlation between metabolic syndrome, neuropathology and microglial activation, targeting these pathways may potentially be relevant for a variety of conditions, from development to aging.

NNP05 | The gut microbiota of environmentally enriched mice regulates visual cortical plasticity

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It is well established that exposing animals to an enriched environment (EE) has dramatic effects on function and plasticity of many different brain areas, including sensory systems. For example, ocular dominance (OD) plasticity, a change in eye preference of cortical cell responses induced by monocular deprivation (MD), present only during a critical period of postnatal development, is observed in adult mice raised in EE. Moreover, EE was found to accelerate several molecular and functional aspects of visual cortical development, indicating that the visual system is highly influenced by EE both during development and adulthood.

Classically, the beneficial EE effects on brain physiology have been explained through mechanisms that arise from the central nervous system itself: increased hippocampal neurogenesis, changes in the excitatory/inhibitory circuit balance, promotion of structural plasticity of dendritic spines, alteration in BDNF levels, microglia rearrangement, chromatin remodeling, and others. In our work, the focus has been shifted to signals coming from the periphery of the body, providing a novel and unexpected link between the gut microbiota and EE-driven plasticity.

The intestinal microbiota is implicated in many processes related to host physiology, such as immune system development and maintenance of metabolic homeostasis; moreover, recent evidence shows that the influence of gut microbes goes beyond the periphery towards the central nervous system. Indeed, studies using germ-free (GF) and antibiotic-treated mice have demonstrated the ability of the intestinal microbiota to influence many brain-related processes: development, neurogenesis, myelination, microglia development, blood-brain barrier (BBB) homeostasis and, finally, regulation of behavioral outcomes.

Despite this evidence, little is known about how intestinal bacteria impinge on neuronal function and especially, nobody has ever explored if there is a link between experience-dependent plasticity and the gut microbiota.

Here we found the EE shaped the intestinal bacteria composition. A longitu-

dinal analysis of the fecal microbiota in EE mice revealed striking differences from standard condition (ST) animals, especially during adulthood. The depletion of the gut microbiota in EE mice using an antibiotic cocktail was able to prevent OD plasticity, to influence spine dynamics and microglia morphology.

Strikingly, transferring the microbiota of EE donor mice to ST mice through fecal microbiota transplant (FT) was able to activate adult OD plasticity in the ST recipients.

Importantly, the gut microbial composition of EE donors showed a significant enrichment in bacteria belonging to *Lachnospiracaeae* family, well-know producers of short-chain fatty acids (SCFA), a signature also observed in ST mice that have received the EE mice microbiota.

Remarkably SCFA treatment alone promoted adult OD plasticity and influenced microglia morphology in ST mice suggesting that SCFA could act as a gut-brain signal to promote EE-driven plasticity.

Overall this evidence shows that experience-dependent changes in gut microbial composition are able to regulate brain plasticity.

NNP06 | Rearrangements of peritumoral tissue that occur along with glioma progression

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Gliomas represent about 80% of all malignant brain tumors. Although there have been recent advances in diagnosis and treatment of the malignancy, the prognosis of gliomas is still poor, especially for those patients with malignant and invasive gliomas.
High-grade gliomas like GBM are known to infiltrate the surrounding brain parenchyma inducing a direct neuronal damage. However, the glioma mass is not an isolated and self-sustained entity; on the contrary, it participates and interacts with the surrounding neuronal tissue. Therefore, in order to develop novel therapeutic approaches, it becomes of extreme importance to understand the alterations that occur in peritumoral tissues along with GBM progression.
This project aims to investigate how the plastic rearrangement of cortical areas occur in glioma-bearing animals. To perform this analysis we took advantage of Thy1-ChR2 Transgenic mice (B6.Cg-Tg (Thy1-ChR2/EYFP)18Cfng/J, Jackson Laboratories, USA), injected with a syngenic line of murine glioma cells (i.e. GL261 cells) into the motor cortex. Thy1-ChR2 mice express the gene encoding for the protein ChR2 under the Thymus cell antigen-1 (Thy-1) promoter, resulting in ChR2 synthesis mainly present in pyramidal, corticospinal neurons. These mice were optogenetically stimulated in 120 points per session (covering the entire extent of forelimb motor representation) at three different time points: baseline (before GL261 cells injection), 14 and 21 days after tumor implantation.
Glioma-bearing mice showed a strong remapping of cortical motor areas, together with an increased threshold required for eliciting a forelimb movement in the peritumoral area.
Immunohistochemical analyses revealed a downregulation of PNNs and of inhibitory markers in the peritumoral cortex. These findings demonstrate that the peritumoral tissue shows biochemical reorganization and transformation along with glioma progression.

NNP07 | Microglia day/night morphological changes in lateral hypothalamus

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Microglial cells are remarkably plastic, constantly scan the microenvironment with their highly motile processes, and can detect disruptions in the homeostasis. While morphological changes of microglia in pathological conditions are well characterized, their plasticity in normal conditions has yet to be fully understood. We recently observed a day/night switch in the excitatory/inhibitory wiring of orexin (OX)-containing cell bodies of the lateral hypothalamus (LH). In the present study, we tested the hypothesis that diurnal changes in synaptic wiring involve local microglial plasticity. The investigation of microglia morphology in the LH was carried out in adult male CX3CR1-GFP transgenic mice, in which microglial cells are tagged with the green fluorescent protein (GFP). Groups of heterozygous ($CX3CR1^{+/GFP}$) or homozygous mice ($CX3CR1^{GFP/GFP}$, in which fractalkine receptor may not be functional) were sacrificed at two time-points (night and day), and video-recorded during the 3 hours preceding sacrifice in order to assess their vigilance states. Immunofluorescence in confocal microscopy was used to visualize cell bodies containing OX-A and GFP-labelled microglia. 3-D reconstructions on a small sample of animals showed day/night changes in microglial cell morphology, with more ramified processes at night (when mice are predominantly awake) than during daytime (the period of sleep predominance). If confirmed by further experiments, such cyclic variations might suggest, during the period of wakefulness, an active role for microglia not only in homeostasis maintenance, but also for the surveillance of the synaptic contacts reached by the highly ramified distal processes. On the other hand, during the sleep period less extensive ramifications might suggest a shift towards a scavenging and, possibly, pruning role of microglia.

NNP08 | Mouse Tracking to Explore Motor Inhibition Processes in Go/No-Go and Stop Signal Tasks

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Response inhibition relies on reactive and proactive mechanisms that exert a synergic control on actions. In studies on inhibitory control, responses are usually recorded by a key-press method. However, the analysis of discrete variables (present or absent response) could be insufficient to capture dynamic features of response inhibition. We used a mouse-tracking procedure to evaluate the movement profiles related to proactive and reactive inhibition, by comparing the performance in a cued Go/No-Go (cGNG) and a Stop Signal Task (SST). The cGNG mainly involves proactive control whereas the reactive component is engaged in the SST. We hypothesize that different movement profiles could be associated with inhibitory failures in these experimental paradigms, reflecting the influence of proactive and reactive mechanisms on motor preparation and execution. 53 subjects performed the c-GNG (high vs. low Go-stimulus probability) and the SST. Velocity profiles extrapolated from mouse trajectories both for responses in the Go conditions and inhibitory failures were evaluated. Movements were classified as *one-shot* when no trajectory corrections were observed. Multi-peaked velocity profiles were classified as non-one-shot. A significantly higher proportion of one-shot profiles was found in the SST (81±9%) compared to the cGNG (21±34% and 30±33% for high and low conditions) for inhibition failures ($p<0.001$). No difference emerged between tasks for Go conditions. Trajectory corrections to the initial motor plan observed for inhibitory failures were less frequent under mainly reactive control. The opposite trend emerged when the inhibitory demand was mainly proactive. Smooth trajectories observed in rapid movements classified as one-shot suggest that the influence of inhibitory control processes on motor planning may be marginal. We hypothesized that proactive control may be responsible for unsmooth profiles in inhibition failures, supporting a differentiation between these tasks.

NNP09 | ATP6V1A silencing affects lysosomal homeostasis and autophagy causing developmental and synaptic defects in neurons

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Vacuolar ATPase (v-ATPase) is a multi-subunit proton pump that governs lysosome acidification. Lysosomes functionality is mostly required in the autophagy process, which is the major degradative pathway of proteins and organelles in cells. V-ATPase in synaptic vesicles (SVs) is responsible for their acidification and neurotransmitter loading. Recently de novo mutations in *ATP6V1A* gene, encoding the A subunit of v-ATPase, have been described in patients with developmental encephalopathies with epilepsy.
Aim: Goal of the project is to study how loss of ATP6V1A impacts on lysosomal physiology, neuronal development and synaptic function.
Methods: We selectively silenced ATP6V1A expression in primary neuronal cultures by shRNA and analysed lysosomal homeostasis, autophagy and morphology by live imaging and immunocytochemistry.
Results: The loss of expression of the ATP6V1A resulted in altered lysosomal homeostasis and impairment of autophagy progression. Silenced neurons also show impaired neurite elongation and loss of excitatory synapses.
Conclusions: Our experiments reveal a crucial role of A*TP6V1A* in maintaining lysosomal homeostasis and regulating autophagy in neurons. Loss of expression causes neurite elongation defects associated with defective synaptogenesis, suggesting that autophagy is a key cellular process for neuronal development and synapse formation and is likely impaired in patients with loss of function mutations in A*TP6V1A*.

NNP10 | Pupillary Response to Real, Illusory and Implied Motion

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Motion perception, generated by real moving objects in the visual scene, is a critical function for interacting with a dynamic environment. Motion perception can be also generated by particular structural features of a visual stimulus (illusory motion) or by static images depicting moving subjects (implied motion). Many cortical areas are involved in motion processing, particularly the medio temporal cortical area (MT), dedicated to the processing of real, illusory and implied motion (IM). Recently, there has been a growing interest in high-level visual processes that have an influence on the pupillary response. However, the effect of processing of different types of motion has not been systematically studied so far, sometimes providing contradictory results. Here, we aim to systematically investigate the effects of real, illusory and implied motion on the pupil size, by using respectively movies with real moving subjects, optical illusions of motion, and photographs depicting IM. We found different pupillary responses depending on the type of motion despite all stimuli had the same physical luminance. Real motion elicited a larger pupillary dilation than IM, that in turn caused more dilation than control photos representing static subjects (No-IM). The pupil resulted to be sensitive even to the strength of IM in the images, as photos with blurred IM resulted in a larger pupil diameter compared to simple IM. The pupil size seemed to depend also on the type of depicted content; indeed, human figures' representations caused larger dilation than objects/animals' depictions. Illusory motion caused a smaller pupil size compared to all the other conditions. Results suggest that the pupil diameter is differently modulated by different types of motion, probably reflecting selective underlying mechanisms, broadening knowledge about top-down modulations of the pupil.

NNP12 | Expression and splicing dysregulation of genes encoding for synaptic proteins in the developing cerebellum as risk factor for autism spectrum disorder

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Autism spectrum disorder (ASD) is a complex neurodevelopmental disease in which both genetic and environmental factors play a role. Mutations in many genes, especially in functional categories related to synaptic structure and function and to developmental signaling pathways, are associated with increased risk of ASD. Notably, ASD-linked genes are strongly activated postnatally in the cerebellum of both human and mice, whereas defects in cerebellar development represent a risk factor for ASD. These observations strongly suggest that the cerebellum is one of the main brain areas involved in ASD. Alterations in gene expression and regulatory process like alternative splicing during brain and cerebellum development in the perinatal window underlie many neurodegenerative and neurological diseases, including ASD. In this regard, we have recently uncovered a dynamic gene expression and splicing program, mainly affecting ASD associated genes, which accompanies post-natal cerebellum development. In the present study, we aimed at investigating the role of this gene expression program in shaping the synaptic connections in the cerebellum and the possible causes that underlie ASD onset during the perinatal window, a sensitive period for ASD. To this purpose, we used a mouse ASD model obtained through a single prenatal administration of valproic acid (VPA) in pregnant mice at embryonic day 10.5 of gestation. Since VPA exposition during pregnancy is associated with cognitive impairment, we assumed that VPA injection in utero during cerebellum development could lead to cognitive and behavioral alterations amenable to ASD. Bioinformatics analyses of the transcriptome of the developing cerebellum highlighted many genes associated with ASD that are developmentally regulated in the cerebellum. Furthermore, we found that several ASD-linked genes are aberrantly expressed or spliced in the developing cerebellum of mice that had been exposed to VPA in utero and we identified altered gene expression of many genes involved in the formation and function of neuronal synapses and whose mutations lead to social behavior defects. Our data suggests that regulation of gene expression and alternative splicing in the developing cerebellum might represent a sensitive risk factor for the establishment of ASD-related phenotypes.

NNP13 | Automatic unbiased classification of mouse behavioural states in an open field environment

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The physiological study of mice models of epilepsy and autism often requires a combined parallel behavioural analysis. Unfortunately, behavioural studies are time consuming and strongly influenced by observer bias. To overcome this problem, specific programs to automatize behavioural analyses are available but they are expensive, they lack versatility and require custom hardware. For these reasons we developed a set-up for the automatic detection of behavioural patterns in mice model freely behaving in an open field arena. The system is assembled from readily available parts and operates by using an infrared webcam and an Arduino board for hardware control. All software was developed in the MatLab environment. The acquisition process identifies the mouse contour by background segmentation and it computes mouse positions and changes in the detected area. From the position we compute locomotion, and from the changes of mouse contour we can identify rest, grooming and sleep. Control of the light cycle is automatised, ensuring minimal interference during uninterrupted recordings that can last for several days. The data analysis module exploits the study of the statistics of the time series of locomotion and contour changes to classify mice activity in exploratory behaviour, grooming/feeding, rest and sleep with no operator intervention. The outputs are matrices that describe the behavioural changes across the recording period, so they are easy to analyse and cluster in different experimental groups. To validate the setup, we analysed the behavioural consequences of the focal ablation of the gene PCDH19 as a mouse model of the human disease Female Clustering Epilepsy across several days and nights. The activity pattern recorded in the first day of each session, demonstrates increased level of anxiety with respect to the control group. We found also different speed, rest duration, and sleep patterns distribution for the two models, especially during the active phase.

NNP14 | Combining Levothyroxine with 3-iodothyronamine (T1AM) improves neurocognitive and neurobiological alterations associated with adult-onset hypothyroidism

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Hypothyroidism is associated with mood alterations and neurocognitive impairment, that point to an underlying disruption of hippocampal function. Levothyroxine (LT4) monotherapy is the gold-standard treatment for hypothyroidism. However, 5 to 15% of patients keep showing hypothyroidism-related dysfunctions, even with thyroid stimulating hormone (TSH) in the normal range. 3-iodothyronamine (T1AM) is an active thyroid hormone metabolite showing pro-learning and anti-amnestic effects. Here, we aimed at investigating whether combining LT4 and T1AM may have beneficial effects in the treatment of neurocognitive and associated neurobiological alterations in a mouse model of adult-onset hypothyroidism. Six-week old C57BL/6J mice underwent induction of hypothyroidism with methimazole and potassium perchlorate followed by replacement treatments through ALZET® pumps. We had the following groups: (1) hypothyroid; (2) LT4; (3) LT4&LT3; (4) LT4&T1AM; (5) T1AM; (6) euthyroid. Mice were tested with behavioural assays to evaluate hippocampus-dependent memory, locomotion, anxiety, and depression. Hippocampal expression of genes involved in neurogenesis was analysed by real-time PCR. Hypothyroid mice showed significant impairment in hippocampus-dependent memory as compared to euthyroid mice. Although LT4 substantially improved the discrimination index, the average best performances were observed in mice treated with LT4&T1AM, while T1AM induced no effect per se. Locomotor activity and anxiety- and depression-related behaviours remained unchanged. We found consistent significant changes in the expression of neurogenesis-related genes, such as *Dll1*, *Jak2*, *Hras1*, *Sox2*, *Shh*, *Smad4* and *Igfr1*. In our model, LT4&T1AM combination had beneficial effects on hypothyroidism-related neurocognitive and neurobiological alterations relative to LT4 monotherapy. Future studies should elucidate whether LT4&T1AM combination may prevent the neurodegenerative consequences of hypothyroidism.

NNP15 | Fast volumetric whole-brain imaging of neural activity in zebrafish larvae at single-cell resolution

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Understanding the neuronal circuits and the brain mechanisms underlying the perception of sensory stimuli and the selection of a motor program accordingly is still an open challenge.
Zebrafish larva, thanks to its small dimensions and optical transparency, in combination with the toolbox of light-based reporter of neuronal activity, represents an ideal model organism to address these questions for a rich panel of innate behaviors. Indeed, this stems from the possibility of reconstructing the neuronal activity across the whole brain at cellular resolution.
Here, we report on a novel hardware design for recording neuronal activity across the whole brain of gel-embedded larvae. With respect to the current state of the art, our design is based on a traditional multiphoton microscope integrating an electrotunable lens. This layout enables functional recording from a volume of 800 x 400 x 200 um^3 at cellular resolution and with acquisition rates that can reach 2 volumes per second when sampling 30 planes. The setup has been tested on 5 days post fertilization (dpf) zebrafish larvae during the presentation of visual stimuli consisting of a series of looming dots, a paradigm typically inducing escape response. To analyze the obtained dataset, we refined a computational pipeline for the automatic segmentation and identification of the activity profiles of the different neuronal components.
In conclusion, our approach allows reconstructing whole-brain activity of the larval brain at cellular resolution and unravelling the circuit dynamics associated with a basic task of sensorimotor integration.

NNP16 | Unravelling the 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. Here, 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. 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 setting up the electrophysiological analysis of gut motility coupled with respiratory rate analysis to evaluate how BoNT intoxication may affect enteric neurons activity, even in absence of severe systemic symptoms. 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 site of action.

NNP17 | An interaction between PRRT2 and Na+/K+ ATPase contributes to the control of neuronal excitability

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Mutations in PRoline Rich Transmembrane protein 2 (PRRT2) cause pleiotropic syndromes including benign infantile epilepsy, paroxysmal kinesigenic dyskinesia, episodic ataxia, that share the paroxysmal character of the clinical manifestations. PRRT2 is a neuronal protein that plays multiple roles in the regulation of neuronal development, excitability and neurotransmitter release. To better understand the physiopathology of these clinical phenotypes, we investigated PRRT2 interactome in mouse brain by a pull-down-based proteomic approach and identified a1 and a3 Na^+/K^+ ATPase (NKA) pumps as major PRRT2 binding proteins. We confirmed PRRT2 and NKA interaction by biochemical approaches and showed their colocalization at neuronal plasma membrane. The acute or constitutive inactivation of PRRT2 had a functional impact on NKA. While PRRT2-deficiency did not modify NKA expression and surface exposure, it caused an increased clustering of a3-NKA on the plasma membrane. Electrophysiological recordings showed that PRRT2-deficiency in primary neurons impaired NKA function during neuronal stimulation without affecting pump activity under resting conditions. Both phenotypes were fully normalized by re-expression of PRRT2 in PRRT2-deficient neurons. Taken together, these results demonstrate that PRRT2 is a physiological modulator of NKA function and suggest that an impaired NKA activity contributes to the hyperexcitability phenotype caused by PRRT2 deficiency.

NNP19 | Maladaptive responses to stress alter glutamate release in gliosomes from rat pre-frontal and frontal cortex

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Stress is a response conserved throughout the evolution and acts as an integral part of any biological system, promoting an adaptive plasticity or maladaptive and harmful effects. Exposure to acute or subacute stress can induce not only rapid but also sustained changes in synaptic function and neuroarchitecture. The aim of the present study was to investigate in the short term the effect of acute stress on the release of glutamate from gliosomes, that represent the perisynaptic areas of astrocytes. Rats were exposed to an acute paradigm of foot-shock (FS) stress and, on the basis of sucrose consumption, they were divided in vulnerable (VUL, subjects with a reduction in sucrose consumption > 25% after FS, compared to the basal consumption) or resilient (RES, subjects with a variation <10%). Differences between the spontaneous and stimulus-evoked glutamate release in these two groups of animals were evaluated in gliosomes obtained from pre-frontal and frontal cortex (PFC/FC) after sucrose test had been administered, 24 hours after FS stress. Results showed that the stimulus-evoked release of [^{3}H]D-Asp, used to label the glutamate gliosomal pools, was significantly increased after FS-stress in VUL rats only. In VUL rats, TFB-TBOA, a glutamate EAAT1 and EAAT2 inhibitor, significantly reduced [^{3}H]D-Asp release; while, the exposure to a calcium-free solution produced a non-significant decrease [^{3}H]D-Asp release. These data suggest that acute FS stress produced an increase of [^{3}H]D-Asp release from PFC/FC gliosomes, possibly increasing the glutamate availability in the synaptic cleft. This increase could be observed only in rat that were VUL to stress application. Glutamate release seems to be supported by reversal of glutamate transporters and, possibly, by exocytosis.

NNP20 | A point mutation in Nerve Growth Factor provides new clues on how the brain creates pain-related memories

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Creating associative memories between painful events and the context in which they occurred is an essential adaptive function of complex organisms, aimed at preserving bodily integrity.
Hereditary Sensory and Autonomic Neuropathy type V (HSAN V) is a rare disorder caused by the R100W mutation in the *Ngf* gene, characterized by partially impaired pain perception, and by the inability to assess the potential harmfulness of a situation, leading to repeated traumas.
To elucidate the neurobiological basis of this peculiar phenotype, we have created and characterized a transgenic mouse model for HSAN V. These animals have normal learning and memory and, thus, represent an opportunity to elucidate how the brain processes pain-related information. To this aim, we used the cued fear conditioning test (CFC). By adjusting the conditioning stimulus intensity, we were able to elicit in HSAN V mice a behavioral response similar to wild type controls. Accordingly, HSAN V mice normally learned the cue-shock association. Strikingly, in the recall phase of CFC, HSAN V mice displayed impaired freezing, indicating a deficit in pain-related memory recall.
These behavioral findings were paralleled by a lower expression of the activity-dependent protein cFos in key brain regions involved in the formation of memories triggered by painful stimuli. On the other hand, innate fear responses of HSAN V mice were normal.
Finally, we found a decrease in the density of somatostatin “fear-on” interneurons in the amygdala, which could provide a circuit basis for the altered recall in fear memories shown by HSAN V mice.
These findings provide new insights into how the brain constructs an essential perception for survival, in addition to providing a novel function of NGF, that extends its role in pain, from a key regulator of peripheral nociceptor development and sensitization to a function of controller of central pain elaboration.

NNP21 | Kinematics analysis and study of the distributed cortical activity emerge in the mouse neocortex during Reach-to-Grasp task.

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Goal-directed movements are among the most complex movements that many animal species perform daily. Coordinated activity across the neocortex has been recently shown to drive complex behaviour in the mouse, while activity in selected areas is canonically associated with specific functions. Reach-to-grasp (RtG) task is known to be dependent upon motor circuits of the neocortex, however, the global activity of the neocortex during these movements has been largely unexplored in the mouse.
The goal of this project is to characterize the neuronal activation patterns distributed throughout the mouse cortex during the execution of RtG. To this aim, we correlated the large-scale cortical activity with the kinematic features of these goal-directed movements.
We performed wide-field imaging of intracellular calcium transients through the intact skull over both cortical hemispheres using transgenic mice (Thy1-GCaMP6f) that express the genetically encoded fluorescent indicator GCaMP6f in a subset of excitatory cortical neurons.
A custom-made hybrid setup allowed detecting cortical activity during the training sessions and video recording the limb that performs the task.
To conduct the kinematic analysis, the trajectories of the limb were manually traced from the videos. For the analysis of cortical activity, was extracted the calcium profile (expressed as fluorescence variation ΔF/F) from the parcellated cortical regions.
During RtG movement planning and execution, we observed the activation of the areas canonically associated with the movement, in addition to sensory, visual, and associative areas. The activation emerged on both hemispheres although being a unilateral task. Finally, while neural activity increases globally during RtG, neuro-kinematic correlations are significant only for the secondary motor cortex.
These results suggest that RtG may involve large neural networks across the cortex while the encoding of kinematic parameters occurs primarily in segregated areas.

NNP22 | PCDH10: A NOVEL SUMO TARGET IN SYNAPTIC FUNCTION AND DYSFUNCTION

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Sumoylation is a post-translational modification essential to the modulation of neuronal functions. Altered sumoylation has been associated with neurodevelopmental disorders. Here, we identified and validated PCDH10 as novel SUMO target in mammalian brain. PCDH10 is an adhesion molecule involved in synapse elimination and etiology of autism spectrum disorders (ASD) in human. Accordingly, mice lacking one copy of the *Pcdh10* gene present sociability deficits as well as increased spine density. To date, the molecular mechanism underlying PCDH10 function in neurons remain obscure. We hypothesize that sumoylation, by controlling PCDH10 function, controls spine morphology and synaptic density. We demonstrated that the unmodified and sumoylated forms of PCDH10 are developmentally regulated. In particular, total PCDH10 increases during the brain development and reaches the plateau in the adulthood. Interestingly, after birth, the expression of PCDH10 gradually switch from a long to a short form suggesting a specific function of these two isoforms during the brain formation. Conversely, the sumoylated form of PCDH10 is almost absent in the adult and embryonic brains and peaks between post-natal day 7 and 16. This period is critical for the development of a proper neuronal network. Similarly, in primary cortical neurons the sumoylated form of PCDH10 peaks during the synaptogenesis and dramatically decreases in mature neurons. We also identified the lysine 831 (K831) as residue targeted by sumoylation in PCDH10 sequence. Interestingly, the K831 is located within the proteasome-interacting region (PIR) which is critical to PCDH10-dependent synapse elimination suggesting that sumoylation participates to this event by controlling the PCDH10-proteasome interaction. Altogether, our results suggest that sumoylation of PCDH10 could contribute to control excitatory synapse elimination.

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NNP23 | 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 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 by large unclear.

Here, through genetic lineage-tracing experiments and 3D reconstructions coupled with mathematical modelling and computer simulations we dissected the transition of striatal astrocytes toward neurogenesis. In the striatum, neurogenic astrocytes are scattered throughout the parenchyma and expand locally, generating clusters of clonally related cells, that we define as striatal niches. These structures are initially composed only of activated astrocytes and transient amplifying progenitors. These latter cells subsequently expand and generate proliferating neuroblasts following a stochastic mode of division and differentiation. Post-mitotic neuroblasts accumulate in the cluster before dispersing as individual cells. Interestingly, striatal astrocytes become activated at a constant rate, resulting in the continuous addition of new striatal niches with time. Nevertheless, the total number of niches does not increase with time indicating that these structures have a transient existence. Thus, continuous striatal neurogenesis occurs through the asynchronous transition of scattered neurogenic astrocytes from quiescence to an active state.

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.

PNPN01 | Rac GTPase in Intellectual Disability: preclinical opportunities from interfering with a Rac1 protein::protein interaction

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Intellectual Disability (ID) is a neurodevelopmental disorder characterized by retarded cognitive maturation. The X-linked forms are hereditary, non-syndromic, and account for 10-20% of all genetic cases of ID. Among them, MRX46 is caused by mutations of ArhGEF6 (hemizygous), loss-of-function mutations in Rac1 (heterozygous) and PAK3 (homozygous), all leading to reduced Rac signaling. Rac1 is a small GTPase of the Rho family and is a key signal-transducing enzyme, linking extracellular signals to the control of cytoskeleton dynamics. Rac1 plays important roles in brain development and is regulated by activating (GEFs) and inactivating (GAPs) proteins, that operate via protein::protein interaction (PPI). Rac is druggable and can be reactivated by preventing the interaction with its negative regulators. We propose to exploit the Rac-specific, brain-specific ArhGAP15 and the ArhGAP15::Rac1 PPI to achieve reactivation of Rac1 in neurons with mutated ArhGEF6 and hypoactive Rac1 by using PPI interfering peptides. We solved the ArhGAP15::Rac1 complex 3D structure using a homology model refined by molecular dynamics and alanine scanning to improve the prediction of the binding hotspots. Two methionine on ArhGAP15 are expected to form strong hydrophobic interactions with two phenylalanine on Rac1. The block of these hotspots should prevent complex formation, halting the activity of the catalytic residue of ArhGAP15 and reactivating Rac1. To test it, we are generating an isogenic human iPSC line with disrupting mutations in ArhGEF6, efficient in neuronal differentiation. We aim to prove that a peptide-based approach, followed by a small-organic compounds screening, can restore normal networking and connectivity in vitro and can alleviate the ID condition in the ArhGEF6$^{-/Y}$ mouse model. The possibility that cognitive deficits can be relieved by remodulating the GTPase pathway has been strongly supported by recent data on a X-linked RhoA hyperactive model of ID.

PNPN02 | 3D Human Cortical Organoids to investigate developmental epileptic encephalopathies

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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. Early Infantile Epileptic Encephalopathies (EIEE), 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. EIEE 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 EIEE 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. Identification the of aberrant pathways associated with epileptic seizures will constitute an invaluable resource to discover novel anti-epileptic drug targets for infantile/pediatric epilepsy.

PNPN03 | Zika virus but not other members of flavivirus determines FOXG1 delocalization from nucleus to cytoplasm

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Zika virus (ZIKV) is transmitted in humans by the *Aedes* mosquito or sexually transmitted. A large percentage of adult individuals with ZIKV infection show mild symptoms, however ZIKV crosses the placental barrier, causing severe congenital malformations in the fetus including microcephaly, brain abnormalities and early epilepsy in children. It is interesting to note that certain characteristics typical of compromised brain development caused by ZIKV can be found in a congenital alteration of the Forkhead box G1 protein (FoxG1) both anatomically (microcephaly) and symptomatically (epilepsy). Indeed, FoxG1 is a nuclear transcription factor only expressed in the telencephalon during embryogenesis, where it slows down staminal cell differentiation. When FoxG1 exits from the nucleus of normal cells these differentiate into neurons.

The present work aims to elucidate the interactions between the ZIKV infection and FoxG1 in IPSC-derived human Neuronal Embrionic Stem (NES) cells derived from human cortex.

Our work shows that after ZIKV infection, FoxG1 migrates from the nucleus to cytoplasm where the transcription factor is degraded. The mechanism underlying FoxG1 migration normally is thought to depend on by the AKT-mediated phosphorylation in Thr271 residue. To probe so, we produced a recombinant FoxG1 protein without the AKT phosphorylation site (T271>A). Our results show that the mutated FoxG1-T271>A does not migrate to the cytoplasm after ZIKV infection in NES cells. These data support the preliminary evidence that ZIKV acts by a pAKT-mediated mechanism to cause FoxG1 delocalization in infected cells.

In conclusion the present work identifies a possibly novel ZIKV induced mechanism of microcephaly caused by the anticipated FoxG1 disruption in NES cells.

ND01 | Discovering multiple ways of action of saffron, an in vivo and in vitro study

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Saffron (Sfr) is one of the most promising neuroprotective natural agent in the treatment on different retinal pathologies such as Age-related Macular Degeneration or Stargardt disease. Experiments on animal model and cell cultures suggest the involvement of multiple ways of action but details on mechanisms and on the real potential of Sfr are still under debate. It was proved that Sfr with slight different chemical composition has different neuroprotective efficacy, accordingly: 1) we compared the most active Sfr selected, named REPRON, with others chemically selected batches. In particular, the efficacy was assessed on in-vitro model of light-induced stress in cells from retinal pigment epithelium (RPE), which play a relevant role in photoreceptors survival. Sfr; 2) we tested the best Sfr composition (REPRON) on two types of spontaneous retinal dystrophic rats (RCS and Fischer). In particular, animals were treated with Sfr 1 mg/kg/day and at the end the retinal healthiness was assessed in terms of function (flash-ERG recordings), photoreceptors survival and expression of trophic (FGF2) or inflammatory factors (GFAP). Results show that REPRON treatment in mild dystrophies like in Fischer rats, can significantly improve both function and morphology of the retina. On the other side, in the severe dystrophy of RCS rats, the spice can barely preserve photoreceptors. In the in-vitro model different types of Sfr can differentially protect ARPE19 cells from death. Interestingly, REPRON has better outcomes compared to the other batches. In conclusion, the chemical composition of Sfr is critical to provide effective neuroprotection but the best results is obtained in degenerative processes where oxidative stress, Muller activation and neuroinflammation play relevant role in the progression of the disease.

ND02 | Ablation of N-Acytethanolamine acid amidase gene affects Zika virus replication

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The central nervous system (CNS) is considered an organ with immune privileges through various barriers, such as the blood-brain barrier (BBB) which prevents pathogen access. However, some viruses are able to cross the BBB and reach the CNS where they cause a wide range of dysfunctions. N-Acytethanolamine acid amidase (NAAA) is an enzyme of 359 amino acids belonging to choloylglycine hydrolase family. NAAA hydrolyses ethanolamides, having palmitoylethanolamine (PEA) as preferred substrate. PEA has analgesic, anti-inflammatory and neuroprotective properties. Here, we want to evaluate whether an intracellular increase in PEA affects the replication of ZIKA virus (ZIKV), an emerging arbovirus of the genus Flaviviridae, implicated in neonatal microcephaly. To explore the role of NAAA in ZIKV replication we generated A549 cells NAAA knock out (KO) using CRISPR/Cas9 system. We observed a reduction of ZIKV infectious particles and a significant reduction in intracellular viral proteins in A549 NAAA KO cells. Consistent with these findings, we observed a significant decrease in viral titer in the supernatants of A549 NAAA KO cells. Moreover, downregulation of NAAA led to high levels of autophagy-related proteins, suggesting a potential degradation pathway that can limit ZIKV replication. Recently, it has been demonstrated that SARS-CoV-2 does not limit its presence to the respiratory tract and frequently invades the CNS. Considering the similarities between ZIKV and SARS-CoV2, we decided to test whether NAAA ablation might affect SARS-CoV2 replication. We observed a 10-fold reduction in viral load in NAAA KO cells. Transmission-electron microscopy showed that SARS-CoV2 was trapped into multi-vesicular bodies, suggesting viral replication impairment. In conclusion, this work explores a novel antiviral mechanism in which the expression level of NAAA can decrease ZIKV replication and can modulate its neurovirulence. Such antiviral activity was also observed for SARS-CoV2.

ND03 | Exploring the possible involvement of LRRK2-PAK6 pathway in ciliogenesis: implication for neuronal physiology and pathology

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Primary cilia are small protrusions that represent a signaling platform for the regulation of crucial cellular and developmental pathways. These organelles detect changes in the extracellular environment and regulate specific pathways. Mutations in genes involved in ciliogenesis are responsible for a group of human disorders called ciliopathies, mostly linked to defective brain development. It has been reported that the presence of functional primary cilia increases the resilience of dopaminergic neurons exposed to toxins that mimic Parkinson's disease (PD) thus exerting a neuroprotective role. Ciliogenesis was recently reported to be regulated by the PD-linked Leucine-rich-repeat kinase 2 (LRRK2), a large multidomain protein whose biological functions are not completely known. Our group demonstrated that the kinase p-21 activated kinase 6 (PAK6) interacts with LRRK2 and modulates its activity. A constitutively active form of PAK6 was found to rescue the G2019S LRRK2-associated neurite shortening phenotype via phosphorylation of 14-3-3γ. 14-3-3s proteins are known to stabilize LRRK2 and to coordinate its subcellular localization and access to substrates. Intriguingly, PAK6-mediated phosphorylation of 14-3-3γ result in loss of affinity between LRRK2 and 14-3-3γ with consequent LRRK2 dephosphorylation, suggesting that PAK6 may control LRRK2 localization in cells. Among LRRK2 substrates, a well-established group is represented by Rab GTPases, master regulators of membrane trafficking. It was recently demonstrated that LRRK2 block primary cilia formation through Rab10 phosphorylation. We here report that in SHSY-5Y cells stably overexpressing PAK6, LRRK2-mediated phosphorylation of Rab10 is strongly reduced and cilia length and number are increased as compared to naïve and PAK6 knockdown cells. In summary, these findings support the fascinating hypothesis that there is an interplay between PAK6 and LRRK2 in modulating ciliogenesis in brain cells.

ND04 | Evidence for glutamate transporter dysfunction in G2019S LRRK2-linked Parkinson's disease

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Parkinson's disease (PD) is characterized by the loss of dopaminergic neurons in the *substantia nigra pars compacta*. Many cell types, e.g. astrocytes, contribute to PD pathophysiology. A key role of astrocytes is the ability to recover glutamate from synapses through specific transporters, avoiding neurotoxicity. The excitatory amino acid transporter type 2 (EAAT2; Glt-1, in rodents) plays a major role in glutamate reuptake and it is mainly expressed in astrocytes. EAAT2/Glt-1 deficit leads to the accumulation of extra-synaptic glutamate inducing excitotoxicity, gliosis and neurodegeneration. Here, we showed a decrease in Glt-1 protein level associated with astrogliosis in mice harboring the PD-linked G2019S mutation in *Lrrk2*. The G2019S mutation in LRRK2 is the most common cause of familiar PD and increases the kinase activity of the protein by 3 folds. A strong reduction of EAAT2 protein and gliosis has been also detected in *post mortem* brains from LRRK2 G2019S patients. Preliminary data on gliosomes (purified astrocytic terminals) indicate a decreased glutamate reuptake in G2019S Lrrk2 mice pointing to a reduction of functional Glt-1 at the plasma membrane. Coherently, voltage clamp recordings in *Xenopus laevis* oocytes co-injected with EAAT2 and LRRK2 mRNA revealed that G2019S LRRK2 reduces the transport current of EAAT2. Using imaging approaches, we revealed that the glutamate transporter redistributes in intracellular Rab4-positive recycling vesicles in Lrrk2 G2019S primary astrocytes. Of note, the pathological phenotype has been reverted by the pharmacological kinase inhibition. Overall, we propose that LRRK2 kinase activity negatively operates in Glt-1 recycling and reduces the functional Glt-1 at the plasma membrane, thus promoting Glt-1 degradation over time. Our results unravel a novel pathway deregulated in PD and open a new therapeutic window for a disease that, at present, has no cure.

ND05 | Dynamic interaction between copper and amyloid-β species.

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Alzheimer's disease is strongly associated to the deposition of misfolded proteins and redox-active metals. The interaction of copper with amyloid-β induces structural and functional alterations, beside the production of reactive oxidative species. The oxidative environment can be also promoted by the intrinsic reactivity of neurotransmitters, as dopamine, to give heterogeneous products, as neuromelanins. In this work, the redox behavior of Cu-Aβ adducts was assayed to characterize the active species involved in the metal redox cycle promoted by catechols. The *N*-terminal domain 1-16 of Aβ shows the dominant Cu ligands and is sufficient to mimic the reactivity of the full-length peptide 1-40/42. In particular, two "resting states" for Cu(II)/(I) interaction were suggested but the structural rearrangement and the energetic barrier between the redox states indicate the existence of some catalytic intermediate. A reactive "in-between state" in the ascorbate oxidation and involving the *N*-terminal amine, aspartate-1 and one histidine as ligands was proposed as transition species. To assay its participation in the catechol oxidation, the catalytic activity of Cu bound to Aβ16 in unprotected or acetylated forms was compared. The redox behaviour of the complexes suggests *N*-acetylation of Aβ enhances the catalytic potential of the adduct, removing a strong Cu^{II} binding site and facilitating its reduction. The oxidative mechanism views an initial step that generates Cu(I)-complex and a second one in which O_2-binding for re-oxidation acts as rate-limiting reaction. Upon the use of point-mutants of Aβ, we can asses the key intermediate is a Cu^{I}-intermediate in which Cu^{I} is linearly stabilized via two His; this geometry is not optimal for O_2 interaction and the substrate binding to give Cu^{I}/Cat/Aβ complex is required for the efficient oxygenation. These mechanistic studies are prerequisite to clarify the biochemical processes associated to AD's progression.

ND06 | A new therapeutic strategy for Spinal Muscular Atrophy symptomatic patients: CPPs-conjugated antisense nucleotides

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Spinal muscular atrophy (SMA) is a motor neuron disease caused by mutations in the Survival Motor Neuron 1 (SMN1) gene, resulting in deficiency of SMN protein. Most of the emerging therapies are based on redirecting the splicing of SMN2, a paralogous gene, to produce a functional SMN protein. In our laboratory, a specific sequence of a Morpholino (MO) antisense oligonucleotide (ASO) against the ISS-N1 region of SMN2 has been successfully tested in pre-symptomatic SMAΔ7 mice. However, our MO10-34 treatment, like other treatments tested for SMA, shows efficient results only when administered in a pre-symptomatic phase. To develop a suitable treatment for symptomatic patients, cell-penetrating peptides (CPPs) can be conjugated to ASOs, allowing the crossing of the blood-brain barrier (BBB). Preliminarily experiments on pre-symptomatic mice led to selection of two peptides, r6 and RXR, for further experiments. To verify the ability of MO-conjugated to CPPs to ameliorate SMAΔ7 pathological features compared to unconjugated-MO, different groups of symptomatic animals were treated at P5 with r6-MO, RXR-MO and unconjugated MO by intraperitoneal injection. The first group was monitored for survival and phenotypical test, while mice of the second group were sacrificed at p10 or p30 to harvest spinal cord, liver, heart, kidney, blood and intercostal and quadriceps muscles, then analysed by immunofluorescence for the presence of motor neurons (MNs), innervated neuro muscular junctions (NMJs), gliosis and for muscle fibers shape and organization. We also evaluated *Igf1* expression in spinal cord and liver, MO biodistribution by ELISA assay and toxicity markers in blood of treated mice, with no significant alterations compared to controls. Our results confirm that both CPPs-conjugated MOs ameliorate the biodistribution of the MO into the CNS increasing significantly phenotypical and neuropathological features compared to unconjugated MO, without causing toxic effects.

ND07 | Tau cleavage in retinal degeneration: translational implications for Alzheimer's Disease.

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Retina and optic nerve are sites of early extra-cerebral manifestations of Alzheimer's Disease (AD). Amyloid-beta (Abeta) plaques and neurofibrillary tangles of hyperphosphorylated tau protein are detected in eyes from AD patients and transgenic animals along with inflammation, synaptic reduction, visual deficits, retinal ganglion cell and axonal loss in optic neuritis. However, the pathological relevance of other post-translational tau modifications -such as truncation with generation of toxic fragments- has not yet been investigated. Moreover, whether the removal of these deleterious tau-derived peptides exerts protective action on both AD retinal and cerebral neurodegeneration remains unclear.

We have recently developed a monoclonal tau antibody (12A12mAb, 26-36 tau aminoacids) which selectively neutralizes the AD-relevant neurotoxic peptide, derived from cleavage of tau protein at its N-term domain, without cross-reaction towards its full-length normal form. 12A12mAb, when intravenously-injected into 6-month-old Tg2576 animal model, significantly improves the AD-like behavioural and neuropathological hippocampal syndrome.

By taking advantage of our well-established tau-directed immunization regimen, we found out that the 12A12mAb admininistration also exerts a beneficial action on some biochemical and morphological signs of ocular injury associated with animals' AD phenotype.

This study has important translational implications in the AD field by showing for the first time that tau cleavage plays a crucial role in triggering the pathological changes occurring *in vivo* in affected retina and vitreous bodies.

ND08 | The effect of extracellular vesicles carrying β-amyloid along the cortico-hippocampal connections

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A major neuropathological hallmark of Alzheimer's disease (AD) is the accumulation of extracellular amyloid-beta (Aβ). Extensive literature implicates Aβ-dependent synaptic dysfunction as an early mechanism affected in AD, that propagates from the Entorhinal Cortex (EC) to connected regions, such as the hippocampus and leading to an aberrant network activity. However, how synaptic dysfunction starts, propagates and causes network alteration is not yet clear. Recent evidence suggests that extracellular vesicles (EVs) secreted from microglia may facilitate delivery of pathogenic proteins, including Aβ, over large distances moving from neuron-to-neuron through their projecting axons. To test the effect of EVs carrying Aβ along the EC-hippocampal connections we performed *in vivo* chronic EEG recording in freely moving mice. All mice were recorded daily during a baseline period and then injected in the lateral EC (LEC) with vehicle, Aβ-EVs, Aβ [1-42], ctrl-EVs or Aβ-EVs + Annexin V (which reduces EVs movement). Moreover, we evaluated the behavioural effect of Aβ-EVs injection either using LEC-dependent associative or hippocampal-dependent non-associative tasks based on object recognition. The results confirmed that Aβ-EVs induced a network dysfunction characterized by increased cortico-hippocampal excitability and the presence of spike-wave discharges, a typical feature of absence epilepsy and early AD. Behavioural testing revealed an impairment in memory that was limited to the LEC-dependent task shortly after the injection of Aβ-EVs but involved the non-associative hippocampal dependent task at later time points. All these effects were reduced by Annexin V vesicles pre-treatment suggesting that Aβ-EVs movement contributes to the disruption of cortico-hippocampal network activity and memory impairment. Our model proposes a new biological mechanism for the spreading of Aβ pathology and offers the opportunity to test treatments targeting the early stage of the disease.

ND09 | Specific miRNAs Shuttled by Exosomes Derived from Mesenchymal Stem Cells Affect the Inflammatory Phenotype of Late Symptomatic SOD1G93A Mouse Astrocytes in Culture

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Amyotrophic lateral sclerosis (ALS) is a motor neuron disease. Despite the significant progress in genetic studies in ALS, the cause of the majority of sporadic cases remains unknown. Currently, epigenetics, also involving miRNA studies, show some promising aspects. We previously reported that the administration of IFNɣ-primed mesenchymal stem cells (MSCs) in the SOD-1^{G93A} mouse model of ALS produced positive effects on disease progression, also modulating astrocytes and microglia reactive phenotypes. We proposed that MSC effects were paracrine, possibly involving exosome-mediated cell communication. We investigated here the activity of nine miRNA, which were found up-regulated in IFNɣ-primed MSCs and shuttled by MSC-derived exosomes, on spinal cord astrocyte primary cell cultures from late symptomatic 120 days-old $SOD1^{G93A}$ mice. We transfected $SOD1^{G93A}$ astrocytes with the single synthetic miRNAs and analyzed their effect on the astrocyte phenotype and the involved inflammatory pathways. Seven out of nine miRNA mimics were able to affect the reactive phenotype of $SOD1^{G93A}$ astrocytes by significantly decreasing the over-expression of GFAP, IL1β, and TNFα, detected by confocal microscopy. We focused on four active miRNAs (466q, 467f, 466m5p, 466i3p), which were overexpressed in both MSCs and exosomes. We selected in-silico their relevant inflammation pathways (p38, TNFα and NFKB) and validated them by determining the miRNA impact on MAP3K8, MAPK-APK2, MAPK11, and TRAF6 in astrocytes by qPCR. Two of them (466q, 467f) strongly reduced MAPK11 mRNA expression, thus inhibiting TNFαformation. We can conclude that miRNAs shuttled by exosomes may represent a way to trigger the paracrine beneficial effects of MSCs.

ND11 | Characterization of microglia and synapses in the early brain of an AD mouse model

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Microglia are the resident macrophages and immune cells of the brain. Besides their immunity role, microglia also orchestrate several processes, critical for proper neuronal functioning. They are implicated in a variety of tasks ranging from removal of neuronal debris to precise refinement of synaptic terminals, contributing to maturation and monitoring of neuronal circuits. In neurodegeneration, such as in Alzheimer's disease (AD), microglia display inflammatory profiles and excessive synaptic phagocytosis, leading to pathological synapse loss. Most of the studies have been conducted to characterize the role of microglia in overt pathological stages. However, whether synaptic pruning by microglia in AD brains initiates long before, e.g. during brain development, has received little attention. To address this question, we have investigated microglia and synapses in the early brain of an AD mouse model, using transgenic mice overexpressing the human Amyloid Precursor Protein (hAPP) carrying the Arctic and Swedish mutations (ArcAb). We used biochemical and histochemical complementary approaches, to assess microglial morphology and density, as well as dendritic spine density and synaptic markers, in the hippocampus of two weeks old mice, to identify early alterations. In addition, lipidomic profile of hippocampal synaptosomes at this age is currently ongoing, and will reveal whether hAPP overexpression affects synapses during brain development. Overall, these data will inform us whether microglial and synaptic alterations are already present at early stages in the brain of an AD mouse model, thus providing susceptibility to neurodegeneration later in life.

ND12 | New insight of meningeal fibroblasts from human brain donors

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Meninges consist of three membrane that surround and protect the central nervous systems (CNS). Primitive meninges play a crucial role in the genesis of cerebral cortex and cerebellum. During adulthood, meninges contribute to the homeostasis of the CNS and the restoration of the blood-brain barrier after injury, releasing trophic factors and extracellular matrix component (ECM). Our goal is to study in-depth the human meningeal fibroblasts derived from brain donors, to characterize cells, and to confirm the presence of neural stem cells, a possible source of new cortical neurons that can partially overcome the senescence and the neurodegeneration. We isolated meningeal fibroblasts (MFs) and skin fibroblasts (SFs) from 6 brain donors. We evaluated the timing of cell appearance, the cell orientation, and the growing rate. We evaluated four protein markers (i.e. Fibronectin, Serpinh1, Beta-III-Tubulin, and Nestin) by immunofluorescence and differentially expressed genes through whole transcriptome analysis in MF compared with SF. MFs show a quicker cell mobility than SFs, and a different cellular orientation, more organized in SFs, probably due to a different tissue function. SFs show a more rapid growth respect to the MFs. Both MFs and SFs show the same expression level of protein markers Fibronectin, Serpinh1, and Beta-III-tubulin, but a different Nestin expression, a neural stem cell marker, more evident in MFs. We found 1145 deregulated RNAs, 52% of which were coding genes. Deregulated pathways show the involvement of different signaling pathways, with an important involvement in ECM organization and the focal adhesion, confirming the cellular differences founded at the morphological level. We can conclude that MFs and SFs are different in term of cell orientation, the grow rate, and the transcriptional profile. The expression of Nestin in MFs will be used for study in aging related pathology and tissue regeneration.

ND13 | Analysis of sphingolipids pattern in microglia after treatment with a remyelinating promoting antibody

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Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system and a major cause of neurological disability in young adults. The adult brain can, to a certain degree, repair damaged myelin however the process is inefficient, and its efficacy lowers during disease progression. Thus, strategies aimed at stimulating myelin repair do represent a valuable therapeutic option. Microglia has an important role in remyelination. It activates after myelin damage and removes myelin debris, which are a non-permissive signal for the remyelination process. Recombinant human IgM22 (rHIgM22) promotes remyelination in mouse models of MS and, although little is known about its mechanism of action, it stimulates microglia clearance of myelin debris. Since we observed that rHIgM22 induces deep changes in the sphingolipid patterns of cells belonging to the oligodendrocyte lineage, we analysed the BV-2 microglial cell line after treatment with rHIgM22. Cells were subjected to a steady-state metabolic labelling with ^{3}H-sphingosine. Radiolabelled lipids were extracted, partially purified, separated by High Performance Thin Layer Chromatography and quantitatively analysed by digital autoradiography. As a result, we observed significant changes in the lipid composition of BV-2 cells respect to control. In particular, we observed an increase of several gangliosides, whose exact identity is currently being determined through ESI mass spectrometry. Literature suggests that the mechanism of action of rHIgM22 might be mediated by the reorganization of sphingolipid-driven signalling complexes at cell surface, in cholesterol and sphingolipid enriched domains. After clarifying which lipids are involved in the rHIgM22 mediated signalling, we will investigate which proteins could be involved in the activity of these signalling complexes and if modulation of the composition of the lipid microenvironment could affect their activity, and thus microglia function.

ND16 | Lithium administration in a mouse model for Globoid cell leukodystrophy

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Globoid cell leukodystrophy (GLD, or Krabbe disease) is a rare and incurable leukodystrophy caused by deficiency of the lysosomal enzyme galactosylceramidase (GALC), which leads to accumulation in the nervous system of the cytotoxic sphingolipid psychosine (PSY).

Autophagy is a crucial lysosomal pathway that allows recycling cellular proteins, damaged organelles and lipids. Recently, impaired autophagy features have been demonstrated in *in vitro* GLD models. Furthermore, two autophagy inducers, Lithium and Rapamycin, could reinstate the healthy phenotype in GLD cells.

Here, we test the effect of Lithium *in vivo*, in a spontaneous GLD mouse model [the Twitcher (TWI) mouse]. We administer Lithium by drinking water and perform motor behavioral experiments (grip strength, wire hang and rotarod tests). Additionally, we quantify the autophagy markers LC3 and p62 in brain and sciatic nerve, to assess the ability of Lithium to stimulate autophagy.

Results show a mild improvement in the ability of the treated mice to perform the grip strength and wire hang tests 5 days after treatment. After 15 days, instead, only wire hang capability shows slight improvement. No improvement in the ability to perform the rotarod test is observed. Unexpectedly, Lithium does not seem to induce autophagy at the concentrations used. We hypothesize, instead, that it can inhibit the activity of the glycogen synthase kinase 3 (GSK-3β), leading to the activation of the cytoprotective b-catenin/Tcf pathway. Further studies to validate this possibility are needed.

These results provide for the first time data about the effect of an autophagy inducer on the TWI mouse, indicating a line along which new therapeutic approaches for GLD might be developed.

ND17 | Synthesis of Novel Hydantoin-based Peptidomimetics as a New Weapon Against Neurodegenerative Disorders

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Peptidomimetic compounds have almost overcome peptide and peptide-derived drugs because they offer better transport properties, resistance to enzymatic cleavage/degradation and even to immune response. These molecules are very attractive in the realm of targeted therapy. The target of our study is Insulin Degrading Enzyme which is involved in the degradation of insulin and other amyloidogenic substrates. By modulation of the action of this enzyme, it is possible to open new therapeutic strategies to treat many IDE-dependent pathologies (like Alzheimer's disease and other neurodegenerative disorders). Among many different molecules, we knew that we needed an easily accessible β-turn mimetic able to offer virtually unlimited screening possibilities due to its ease of modification. We then demonstrated that the chemical structure needed to achieve the previously mentioned benefits was a molecule containing an hydantoin core. This scaffold showed to be very important since it can be properly functionalized, allowing the molecule to adapt to a wide range of kinetically and thermodynamically accessible conformations, that can subsequently mimic selected secondary protein structures. The mimicry of peptide secondary structures and their adjustment to hot spots is crucial, since the modulated interaction can lead to major or minor conformational changes of the protein or its parts, resulting in beneficial effects. The synthetic sequence implies the formation of an isocyanate that will react with an amine to form a urea. This urea is the key intermediate for the preparation of the hydantoin core, since it is involved in an intramolecular cyclization in basic conditions. We synthesized three candidates for biological evaluation and we supported our study with Molecular Mechanics calculations in order to verify the possible conformations that our molecules will adopt.

ND18 | Expansion and characterization of human adipose derived stem cells inside the 3D micro-niche Nichoid

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Stem cells (SCs) have shown great promises in the treatment of various diseases, especially neurodegenerative ones. Human Adipose Derived Stem Cells (hADSCs) represent a promising source for cell therapy, especially as their isolation is less invasive compared to the isolation from other organs or tissue. In the body, SCs reside in a specialized microenvironment, characterized by a unique combination of biophysical and biochemical properties. Together, these features allow the maintenance of stemness. Recently, biomaterials have been used to create 3D micro-scaffolds, which mimic the biomechanical characteristics of SC niches. In this context, the micro-niche "Nichoid" has shown the ability to induce pluripotency in SCs. The aim of this study was to investigate the proliferation and stemness properties of hADSCs expanded in this engineered niche.

Nichoids were fabricated by laser two-photon polymerization onto glass coverslips using a biocompatible photoresist. hADSCs were isolated from lipoaspiarate adipose tissue obtained by elective liposuction performed in voluntary subjects with full knowledge of the events. Cells expanded inside the Nichoid have been characterized by viability assay, Real Time PCR and immunofluorescence analysis. Furthermore, adipose and neural differentiation were investigated.

hADSCs grown inside the Nichoid show a significantly higher cell viability than in standard conditions. Furthermore, the expression of pluripotency markers was upregulated inside the Nichoid, suggesting that the 3D micro-scaffold permits the potentiation of stemness features.

Together, these results demonstrate that the Nichoid drives stem cell pluripotency maintenance without any chemical media. Our findings represent a great promise for the Nichoid's possible application in the field of regenerative medicine, in particular in cell transplantation in preclinical animal model of Parkinson's disease and Spinal Cord Injury.

ND19 | Genetic modulation of anti-oxidant nutraceutical molecules slows down retinal degeneration in a mouse model of Retinitis Pigmentosa

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Rhodopsin (RHO) mutations are responsible for 25-40% of the dominant cases of Retinitis Pigmentosa (RP), with distinct amino acid substitutions causing different RP severity and progression rates. Tvrm4/+ mice, heterozygous for a I307N dominant mutation of RHO, display a normal retinal phenotype when raised in ambient light conditions, but undergo photoreceptor degeneration when briefly exposed to strong white light. Then, this animal model presents a typical rod-cone degeneration in an area concentric to the optic disc. Here, Tvrm4/+ mice will be pre-treated with 100mg/kg/day of Naringenin or Quercetin or vehicle (DMSO 0.25%) in the drinking water for 30 days. On the 30th day retinal degeneration will be induced by exposure for 1 minute to a white light of 12000lux intensity and the treatment will continue for another 5 days. The treated animals will then be compared with a group of healthy controls. At the end of the protocol retinal functionality was tested by recording ERG flash response in both scotopic and photopic conditions. Retinal tissue was collected from recorded mice and the tissue was used for further analyses including biochemical and molecular biology assays. The data obtained show that treatment with nutraceutical molecules is effective in counteract retinal degeneration by preserving the functionality of photoreceptors and increasing the antioxidant defenses of cells. The profile of the genes involved in oxidative stress is visibly modulated by the molecules administered, highlighting, especially for quercetin, a profile almost superimposable to that of healthy controls. As shown in our previous work, on an animal model of autosomal recessive RP (rd10), the data shown here confirm that molecules of natural origin are able to slow photoreceptor degeneration in a mutation-independent way by modulating the antioxidant response of the retina at the genetic level.

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ND20 | Targeting mitochondrial calcium to fight neurological deficits: role of the MCU in the pathogenesis of Alzheimer's disease and Status Epilepticus

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Excitotoxicity, Ca^{2+} homeostasis impairment and mitochondrial dysfunction are crucial events associated with several neurological conditions. The Mitochondrial Calcium Uniporter (MCU) is a specialised channel responsible for Ca^{2+} uptake into the mitochondria; however, the authentic connection between mitochondrial Ca^{2+} overload, through the MCU, and neurological disorders has never been directly addressed. Here, we showed that ablation of *MCU* significantly diminished mitochondrial Ca^{2+} uptake and neuronal death in response to NMDA-induced excitotoxic apoptosis and necrosis, but not in response to a milder and transient NMDA overactivation. Next, mRNA expression of selected MCU complex components resulted increased over time in response to NMDA-induced excitotoxic apoptosis *in vitro* and they were significantly upregulated in transgenic familial AD mouse models (PS2APP and PS2KO), *in vivo*, at 1.5 months of age compared to their WT controls. Moreover, Ca^{2+} dynamics studies in hippocampal slices *ex vivo* showed significant NMDA-induced intracellular Ca^{2+} alterations during and post-excitation in 1.5 months old FAD mice, and we observed significant up- and down-regulation in several genes involved in the major cell death pathways and in both inhibitory and excitatory neurotransmission in 1.5 months old FAD transgenic mice. Finally, we explored the effect of *MCU* deficiency in kainite acid (KA)-induced status epilepticus, demonstrating, for the first time, that *MCU* ablation protects against seizure *in vivo*. In summary, our results suggest that a substantial rearrangement of gene expression occur early in FAD mice, especially of those involved in Ca^{2+} homeostasis and cell death regulation, suggesting their potential as neurotherapeutic targets towards the treatment of AD. Furthermore, we provided important new insights into the functional role of MCU, and specifically, in excitotoxicity and status epilepticus-mediated neuronal injury.

ND21 | Boosting peripheral nerve regeneration in ALS by CXCR12-CXCR4 axis

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative condition characterized by the loss of contact between nerves and muscles due to motor neurons death. Both the aetiology and the pathogenesis are unclear; however, the role of the neuromuscular junction (NMJ) as the site where the molecular changes leading to the pathology begin is emerging: the loss of the correct signalling and the loss of the homeostatic plasticity at the NMJ may be fundamental steps in the pathology progression. A focus on this aspect may offer a novel paradigm to treat neuromuscular disorders. Here we show that the recently discovered molecular axis CXCL12-CXCR4, involved in NMJ repair and nerve regeneration may be a strong candidate to target ALS. The receptor CXCR4 is re-expressed in the nerve at the NMJ in the Soleus and EDL muscles of $SOD1^{G93A}$ mice. This is already evident at early stages when no major phenotype is observed, but the expression fails with the pathology progression with a stronger impact on the EDL. Thus, we propose CXCR4 as marker of regenerative capacity and its failure may be at the basis of the greater vulnerability of fast muscles. Moreover, we exploited a novel synthetized agonist of the receptor, named NUCC-390, which was found to be effective in promoting peripheral nerve regeneration. We are now testing the ability of NUCC-390 to delay the degeneration that characterizes the pathology via behavioural and functional analysis. This includes the measurement of the compound muscle action potential and respiratory rate, as well as motor neurons survival. In addition we are interested in the possible mechanisms involved in NUCC-390 protective activity which may have a role in retrograde axonal transport. In conclusion we propose NUCC-390 as possible therapy to sustain NMJ plasticity and boost its regenerative capacity to delay motor neurons death.

ND22 | Exploring the role of astrocytic Ca2+ signaling in Alzheimer's Disease

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Alzheimer's disease (AD) is an incurable neurodegenerative disease, characterized by progressive memory loss and cognitive dysfunctions. Nowadays research on neurodegenerative diseases is giving attention also to cell types other than neurons, such as astrocytes and microglia. We studied astrocytic Ca^{2+} dysfunctions along the progression of AD, to clarify whether these dysfunctions precede or follow Aβ plaque deposition. To this aim, we employed three FAD mouse models, PS2.30H and APPSwe, which express the human PS2-N141I and APP Swedish mutation alone respectively, and the PS2APP model that expresses both mutants. The experiments were carried out in mice at 3 and 6 months of age, before and after, respectively, the onset of plaque deposition in PS2APP mice. We carried out experiments in brain slice and in *in vivo* preparations from somato-sensory cortex (SSCx). We found that astrocyte Ca^{2+} activity in PS2APP mice exhibits a sequence of changes: at 3 months of age, spontaneous activity is significantly increased, while at 6 months of age both spontaneous activity and the responsiveness to different metabotropic agonists are drastically reduced. These alterations are not present in APPSwe and PS2.30H mice, thus the expression of the two mutants alone is not sufficient to fully recapitulate the Ca^{2+} defects observed in PS2APP mice. We investigated the intracellular mechanism responsible for these alterations and we found a significant decrease in ER Ca^{2+} content only in 6-month-old mice. Although these defects start in concomitance with Aβ plaque deposition, astrocytic hypoactivity is unrelated to plaque proximity. We investigated the consequences of those defects in synaptic plasticity and we found a significant impairment of a specific astrocyte-dependent long-term potentiation in 6-month-old PS2APP mice. Our future goal is to demonstrate that astrocytic Ca^{2+} dysfunctions impact also on the behavioural phenotype of this AD model.

ND23 | Nuclear SOD1 in PBMCs of sporadic ALS patients modulates activation of protective pathways

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease caused by loss of motorneurons. It can occur sporadically, without any family history (sALS; 90-95% of patients), while a small percentage is considered familial (fALS; 5-10%). SOD1 is a gene reported in ALS pedigrees with a strong pathogenic role when mutated. Also the involvement of wild-type SOD1 in sporadic cases has been investigated and it was demonstrated that depending on SOD1 localization, sALS patients can be divided into two subgroups: those where the protein aggregates in cytoplasm and those where it relocates in nuclear fraction. The protein re-localization as aggregates in insoluble fraction generates oxidative stress leading to DNA damage in contrast with the protective role that SOD1 acquires in the nucleus, preventing DNA damage. RNA metabolism is relevant in ALS disease etiology. Issues in RNA processing have been associated to ALS and in fact changes of gene expression in patients have been demonstrated. We investigated pathways activated by nuclear SOD1 (nSOD1) in Peripheral Blood Mononuclear Cells (PBMCs) of sALS patients by dividing them depending on the “high” (n=8) or “low” (n=10) concentration of nSOD1 through RNA-seq experiments. Pathways activated in high nSOD1 are related to the up-regulation of HSP70 ensuring the correct protein folding. HSP70s up-regulation has been associated to mutant SOD1 aggregation suppression and their involvement in molecular network of ALS also includesTDP-43 clearance. In low nSOD1 group the up-regulation of KDM4C and S100B may be responsible for loss of DNA damage sensing and increased neuroinflammation. Our findings highlight the importance of subcellular localization of soluble SOD1 in ALS patients. We observed different behavior of RNA regulation in the two groups of patients, leading to pathways conferring “protection” where nSOD1 was high, and “perturbation” in crucial biological systems where nSOD1 was low.

ND24 | Astrocyte-derived extracellular vesicles from nigrostriatal brain regions differentially exert dopaminergic neuroprotection

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Astrocytes (AS) are key players in the regulation of dopaminergic (DAergic) neuron homeostasis both in health and disease, such as Parkinson's disease (PD). PD is a neurodegenerative disorder affecting neurons in the Substantia Nigra pars compacta (SNpc) within the midbrain (VMB), and their axons which project in the striatum (STR), with the consequent dopamine depletion. Our previous work demonstrated that AS activated by the chemokine CCL3 exert a robust DAergic neuroprotection/regeneration against the PD neurotoxin MPTP, both *in vitro* and *in vivo*. The mechanism(s) underlying the complex cross-talk between AS, neurons and neural stem cells is still unknown. In this context, the extracellular vesicles secreted by AS (AS-EVs) may be involved in the signalling mechanism with the inflamed environment. We characterized AS-EVs from both VMB and STR, before and after the CCL3 treatment. We found that: (i) AS-EVs are enriched in small-EVs; (ii) VMB-AS release more EVs than STR-AS; (iii) CCL3 affects EV secretion in a brain area-dependent fashion. These data suggest nigrostriatal-specific differences of AS-EV secretion, with potential functional implications. For this purpose, we applied AS-EVs on differentiated SH-SY5Y cells used as a model of target cells. Firstly, we assessed AS-EV internalization by SH-SY5Y cells, thus supporting the potential transfer of AS-derived cargoes to target cells. Then, we tested the effects of EVs under oxidative stress condition (H_2O_2) and mitochondrial functionality inhibition (MPP^+). We combined different approaches to evaluate viability, cytotoxicity and caspase activation of SH-SY5Y cells in response to the different AS-EVs (i.e. VMB vs. STR and basal vs. CCL3). Our results confirmed that both brain area and CCL3 treatment differentially affect the neuroprotective capacity of AS-EVs. Overall, our findings suggest a possible role for AS-EVs in facilitating glial-neuron communication and possibly their reparative/regenerative potential.

POSTER SESSION 2

NI16 | Microglia-derived extracellular vesicles promote brain repair and functional recovery after stroke

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Microglia, the resident immune cells of the central nervous system, acquire multiple activated phenotypes following ischemic brain injury, contributing to both harmful and regenerative processes. In particular, during the early phase after stroke microglia exert protective functions, while at late stages they acquire a prominent detrimental phenotype that has been suggested to hinder the spontaneous reparative response sustained by GPR17-expressing oligodendrocyte precursor cells (OPCs), a subpopulation of glial cells that reacts to brain injury and differentiate to replace damaged oligodendrocytes. In this respect, extracellular vesicles (EVs) released by activated microglia have been recently highlighted as key players in the communication between microglia and OPCs. However, if and how microglia-derived EVs could affect the regenerative process after brain ischemia is still not known. Here, we investigated the effects produced by EVs isolated from primary cultured microglia on brain repair and functional recovery in an experimental model of stroke. Briefly, middle cerebral artery occlusion has been performed in GPR17-iCreER^{T2}:-CAG-eGFP mice to follow the fate of GPR17-expressing OPCs thanks to GFP synthesis. Then, either inflammatory or regenerative microglia-derived EVs have been infused in the ipsilateral corpus callosum of ischemic mice to evaluate their impact on GFP$^+$ OPC differentiation, neuroinflammation, and post-stroke recovery. Results show that infusion of regenerative microglia-derived EVs restores protective microglia/macrophages functions and enhances the maturation of GFP$^+$ OPCs at lesion borders, resulting in improved functional outcome. Interestingly, transcriptomic profiling of primary cultured OPCs exposed to inflammatory or regenerative microglial EVs showed that specific molecular pathways are significantly modulated by EVs, unveiling novel putative targets for remyelinating therapies.

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NI17|Adrenergic signals to β3-adrenergic receptor expressing stromal cells instruct bone marrow and thymus to increase newly-generated T lymphocytes in a mouse model of multiple sclerosis

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In bone marrow (BM), mesenchymal stem cells (MSC) maintain the homeostasis of hematopoietic stem cell (HSC). Sympathetic nervous system (SNS) signals activate β3-adrenergic receptors (B3AR) in MSC, inducing HSC mobilization, proliferation and myeloid differentiation in mouse models of diabetes, stroke and stress. Also the thymus is innervated by adrenergic SNS fibers and contains MSC. However, the expression of B3AR and the effect of its activation has never been investigated in thymus.
We speculate that in experimental autoimmune encephalomyelitis (EAE), a T-cell-mediated model for multiple sclerosis, adrenergic signals may activate B3AR in BM and thymus MSC, promoting lymphoid hematopoiesis.
We performed a confocal, gene expression and FACS analysis of BM and thymus at several times upon immunization for EAE in mice administered or not with selective agonist and antagonist of B3AR.
We found that early upon disease induction, SNS transmission increased in BM and thymus. In BM, B3AR are activated and lymphoid differentiation and mobilization of HSC was promoted. Toward the elucidation of molecular mechanism through which MSC control HSC homeostasis, we performed a RNA-seq analysis of BM stroma, revealing new factors involved in cell adhesion and lymphoid differentiation that were modulated by EAE induction in a B3AR-depending way.
Concomitantly with HSC mobilization, lymphoid precursors increased in thymus and sustained T-cell maturation. We demonstrated that the activation of B3AR in thymus stromal cells promoted the release in the blood system of T-lymphocytes. Inhibition of B3AR in EAE mitigated lymphoid differentiation of HSC, their mobilization toward the thymus, as well as the release of newly-generate T lymphocytes from thymus.
Overall, our results indicate that a neural pathway involving MSC is activated by EAE induction and instructs BM and thymus to promote the generation of immune cells relevant for disease establishment.

NI18 | Crosstalk between Adipose Tissue and Brain in response to a Western Diet

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Dietary fats and sugars were identified as risk factors for overweight and neurodegeneration, especially in middle-age, an earlier stage of the aging process. Therefore, our aim was to study the metabolic response of both white adipose tissue and brain in middle aged rats fed a typical Western diet (high in saturated fats and fructose, HFF) and verify whether a similarity exists between the two tissues. Specific cyto/adipokines (tumor necrosis factor alpha (TNF-α), adiponectin), critical obesity-inflammatory markers (haptoglobin, lipocalin), and insulin signaling or survival protein network (insulin receptor substrate 1 (IRS), Akt, Erk) were quantified in epididymal white adipose tissue (e-WAT), hippocampus, and frontal cortex. We found a significant increase of TNF-α in both e-WAT and hippocampus of HFF rats, while the expression of haptoglobin and lipocalin was differently affected in the various tissues. Interestingly, adiponectin amount was found significantly reduced in e-WAT, hippocampus, and frontal cortex of HFF rats. Insulin signaling was impaired by HFF diet in e-WAT but not in brain. The above changes were associated with the decrease in brain derived neurotrophic factor (BDNF) and synaptotagmin I and the increase in post-synaptic protein PSD-95 in HFF rats. Overall, our investigation supports for the first time similarities in the response of adipose tissue and brain to Western diet.

NI19 | The multiple sclerosis drug, monomethyl fumarate, signals through hydroxycarboxyl receptor 2: possible implications for gastro-intestinal side effects.

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We previously demonstrated that monomethyl fumarate (MMF), the bioactive metabolite of dimethyl fumarate (DMF), a disease-modifying drug for multiple sclerosis, modulates microglia activation through a new pathway mediated by hydroxycarboxylic acid receptor-2 (HCAR2) that inhibits NF-κB, via the AMPK/Sirt1 axis. Treatment with DMF is associated with gastro-intestinal side effects, and we have speculated that MMF could signal in intestinal epithelial cells (IEC) through the pro-inflammatory prostaglandin/cycloxygenase-2 (COX2) pathway, such as occurs in keratinocytes, upon skin flushing, another common side effect of DMF treatment. HCAR2 is also a receptor for butyrate, an anti-inflammatory commensal metabolite, and we propose that butyrate could signal through the novel AMPK/Sirt-axis and that competition of butyrate with MMF for HCAR2 could therefore attenuate the gastro-intestinal side effects associated with DMF treatment.
We found that MMF,not butyrate, has an inflammatory effect in vitro on IEC isolated from wild-type (WT) mice, increasing the expression of Tnf and COX2; such an effect was not observed in HCAR2-KO mice, confirming the dependence of the signal on HCAR2. In in-vitro-activated IEC, both ligands exert an anti-inflammatory effect, albeit HCAR2-dependent only for MMF. To confirm the pro-inflammatory effect of MMFin vivo, we isolated IEC from WT and HCAR2-KO mice affected with experimental autoimmune encephalomyelitis (EAE), the animal model for multiple sclerosis, and treated or not with DMF. We found that DMF administration induces an HCAR2-dependent increase in the expression of pro-inflammatory cytokines on IEC, albeit through the activation of the ERK1/2 pathway, rather than the COX-2 pathway we might have expected. We show that DMF treatment does not worsen morphological and immunological alterations induced by EAE at intestinal level. Altogether these data suggest a cell- and environment-biased activation of different pathways in HCAR2 signaling.

NI20 | Glial cell activation and altered metabolic profile in the spinal-trigeminal axis in a model of multiple sclerosis-associated trigeminal pain

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Trigeminal (TG) pain is one of the worst multiple sclerosis (MS)-associated neuropathic pain syndromes. Its severity often does not correlate with the severity of the disease, suggesting that pain and clinical signs in MS are triggered by parallel but independent mechanisms. Our aims were to study the development of spontaneous TG pain in a rat model of Experimental Autoimmune Encephalomyelitis (EAE), and to analyze the activation of glial cells and metabolic changes in the spinal-TG system.

EAE was induced in Dark Agouti male rats by intra-dermal injection of recombinant MOG_{1-125} protein fragment in Incomplete Freund's Adjuvant and sodium acetate. Motor symptoms were monitored on a scale of ascending paralysis and the development of TG pain was evaluated by von Frey's hairs. At day post immunization (DPI) 21 or at the onset of EAE symptoms, animals were sacrificed, and the brainstem, TG ganglia and nerves were collected for analyses.

MOG-injected rats developed relapsing-remitting EAE, with a first peak after DPI 13, a remission stage from DPI 16 and a second peak at DPI 21. Interestingly, orofacial allodynia developed from DPI 1, well before the onset of EAE, and worsened over time, irrespective of the disease phase. Activation of glial cells both in the TG ganglia and in the brainstem, together with over-expression of glial purinergic receptors involved in pain transmission, was observed along with metabolic alterations in the TG ganglion, with no signs of demyelination in the brainstem. At EAE onset, brainstem glial cells were already activated and overexpressed the A_3 adenosine receptor subtype.

The spontaneous development of TG pain before the onset of EAE confirms the existence of parallel mechanisms controlling motor symptoms and orofacial pain, in which central and peripheral glial cell activation and metabolic alterations in the TG ganglia contribute to trigger and to sustain neuronal sensitization, thus representing interesting targets to manage pain in MS.

NI21 | Pro-inflammatory changes in the brain of the CNTNAP2 mouse model of Autism Spectrum Disorders

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Autism Spectrum Disorders (ASD)s are a heterogeneous group of neurodevelopmental disorders associated with social communication deficits, repetitive behaviours, as well as sensory deficits. These symptoms affect children from early childhood and produce clinically significant developmental impairments. Many reports have focused on the identification of genes associated with ASD. Despite this, considerable work is still needed to identify effective medical treatments. Immune dysfunction has emerged as a major contributor to ASD. Indeed, pro-inflammatory cytokines such as IL-1β, IL-6, TNF, IFNγ and IL-17 were found to be elevated in plasma of medication-free and healthy ASD children. Enhanced expression of neuroinflammatory markers was additionally present in post-mortem human ASD samples. Thus, evidence exists that immune dysregulation and inflammation play a key role in ASD. In the current project, we took advantage of an established animal model of ASD, i.e. contactin associated protein-like 2 knockout (Cntnap2-/-) mice. These mice develop social behaviour deficits, epilepsy and other ASD-related features, and recapitulate a syndromic form of ASD. To assess the expression of pro-inflammatory markers, cerebellum, hippocampus and cerebral cortex were dissected from 6-9-month old Cntnap2-/- and wild-type (WT) mice. After performing RT-qPCR experiments, mRNA levels of pro-inflammatory cytokines IL-6, IL-1β and IFNγ were found to be over-expressed in the cerebellum of Cntnap2-/- mice, when compared to WT littermates. In addition chemokines CCL3, CCL5 and CCL8, known to be involved in the recruitment of immune cells into inflamed tissues, increased in the cerebellum of mutant mice. Interestingly, no differences were observed in the cerebral cortex and hippocampus. Altogether, these data report for the first time that a pro-inflammatory environment may be present in the cerebellum of Cntnap2-/- mice, which may contribute to ASD-like behaviours in this mouse model.

NI23 | Effects of six months probiotic supplementation on the inflammatory profile of an Italian sample of preschoolers with Autism Spectrum Disorders

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Background There is increasing evidence supporting the use of probiotics in subjects with Autism Spectrum Disorder (ASD) as a treatment that potentially improves gastrointestinal (GI) symptoms and socio-behavioral functioning, possibly acting on the inflammatory status. Despite the growing research on this topic, existing data on effects of probiotics on systemic inflammatory biomarkers in ASD are contradictory.

Methods During a double-blind randomized, placebo-controlled trial, we compared the effects of a 6-months probiotic (De Simone Formulation) versus placebo supplementation on plasmatic levels of some inflammatory biomarkers (Leptin, Resistin, PAI-1, MCP-1, TNF-α, and IL6) in a sample of 63 preschoolers with ASD (mean age, 4.2 years; 81% boys), comparing the "Probiotics" (PRO) vs "Placebo" (PLA) groups i) in the whole sample ii) within the subgroup with GI symptoms (GI-PRO versus GI-PLA).

Results All biomarkers showed basal values lower than those reported in previous studies on children with and without ASD with systemic inflammatory conditions: TNF-a 0.74-16.09 pg/ml; IL-6 0.80-104.00 pg/ml; MCP-1 26.36-451.00 pg/ml; Leptin 0.03-4.83 pg/ml; Resistin 0.59-8.13 ng/ml; PAI-1 0.73-5.51 ng/ml. After 6-months of placebo or probiotics treatment, no significant changes in any one of the measured biomarkers in the PRO vs PLA groups were reported, neither in the GI-PRO versus GI-PLA subgroups.

Conclusions In the ASD children under study, a systemic inflammatory state supported by the measured biomarkers, was not observed and 6-months treatment with probiotics did not modify their levels compared with placebo, also in children with GI symptoms. In our sample, the hypothesis that ASD could be associated with a systemic inflammatory condition is not supported, and probiotics do not seem to act directly on these biological pathways. To our knowledge, this is the first report on the effect of probiotics on systemic inflammatory biomarkers in ASD.

NI24 | Tools for large specimen clearing: applying SOCRAT to the auditory system of small and large mammals

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The field of anatomy is experiencing a major revival due to the introduction of techniques, such as tissue clearing and lightsheet microscopy, which allow the visualization of macroscopic structures at microscopic detail (mesoscale imaging). This is particularly important in neuroanatomy, where mesoscale imaging is now routinely applied in rodents. Tissue availability from brain banks would allow mesoscale imaging of human structures as well; however, clearing and imaging human specimens presents several challenges. First, large animals display age-related accumulation of opaque molecules, higher myelinization, lipidome heterogeneity, and denser connective tissue; the SHANEL (small-micelle-mediated human organ efficient clearing and labeling) clearing protocol has been created to overcome these problems. In addition, if the structure to be cleared includes bone, decalcification is necessary, and it can be very time-consuming (several months).
In our lab, we have previously set up a protocol for clearing the rat auditory system from the bone-encased inner ear to the brainstem, based on iDISCO (immunolabeling-enabled 3D imaging of solvent-cleared organs) and adding temporal bone decalcification. In the rat, complete EDTA-based decalcification step requires 3-4 weeks @RT but only 2-3 days in a temperature-controlled histology-grade microwave. We therefore started optimizing clearing protocols, aiming at mesoscale imaging of the human auditory system, obtaining SOCRAT (Small micelle-mediated Organ Clearing with RApid decalcification Treatment).
To assess SOCRAT efficiency, we compared it with iDISCO clearing of the rat auditory system. Moreover, to test efficiency on large animal samples, we performed SOCRAT clearing on pig brain and bone fixed samples, obtained from animals used for abdominal surgery. To minimize costs, labeling in the larger pig samples was performed with TOPRO rather than antibodies.

NI25 | What does the microanatomy of the choroid plexus tell us on its function?

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The ventricular choroid plexus (ChP) has recently emerged as an important mediator of extracellular signaling within the brain. ChPs act as both source and target of signal molecules which are exchanged with the brain parenchyma, but the functional details of these exchanges are largely unclear. ChPs release important factors for neurogenesis through volume transmission, which is affected by the relative position of ChP and ventricular surface.
Especially in the IV ventricle, the ChP displays villus-like “fronds” which extend in the ventricle and, in some places, directly contact the ventricular surface. These places could represent privileged sites for signal exchange; however, no data are so far available on them, because their microscopic size hinders their imaging with available *in vivo* techniques while their structural fragility biases conventional histological approaches.
On the other hand, lightsheet imaging of cleared intact brains allows microscopic reconstruction of these points of contact without cutting artefacts. In our lab, we have previously observed “foot-like” contacts between the IV ventricle ChP and dorsal cochlear nucleus surface using the iDISCO (immunolabeling-enabled 3D-Imaging of Solvent Cleared Organs) clearing method; our current aim is to expand the study to highlight contacts between ChP and other IV ventricle-facing nuclei. Therefore, we have annotated available 3D atlases, finding several ChP-accessible nuclei conserved from mouse to human. The most exposed structures are the dorsal cochlear nucleus, superior and medial vestibular nuclei, dorsal raphe nucleus and cerebellum. We are currently mapping the microscopic contact points of the ChP with all these structures, as a morphological basis for subsequent investigation of the “on-demand” neurogenesis observed in the hindbrain.

NI26 | Role of microglia in synaptic dysfunction in the Fragile X Syndrome mouse model

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Fragile X Syndrome (FXS) is a genetic disorder caused by increased CGG triplets in the *fmr1* gene, which results in silencing the Fragile X Mental Retardation Protein (FMRP), a RNA binding protein particularly important in the brain development. The Fmr1KO mouse is a commonly used model for autism spectrum disorders (ASD). Both FXS and ASD patients and mouse models present abundant and immature dendritic spines, which pointed to a neuronal phenotype for the diseases. However, Fmr1KO mice lack FMRP in all brain cells, including microglia. Moreover, altered microglia-mediated synaptic pruning has been previously reported in Fmr1KO mice. Given the central role of microglia in eliminating synaptic contacts and shaping neuronal circuits, we aim to explore the mechanisms underpinning the aberrant microglial function in FXS.
Our preliminary results show that Fmr1KO mice at P20 (a period of intense synaptic pruning in the hippocampus) display a significant increase in the density of the post-synaptic protein PSD-95 compared to age-matched WT, accompanied by a decrease in gene translation. P20 Fmr1KO mice, compared to controls, also display a significant decrease of Iba1-positive microglial cells in the hippocampus, along with an increased expression of the lysosomal marker CD68. To explore whether excessive PSD-95 density in Fmr1KO mice results from altered synaptic pruning, we evaluated the volume of engulfed synaptic proteins within CD68 in hippocampal sections of mutant or WT brains. We observed a significantly increase amount of PSD-95 in CD68-positive structures in P20 Fmr1KO hippocampus compared to controls. These data suggest that Fmr1KO microglia may be unable to properly degrade engulfed synaptic material, thus resulting in a defective refinement of excessive synapses. The involved molecular mechanisms and how these processes affect behavior and synaptic plasticity in Fmr1KO mice is presently under investigation.

NI27 | Mechanism of regulation of neurosteroidogenesis under the influence of proinflammatory factors in chicken pinealocytes in vitro

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The pineal gland is multifunctional structure. It synthetizes melatonin (MEL), a hormone regulating the rhythms of various physiological processes, at night. Moreover, the pineal gland is the one of the three equivalent avian central biological clocks. Bidirectional communication between the pineal gland and immune system have been found in all vertebrate classes investigated. Investigations also have shown that pineal gland is also a major organ responsible for neurosteroidogenesis. The pineal neurosteroids regulate myelination of fibers, organogenesis, reproductive behavior, and locomotion activity. Our recent experiments demonstrated that peripheral inflammation decreases the level of transcription of three genes (*Hsd3b2*, *Srd5a3*, *Akr1d1*) encoding enzymes of neurosteroid's biosynthesis pathway in the pineal glands *in vivo*, but mechanism underlying this process is unclear. We hypothesized that information about developing inflammation directly reach the pineal gland *via* Toll-like receptors (TLR) localized in pinealocytes cell membranes. This cause an activation of the intracellular signaling pathways: NF-κB and MAPK. As a consequence, the transcription factors connected with the modulation of immune response may be activated. The aim of this study was to investigate influence of lipopolysaccharide, zymosan and recombinant flagellin on (1) expression of genes of neurosteroidogenesis, using droplet digital PCR; (2) changes in the level of effector proteins of the NF-κB and MAPK signaling pathways using Western blot in pinealocytes *in vitro.* We found that the most sensitive to proinflammatory factors is *Cyp11a1*, the first gene of the biosynthetic pathway. Moreover, three of tested genes *Akr1d1*, *Srd5a1* and *Srd5a3* are under constitutive positive control of NF-kB pathway. The MAPK signaling pathway is not involved in signal transduction, but proteasome's inhibition activates the MAPK. The study was supported from grant UMO-2016/21/B/NZ3/00364.

NI28 | Neuroprotective effect of PEA-OXA on oxaliplatin-induced neuropathic pain

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Chemotherapy-induced neuropathy is a common, dose-dependent adverse effect of several antineoplastic, like oxaliplatin (oxa). N-Acylethanolamines (NAEs) involve a family of lipid molecules that arouses great attention owing to its anti-inflammatory, analgesic, and neuroprotective activities. The aim of the present work was to evaluate the potential beneficial effects of 2-pentadecyl-2-oxazoline (PEA-OXA), compared to PEA ultramicronized (PEAum) alone, in a model of oxa-induced peripheral neuropathy (OIPN). Chemotherapic pain was induced by an intraperitoneally injection of oxa in rats on 5 consecutive days (D0–4) for a final cumulative dose of 10mg/kg. PEA-OXA and PEAum , both 10mg/kg, were given orally 15-20 min prior oxa and sacrifice was made on day 25.
Our results demonstrated that PEA-OXA, more than PEAum, reduced the development of hypersensitivity in rats; this was associated with the reduction of hyperactivation of glia cells and the increased production of pro-inflammatory cytokines in the dorsal horn of the spinal cord, accompanied by an up-regulation of neurotrophic factors in the dorsal root ganglia (DRG). Moreover, we showed that PEA-OXA reduced oxa-damage by the reduction of NF-κB pathway activation and modulating Nrf-2 ones. Together our findings identify PEA-OXA as a therapeutic target in chemotherapy-induced painful neuropathy, throughout the biomolecular signaling NF-κB/Nrf-2 axis. Therefore, we can consider PEA-OXA as a promising adjunct to chemotherapy to reduce chronic pain in patients.

NI29 | Towards an NGF-Based therapy for Rett Syndrome

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Rett syndrome (RTT) is a rare neurodevelopmental disease, affecting 1 over 10,000 females born worldwide. The main cause is a series of sporadic genetic mutations in the methyl-CpG-binding protein 2 (MECP2) gene, located in the long arm of the X chromosome. Astrocytes – by definition, the supporting elements of the central nervous system – have been shown to be deeply involved in the pathogenesis of RTT, to the point that selective re-expression of MeCP2 only in these glial cells allows for great behavioral rescue in MECP2KO mice. Despite the great scientific effort in discovering the molecular and cellular basis of the disease, there are still no pharmacological treatments against RTT. In this study, we lay the groundwork for exploring the therapeutic potential of a specific mutein of the Nerve Growth Factor (NGF), called human NGF painless (hNGFp), in the context of RTT. This neurotrophin maintains the same neurotrophic properties compared to wild-type NGF, but has a tenfold reduced pain-inducing effect. Taking advantage of the published positive properties of hNGFp on astrocytes in mouse models of Alzheimer's disease, we have evaluated the effect of hNGFp on MECP2KO astrocytes. Here we report that, *in vitro*, primary astrocytes obtained from MECP2KO brains present an asthenic phenotype and decreased levels of both NGF receptors, TrkA and p75NTR. Moreover, the same atrophic morphology in astrocytes can be found, *in vivo*, in the hippocampus of MECP2KO mice. Interestingly though, a 1-month intranasal hNGFp treatment rescues the morphological anomalies of hippocampal astrocytes and increases the expression of the postsynaptic marker Homer in 4 months old MECP2KO mice. Finally, these molecular changes are accompanied by a partial rescue of the behavioral phenotype of MECP2KO mice. Conclusively, hNGFp can be viewed as a promising therapeutic candidate for RTT, possibly via its action on astrocytes.

NI30 | Positive allosteric modulation of CB1 and CB2 cannabinoid receptors enhances the neuroprotective activity of dual CB1R/CB2R orthosteric agonist

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The Endocannabinoid System (ECS) takes part in the regulation of neurodegeneration and, during the last few decades, significant advances have been made in the understanding of the cannabinoid receptors (CBRs, CB_1R and CB_2R) role in neuroprotection. In particular, the endocannabinoid signaling may be beneficial because of its impact on attenuating neurotoxicity through neuronal CB_1R, limiting glutamate release. The other prominent target for the neuroprotection is the CB_2R, whose activation was proved to decrease microglial-derived neuroinflammation, modifying the rate between pro- and anti-inflammatory cytokines released by these cells. However, the chronic use of CB_1R and CB_2R orthosteric agonists has several disadvantages, limiting their usefulness as clinically relevant drugs. Indeed, CB_1R orthosteric agonists induce psychotropic effects, strong mood alteration, acute psychosis, and cognitive and motor impairments. On the contrary, CB_2R ligands do not produce psychotropic effects, nevertheless, their predominance on immune cells might cause immunosuppression. Positive allosteric modulators (PAMs) might represent an appealing approach to achieve the potential therapeutic benefits of orthosteric CBRs agonists increasing their activity and limiting their adverse effects. The goal of the present study was to determine whether the PAMs Gat 229 and EC21a of CB_1R and CB_2R respectively, can modulate the activity of the potent dual CB_1R/CB_2R orthosteric agonist FM-6b on excitotoxic damage by excessive glutamate release and neuroinflammation. The obtained results indicate that the combination of FM-6b with both CBRs PAMs could represent a promising therapeutic approach for the treatment of neurodegenerative disorders.

NO07 | Microcephaly gene inhibition induces mitotic catastrophe in brain tumors

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Medulloblastoma (MB) is the most frequent high-grade brain tumors in children. The standard treatment for these tumors consists in surgery, followed by radiotherapy and chemotherapy. Despite the improvement in patient survival, these therapies are only partially effective. Many patients still die and those who survive suffer from neurological and endocrine disorders. Therefore, more effective therapies are needed Primary microcephaly (MCPH) is a rare disorder caused by mutations in 20 different genes, specifically implicated in the developmental expansion of neural progenitors. Centromere-associated protein E (CENPE) heterozygous mutations cause the MCPH13 syndrome. CENPE is a microtubule plus-end-directed kinetochore motor protein important in chromosome congression, spindle microtubule capture at kinetochores and spindle checkpoint activation. As for other MCPH gene, CENPE is required for normal proliferation and survival of neural progenitors, but has limited effects on other tissues. Since there are evidence that MB shares many molecular features with neural progenitors, we hypothesized that CENPE could be an effective target for MB treatment. In ONS-76 and DAOY, CENPE knockdown increases both mitosis defect and apoptosis. Indeed, CENPE's siRNA prolongs cells metaphase inducing mitotic catastrophe via p53 or p73 signaling pathway. To consolidate CENPE as a target for MB treatment, we tested GSK923295, an allosteric inhibitor that binds the ATPase pocket already in clinical trial for other cancer type. GSK923295 induces same effect of knockdown, but with higher penetrance, at nM level in MB cell lines. These results suggest that CENPE's chemical inhibition could be a useful target for MB treatment.

NO08 | Inter and intratumoral heterogeneity analysis in pediatric high-grade gliomas through the application of mass cytometry

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Pediatric Glioblastoma (pGBM) and Diffuse Intrinsic Pontine Glioma (DIPG) are aggressive tumors that affect the central nervous system (CNS) of children and young adults, without no effective treatment. They are highly heterogeneous malignancies, arising in different anatomical sites of CNS with distinctive molecular alterations and clinical outcomes (inter-tumor heterogeneity). Moreover, the molecular profiling highlighted the existence of genetically and phenotypically distinct subpopulations that coexist within the same tumor mass, cooperating in promoting tumorigenesis (intra-tumor heterogeneity).

In order to dissect the intra and inter tumor heterogeneity, we used the mass cytometry technique, one of the most advanced technology of the "-OMICS" era that, by using antibodies conjugated to metals, allows the simultaneous measurement of up to 40 markers at single-cell level. To this end, we adopted a panel of 8 primary cell lines derived from pGBM and DIPG patients and belonging to different molecular and anatomical subgroups (2 cell lines/molecular-locational subgroup). The antibody panel that was used to capture heterogeneity allowed to highlight important differences in the expression of the considered antigens. It has been specifically set to recognize antigens expressed by brain and pHGG tumor cells and it includes two antibodies that we successfully customized in-house (H3K27M and H3.3G34R) to specifically recognize tumor cells from non-tumor ones.

Our study is the first to demonstrate, through the application of mass cytometry technique, a significant intra and intertumoral heterogeneity strongly dependent on the molecular classification.

NO09 | Role of Calcium Activity in Cell Invasion and Migration by Intravital Two-photon Imaging of Glioblastoma Mouse Models

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Glioblastoma multiforme (GBM) is a malignant form of brain tumor, which is characterized by extensive cellular and genetic heterogeneity, and poor prognosis. Calcium signaling has been proposed to be directly involved in cancer proliferation and invasion, but there are no demonstrations *in vivo* of the correlation between GBM cell motility and calcium signaling. In light of this idea, we have produced a strain of mouse glioma cells (GL261) expressing a red fluorescent protein and a genetically encoded calcium sensor (GCaMP6s). Two photon imaging data that we acquired demonstrate that most of the tumor volume, i.e. the core, is occupied by tightly packed spherical cells characterized by little or no motility and low Ca activity. This core is surrounded by sparse cells displaying a very polarized morphology and migrating in a coordinated way. These cell streams in the peripheral region are characterized by very active Ca signaling, with Ca waves propagating within cells and between distinct ensembles of cells based on their common activation. This phenomenon was strengthened by observing global Ca activity at a mesoscale via wide field imaging. We noticed clustered Ca activity that were generated by small groups of cells. We postulate that these regions represent the infiltrating component of the tumor. Finally, by means of *in utero* electroporation we transfected a small population of glial precursors with the active mutant of Ras (HRasV12), GCaMP6 and a red reporter, that subsequently generates a spontaneous glioma model. Two photon imaging demonstrated that, within 10 days from the electroporation, the brain is gradually invaded by hypertrophic cells characterized by elevated motility and infiltrative potential. Similarly to the GL261 model, the infiltration is organized in cell streams endowed by very elevated Ca activity.

NO10 | Surgical washing from Cavitron Ultrasonic Surgical Aspirator (CUSA) as reservoir of patient-derived glioblastoma stem cells and extracellular vesicles.

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One of the most specific features of glioblastoma (GBM) is represented by its complex heterogeneity, which makes therapies increasingly challenging. This also results from the presence, in the tumor, of GBM Stem Cells (GSCs) which have the intrinsic ability of self-renewing, as well as modulating the tumoral immune response and invasion.
In order to investigate GSC molecular and cellular heterogeneity in different tumor microenvironments, we generated GSC primary cell lines from either Cavitron Ultrasonic Surgical Aspirator (CUSA) washings or bulk tumors. As CUSA aspiration is used during surgery to remove the tumoral edge-zones, stemness and self-renewal capacity of paired (edge vs tumor core) GSC lines from the same patient can be investigated.
Extreme Limiting Dilution Analysis (ELDA) showed differences in stemness and self-renewal profiles; GSCs derived from tumor edge-zone (GSC-edge) displayed a higher self-renewal capacity compared to GSCs derived from the tumor core (GSC-core).
Furthermore, extracellular vesicles (EVs) released from GSC-edge or GSC-core promoted tumor cell migration and invasiveness at a different extent, with exosomes (EXOs) from CUSA washing being more potent in promoting tumoral cell migration compared to GSC-derived EXOs.
These data have important implications for the understanding and characterization of GSCs in different tumoral microenvironments. In addition, our results point out the relevance of the use of CUSA surgical washing as a valuable source of patient's GSCs and EVs.

NO11 | TBK1 inhibitor exerts anti-proliferative effect on glioblastoma multiforme cells

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Glioma are common malignant brain tumors, among which glioblastoma multiforme (GBM) has the worst prognosis. Previous molecular analysis of GBM revealed enrichment of nuclear-factor-κB (NF-κB) target genes showing that NF-κB inhibition attenuated tumor proliferation and prolonged cell survival. TBK1 (TANK {TRAF (TNF (tumor-necrosis-factor) receptor-associated factor)-associated NF-κB activator}-binding kinase 1) is a serine/threonine-protein kinase, a member of the IκB kinase (IKK) family that show ubiquitous expression. Binding of IκB kinase subunit epsilon (IKKε), a key component of NF-κB signaling), to the TBK1 leads to the activation of NF-κB, interferon-regulatory-factor (IRF) signaling pathway, autophagy and various cancer-driving factors including, protein-kinase B (Akt), tumor necrosis factor receptor (TNFR) associated-factor-2 (TRAF2). TBK-1 has been shown to increase in solid tumors, denoting the importance for tumor angiogenesis, apoptosis induction and cell proliferation. Although TBK-1 role in the pathogenesis of cancer has been hypothesized, limited evidences have been provided using TBK-1 inhibitor in brain tumors. Thus, the effects of BX795 an inhibitor of TBK-1 were investigated in an in *vitro* model of glioblastoma. Different GMB cell lines (U87 and U138) and primary GMB cells were treated with different concentrations of BX795 at different time-points (24, 48 and 72h) and cell viability, autophagy, inflammation and apoptosis were observed. BX795 showed a significant anti-proliferative effect, reducing cell viability and increasing apoptosis, as demonstrated by activation of pro-apoptotic Bax, p53, caspase-3 and caspase-9. BX795 was able to reduce angiogenesis factors, autophagy and inflammatory process. In conclusion, BX795 could act as tumor suppressor let us hypothesize the role played by TBK-1 in neuroinflammation and autophagy associated to GMB. The association of this inhibitor with the available chemotherapy could decrease the side effects.

NNP25 | Are astrocytes the mediators of NGF homeostatic activity on the cholinergic system?

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Astrocytes are the most numerous population of the CNS and represent scaffold and support to neurons and their connections. Their ability to elicit rapid calcium signals in response to a variety of neuromodulators and their direct access to synapses makes them interesting substrates of brain state activity integration. Acetylcholine (ACh), a neurotransmitter released during wakefulness by long-range cholinergic fibers from the basal forebrain, has been shown to activate astrocyte networks and promote neuronal modulation. The neurotrophin Nerve Growth Factor (NGF) is intimately connected with the cholinergic system. In the CNS, NGF is required for the survival and function of the basal forebrain cholinergic neurons (BFCNs), to an extent that disruption of a single allele of the NGF gene or the neutralization of this neurotrophin by recombinant antibodies result in atrophy of cholinergic neurons accompanied by memory and learning deficits. Apart from its neurotrophic properties, NGF also behaves as a neuromodulator of cholinergic activity, enhancing ACh release from BFCNs in culture. Thus, NGF modulation of cholinergic activity has in theory the ability to deeply affect cognitive function, though there is little literature *in vivo* on the activity of NGF in cortical circuits. Here we show that, *in vitro,* astrocytes possess the receptors and the signaling machinery to respond to NGF and that they respond to acute functional neutralizations of NGF via calcium transients. *In vivo,* NGF receptors can also be found and, interestingly, by imaging calcium activity in astrocytes, we observe the same increase of their activity in response to NGF deprivation. Moreover, NGF deprivation was accompanied by a decreased cholinergic tone and somatostatin interneurons activity. Further studies are required to demonstrate whether we have uncovered a previously unacknowledged homeostatic mechanism in which astrocytes can modulate the cholinergic tone in the cerebral cortex.

NNP26 | Structure/function study on de novo mutations in ATP6V1A causing developmental encephalopathy with epilepsy

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Vacuolar ATPase (v-ATPase) is a large protein complex that acts as an ATP-dependent proton pump. It is the major responsible for acidification of intracellular organelles and maintenance of endo-lysosomal pH homeostasis. Recently, we described *de novo* mutations in a specific subunit of v-ATPase (*ATP6V1A*) in patients with developmental and epileptic encephalopathy.

Aim: the goal of the project was to uncover the impact of *de novo* mutations on ATP6V1A structure, expression and lysosomal function.

Methods: We mapped the mutations onto the recently published 3D structure of the rat ATP6V1A (Abbas 2020; PDB accession code 6VQ9). We performed functional experiments in fibroblasts from four patients affected by different mutations leading to severe or mild epileptic phenotype. ATP6V1A expression was analysed by western blot. Lysosomal content and acidification were analyzed by immunocytochemistry and Lysotracker labeling.

Results: When mapped in the rat structure, most of the pathogenic mutations cluster either near the P-loop involved in ATP binding or at the interface between subunits A and B. The remaining mutations appear to alter local amino acidic interactions, possibly resulting in decreased protein stability. Expression experiments showed decreased levels of ATP6V1A in one proband and no significant differences in the three others. Fibroblasts from the two severe patients presented lower Lysotracker signal accompanied by a decreased LAMP1 staining. Fibroblasts from the patients with mild phenotype showed an increased Lysotracker signal with no variation in LAMP1 levels.

Conclusions: Our experiments suggest that de novo ATP6V1A mutations may affect v-ATPase function altering either ATP hydrolysis, A/B interaction or protein stability. The functional experiments revealed an alteration of lysosomal homeostasis, with diverse impact depending on the clinical phenotype.

NNP27 | Different systems support time and numerosity perception in extrapersonal space

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The representation of space, time and number is believed to rely on a common encoding system developed to support action guidance (A Theory of Magnitude). While the ecological advantage of such a shared system is evident when objects are located within the region of space we can act on, known as peri-personal space, it is less obvious in the case of objects located beyond our arms' reach. In the current study we investigated whether and to what extent the distance of the stimuli from the observer affects the perception of duration and numerosity. Adult participants were required to perform a duration reproduction task or a non-symbolic numerosity estimation task for stimuli of different sizes displayed in the peri- or extra-personal space. Results show that, independently of physical size, duration estimates were overestimated when visual stimuli were presented in the extra-personal space. A similar effect was also found for numerosity perception, but overestimation for far stimuli was much smaller in magnitude and, most importantly, completely accounted for in terms of interactions between the perception of stimuli size and numerosity. Overall these results suggest that, while the processing of temporal information is robustly affected by the position of the stimuli in either the peri- or extra-personal space, numerosity perception is independent from stimulus distance. We speculate that, while time and numerosity may be encoded by a shared system in the peri-personal space (to optimize action execution), a different mechanism may underlie the representation of time and numerosity in extra-personal space.

NNP28 | Longitudinal multifactorial evolution after stroke in mice

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Stroke is one of the leading causes of adult disability, the degree of impairment is influenced by the size of the lesion, its location and the extent of damage to ascending and descending pathways. Post stroke changes involve cells network modification and cortical reorganisation, coordinated by cellular and molecular mechanisms. Understand how those changes affect the motor recovery could lead us to understand how to modulate a clinical intervention. Results coming from clinical trials need to be taken carefully first, for the high inter-variability of treatments, patients (in lesions, impairments and age) and outcomes; second, for the high intra-variability. In my PhD project I plan to use a mouse model of ischemic injury to identify the longitudinal evolution of multiple parameters to distinguish subjects with good or poor recovery prognosis. Using a Middle Cerebral Artery occlusion (MCAo) model to better represent human stroke characteristics and variability, I will evaluate changes in motor impairments, corticospinal tract (CST) integrity, corticomuscular coherence (CMC) and motor maps organization. Identifying the plastic changes that occurs after an ischemic lesion is the first step to assess a clinical intervention. Thy1-ChR2 mice will be tested before and after the MCAo lesion, motor impairment will be followed with motor tests. Optogenetic stimulation will be used to trigger the activation of layer V corticospinal neurons to evaluate the CST integrity through Motor Evoked Potentials evocation and electromyographic (EMG) readings; the same tool will be used to monitor forelimb cortical map reorganization. Electroencephalography (EEG) apparatus will be coupled with EMG to control CMC. Further steps are the assessment of the optogenetical stimulation effects on CST integrity, CMC and motor maps. For a translational approach, Short Interval Cortical Inhibition can be used to evaluate intracortical excitability.

NNP29 | Union is strength: observed drinking action facilitates the same behaviour in mice.

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The observation of an action made by a conspecific can lead, in some cases, to the repetition of the same action. This process, called social facilitation, seems to be at the basis of social interaction and communication within the members of the same group or species. It has been demonstrated that this process occurs mainly in species that live in groups such as birds, primates and rodents.
In the present study, we implemented a novel behavioural paradigm to investigate the social facilitation process in mice, focusing on how the presence of a feeding (drinking) conspecific is able to affect motivation and behaviour in the observer.
We demonstrated that the observation of an highly motivated drinking mouse strongly facilitated the drinking behaviour in a head-fixed observer. In fact, the time spent drinking, was significantly higher if a drinking conspecific has been observed compared to the observation of a non-drinking conspecific or the mere presentation of the drinking bottle. Moreover, we found that the observation of a drinking subject directly triggered the beginning of drinking by the observer. For each session, we considered the temporal distribution of the actor's and observer's drinking intervals and we found a higher probability that the observer began to drink if the actor has already started to.
These results suggest that feeding behaviour in mice could be influenced, in particular enhanced, by the observation of a conspecific performing the same action. In an evolutionary perspective, our data provide information on the possibility of the existence in mice of a system involved in understanding behaviours with important social meaning.

NNP30 | In vitro modelling of Joubert syndrome

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The advent of Next generation sequencing (NGS) techniques led to an impressive increase in the discovery of genes responsible for rare genetic disorders. Functional validation is then needed to understand the biological function of novel genes, and how it is disrupted by the identified mutations. Our project focuses on Joubert syndrome (JS), a rare neurodevelopmental disease affecting between 1 in 80.000 and 1 in 100.000 patients (the prevalence is still changing since more causative genes and a broader range of phenotypes are identified), and aims to generate innovative in vitro models based on the use of patient-derived induced pluripotent stem cells (iPSCs), which can be differentiated towards the cerebellar lineage while maintaining the full genetic background of the patient, in order to investigate the molecular basis of the disease. JS is a genetically heterogeneous congenital cerebellar ataxia with recessive inheritance, characterized by a peculiar cerebellar and brainstem malformation, the so-called “molar tooth sign” (MTS). All known JS-linked genes encode proteins of the primary cilium, a subcellular organelle widely present in embryonic and adult tissues, qualifying JS as a ciliopathy. There is clinical and genetic overlap among ciliopathies because the same gene may be responsible for distinct phenotypes, and the same clinical manifestation can be caused by many genes. The experimental approach involves differentiation of iPSCs towards neural stem cell (NSC) and cerebellar lineages, and characterization of the cellular and transcriptional phenotype in patients carrying mutations in distinct JS genes.

NNP31 | Cell proliferation and amount of doublecortin-positive neurons in the dentate gyrus of different mammalian species

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Adult neurogenesis extension, rate, time course and functions can be quite heterogeneous among mammals. No systematic, fully comparable analyses are available on a wide range of animal species. Knowledge is restricted to the “extremes”: high rate of neurogenesis in small-brained rodents versus reduced/vestigial in large-brained humans and dolphins. A debate is ongoing due to reports with different conclusions about the rate and temporal extension of adult human hippocampal neurogenesis; in all cases, doublecortin-positive (DCX+) “immature” neurons were observed during adulthood, while no substantial cell proliferation was detectable. Here, the optical Fractionator with StereoInvestigator software was used to estimate the number of granule cells, DCX+ and Ki-67+ cells in three adjacent sections of the dentate gyrus (selected along rostro-caudal axis - one in the anterior, one in the central and one in the posterior part) in 3 specimens of mouse, rabbits, cats, and sheep. The ratio DCX+/Ki67+ cells was evaluated as a proxy neuronal differentiation: preliminary data indicate that in sheep, the animal with largest and more gyrencephalic brain here considered, it is significant higher than in other mammalian species. These data suggest that in gyrencephalic brains hippocampal immature neurons might persist for a long time in a state of protracted maturation. Previous studies carried out in the hippocampus of sheep and monkeys showed that newly born neurons mature far slower than in rodents (3 to 6 months). This trend is reminiscent of what has been described in adult humans, and might be shared by newly generated and non-newly generated immature neurons in large-brained mammals.

NNP32 | A new transgenic mouse for mitochondrial calcium ratiometric imaging

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Spatial and temporal organization of intracellular calcium (Ca^{2+}) signals is crucial for the correct function of all tissues. Among other functions, mitochondria play a relevant role in Ca^{2+} homeostasis and their dysfunction has been correlated to several pathological conditions. In the past years, several Genetically Encoded Ca^{2+} Indicators (GECIs) have been developed to study Ca^{2+} dynamics inside mitochondria. In particular, ratiometric, FRET-based GECIs such as Cameleons are largely used in cell cultures. However, the study of Ca^{2+} dynamics in organelles *in vivo* or in *ex vivo* preparations demands invasive techniques, such as viral injection.

The aim of this project is to develop and characterize a transgenic mice line expressing a mitochondria-targeted Cameleon probe. To achieve this goal, we engineered the Rosa26 mouse genomic locus by inserting the sequence of the mitochondria-targeted Cameleon preceded by a LoxP-STOP-LoxP sequence. The probe can be easily expressed in a tissue-specific manner upon Cre recombinase-mediated excision, obtainable by a single cross.

We exploited a CAG–Cre mice line to drive the expression of the Cameleon probe in all tissues, included the brain. Preliminary data demonstrates the good expression of the Cameleon at different mice age analyzed. Moreover, two-photon Ca^{2+} imaging experiments, performed in *ex vivo* brain slices and in cell culture, confirmed the functionality of the probe.

The new transgenic mice line we generated will allow the study of Ca^{2+} dynamics in different tissues under physiological and pathological conditions *in vivo*, without the need of invasive delivery procedures.

NNP33 | Brain at altitude: pieces of evidence from two Himalayan expeditions

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Altitude hypoxia detrimentally affects a broad spectrum of cognitive functions. Adaptations may be due to brain damages. Ethnogenetic and cultural variations may be involved. To address this topic, two diverse studies at altitude were conducted. For the first one, the experimental group (EG) was composed by 12 healthy adults (6m and 6f); they completed 12 days of trekking at moderate and high altitude and were compared to a matched control group (CG). Participants were tested for anxiety, mood, short-term memory and verbal skills; neuroimaging measures were acquired before and after the expedition for investigating cerebral blood flow and brain microstructural changes. The EG had a lower trait anxiety and conscientiousness at the baseline than the CG. State anxiety and depression reversed after the trek. Verbal fluency increased after the trek, particularly in the females of the EG, and 2-back response time was faster during the expedition. The second study aimed to determine if diverse ethnic groups would show different performance in spatial abilities in response to hypobaric hypoxia. 22 healthy adults (5 Italian trekkers, 6 Nepalese porters, 5 highlander and 6 lowlander Sherpas, all males) were tested with a building photo recognition task, a map orienting task and a mental rotation task. Nepalese performed worst, but the difference was mitigated after correcting for schooling. Participants took more time to respond at low than at high altitude; in the map task, participants performed with greater accuracy at low altitude. Anxiety-related disorders due to high altitude exposure occur, whereas such environmental condition does not entail detrimental effects in short-term memory, concentration, updating skills, verbal fluency and brain structure if altitude sickness does not emerge. Altitude hypoxia elicits a cognitive impairment in spatial tasks, possibly due to an inadequacy to acquire new unfamiliar patterns or to manage a higher cognitive workload.

NNP34 | Antioxidant supplementation and physical exercise: beyond the ordinary notion

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In the recent years the market of dietary supplement obtained a conspicuous growth, particularly between athletes; but the perception that the sum of two healthy factors would double the beneficial effects could be erroneous. Physical exercise induces a wide range of beneficial effects on brain health at different levels. A relationship between aerobic capacity, hippocampal plasticity and memory is widely recognised and accepted, and physical exercise improves and maintains cognition in old individuals. It is worth noticing that running has been shown to reinstate juvenile-like plasticity in the visual cortex of adult rats through a reduction of the intracortical inhibitory tone. Very recently, this approach has been successfully applied to restore plasticity also in adult human subjects. However, despite the increasing number of studies investigating the effects of physical activity on the brain, the peripheral mechanisms that drive these beneficial events remain unclear. In particular, very little is known about the physiological processes underlying the translation of general muscles activation into enhancement of brain molecular pathways involved in neural plasticity. At the peripheral level, physical exercise has been shown to exert a positive outcome in different systems: it prevents cardiovascular diseases and osteoporosis, improves glucose metabolism and decreases the chance to develop cancer. Strikingly, it has been demonstrated that the beneficial effects of physical activity on muscles and glucose metabolism in humans is counteracted by a moderate intake of antioxidants. Altogether, these evidences depict the prospect that incautious vitamin supplement could represent a detrimental factor for human health; these concepts, however, have not been tested in the functional domain of brain plasticity, and the underlying molecular mechanisms remain obscure.

NNP35 | Can the cellular interference hypothesis find an evidence through single unit analysis?

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Early Infantile Epileptic Encephalopathy is a neurodevelopmental disease characterised by the mutation of the protocadherin 19 (PCDH19) gene, encoding a cell-adhesion protein supposed to mediate synaptic connection. Genetic mosaicism underlies the pathological phenotype as, according to the cellular interference hypothesis, the co-existence of two distinct neuronal populations is believed to hinder cell-cell communication and network wiring. Thus, characterisation of the activity pattern of the two networks can provide strong insights into the physiopathology of this model. Single unit (SU) analysis is an ideal tool to compare electrical activity of different cell populations, as well as to relate activity of one neuron to the overall cortical dynamics, because it aims to identify individual neurons whose activity is sampled in local field potential (LFP) recordings. Since the major contribution to LFP signal is given by slow synaptic events and the brain impedance dampens down the high frequencies, detection of spikes is not immediate and is confined to the few neurons surrounding the electrode. Here, we provide a customizable algorithm to detect and classify single units in three steps: detection, feature extraction, and clustering. Spikes detection is performed by applying a threshold to high pass filtered data, and by selecting appropriate temporal windows we can merge suprathreshold peaks belonging to the same event. Feature extraction computes a dimensionality reduction: it finds those components that most reliably describe the spike population. Eventually, we compute what single units really are: using clustering algorithms, each event described by the features is assigned to a specific group that should corresponds to a single neuron. By applying SU analysis on LFP recordings from a PCDH19 mosaic mouse model, we expect to describe two separate neuronal populations providing an electrophysiological evidence for the cellular interference hypothesis.

NNP36 | NURR1 deficiency in mice is associated with sex-dependent altered behavioral phenotypes

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The transcription factor NURR1 regulates the dopamine (DA) signaling pathway and exerts critical role in the development of midbrain dopaminergic neurons (mDA). NURR1 exerts also an anti-inflammatory function in microglia, which protects mDA from inflammation-induced death. Given its crucial functions, altered NURR1 expression is implicated in DA-associated brain disorders, including Parkinson's disease (PD) and schizophrenia. NURR1-knockout (NURR1-KO) mice have been reported to exhibit increased spontaneous locomotor activity, but their complete characterization is still lacking.
In the present study a wide-ranging test battery was used to perform a comprehensive analysis of the behavioral phenotype of adult and old male and female NURR1-KO mice. As a result, the hyperactive phenotype was confirmed only in NURR1-KO male, while the impulsive behavior was reported in both sexes for the first time. NURR1-KO female mice also reported sociability defects. On the other hand, no anxiety and alterations in motor coordination and spatial memory were observed in both sexes of NURR1-KO mice. The behavioral alterations reported were maintained also in old NURR1-KO mice. In order to detect the consequences of NURR1-deficiency in mice, biochemical and histological analysis were performed. Specifically, the number of mDA expressing tyrosine hydroxylase, a rate-limiting enzyme of catecholamines biosynthesis, and DA level in brain and in plasma were not impaired in NURR1-KO mice. Notably, NURR1-KO mice showed a reduced number of Iba1+ microglia cells in substatia nigra in comparison to their wild-type mice. The study suggests that the NURR1 deficient mouse model may be a satisfactory model for studying some sex-dependent behavioral phenotypes characteristic of attention-deficit hyperactivity disorder (ADHD), such as hyperactivity and impulsivity. The data also support the novel finding in which glia functionality could cooperate to the pathogenic mechanisms of psychiatric disorders.

NNP37 | Stress-induced neurobehavioral dysfunctions in obese mice: a translational model for neuroprotective nutraceutical strategies

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Depression, anxiety and memory impairment caused by psychosocial stress (PS) in adult obese are global public health issues. However, an effective neuroprotective treatment is still awaited. Although food intake is central to the problem of obesity, nutrition could also play an important role in neuroprotection. Functional foods exert established health benefits apart from their nutritional properties due to the activity of specific bioactive compounds. While the dietary supplements demand is widely increasing, studies targeting the food neuroprotective compounds development are missing due to lack of clinically relevant animal model recapitulating hallmarks of neurobehavioral impairment induced by PS combined with obesity. To achieve this purpose, male adult C57BL/6J mice were fed a high-fat diet (HFD) for 18 weeks and exposed to Resident-Intruder Paradigm during the last 2 weeks of diet to spark off PS (Ob+PS, n=4). In the 8^{th} week of the HFD, the diet of an additional group of mice was supplemented with 3% of barley β-D-Glucan, a natural cardioprotective agent, and PS was superimposed (Ob+β+PS, n=5). Age-matched mice fed a standard diet and unstressed served as controls (SD, n=4). The onset of anxiety-related traits, depression-like behavior and spatial memory deficit was detected in Ob+PS group using Elevated Plus Maze, Open Field and Y-maze test. Perfused hippocampal slices of the same animals have shown smaller dentate gyrus volume and a reduction in neurogenesis ($BrdU^+$ cells), synaptic plasticity (PV^+ interneurons) and astrogliosis (GFAP levels) phenomena. Conversely, β-D-Glucan supplementation in the HFD significantly counteracted the onset of anxious traits and the spatial memory decay, simultaneously with a lower decrease of PV^+ interneurons. In conclusion, our murine model recapitulates hallmarks of stress-induced neurobehavioral dysfunction in obesity and is effectual in testing neuroprotective efficacy of functional food compounds, such as β-D-Glucan.

NNP38 | Associative learning and synaptic plasticity in the Lateral Entorhinal Cortex

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The entorhinal cortex (EC) represents a major hub between the hippocampal formation and polymodal associative areas and, based on cytoarchitecture, connectivity and function, it can be subdivided into two main subregions: the lateral entorhinal cortex (LEC) and the medial entorhinal cortex (MEC). Interestingly, the LEC has been shown to play a role in the formation of object-place-context associative learning. However, changes in synaptic plasticity induced by associative learning in the intrinsic circuitry of the LEC have not been investigated. In this work, we used the object-place-context recognition test (OPCRT) as a behavioural paradigm and we characterized the time course of this specific memory performing the sample trials at time 0 and presenting the test trial 1h, 6h, 12h or 24h following the presentation of the sample trials. Moreover, we recorded field excitatory post-synaptic potentials (fEPSPs) in LEC superficial layers using acute brain slices obtained from mice subjected to OPCRT and we found that the main forms of synaptic plasticity, namely LTP and LTD, were altered following behaviour. Specifically, LTP was reduced in mice subjected to OPCRT compared to controls (97 ± 6% of baseline, n =4 slices from OPCRT mice $p < 0.05$ *vs* 127 ± 7%, n = 9 slices from control mice, 2-way ANOVA RM), while LTD was enhanced (52 ± 6% of baseline, n= 4 slices from OPCRT mice $p<0.05$ *vs* 85 ± 5% of baseline, n = 10 slices from control mice, 2-way ANOVA RM), suggesting that the formation of an object-place-context associative memory is able to induce changes in the intrinsic circuitry of LEC superficial layers and that it could induce a shift in the threshold for synaptic modifications. These results confirm the involvement of the LEC superficial layer plasticity in the OPCRT paradigm and encourage us to further investigate the specific role of LEC neurons in the formation and retrieval of associative memories.

NNP39 | Active training promotes visual functions recovery in adult amblyopic rats

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Amblyopia is the major cause of visual impairments in infant and young children, arising from an unbalanced stimulation of the two eyes during postnatal visual development. Current treatments such as eye patching are ineffective in adult subjects, due to the drastic decline in cortical plasticity accompanying the transition from youth to adulthood. Nevertheless, recent studies have started to reveal the possibility to enhance adult visual cortical plasticity and to promote visual acuity recovery. We investigated the effect of two non-invasive active training procedures, i.e. voluntary physical exercise and visual perceptual learning, on visual functions recovery in adult amblyopic rats with unrestricted binocular vision. We proved that active training induces a marked and long-lasting recovery of both visual acuity and depth perception abilities. The beneficial effects of voluntary physical exercise were also linked to the activation of a distinct GABAergic circuit in the primary visual cortex.

NNP40 | Pupil fluctuations as a biomarker for CDKL5 disorder

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CDKL5 deficiency disorder (CDD) is a neurodevelopmental disorder characterized by a global developmental delay, seizures and visual abnormalities. Preclinical efficacy assessment of treatments depends on the availability of noninvasive biomarkers that are still missing. The aim of the present study was to establish a first background for the use of pupillometry as a biomarker for CDD. We performed pupillometry in fully symptomatic male and female CDD mutant mice. We implemented a machine learning algorithm to analyze average pupil diameter in different behavioral states (run/resting) through the use of a discoid treadmill and virtual reality. A movement sensor was used to record locomotor activity. To correlate alteration in pupil dynamics with behavioral abnormalities, we performed an appetitive conditioning (AC) task, to assess different aspect of learning and cognition. Lastly, we recorded visual responses by Imaging of the Intrinsic Optical Signal (IOS), a method that rely on activity-depedent changes in blood oxygenation, to assess cortical circuit function. We found, in mutant mice, an overall reduction in pupil size, but also longer period of running and dilated pupil, compared to wild-type. During the AC training mutant mice, despite a normal learning curve, performed significantly more trials with shorter reaction times. Conversely, during the test, mutant mice fail to acquire the new strategy. We found a strong correlation between the severity of behavioral (low number of trials performed during the test), visual cortical impairment and the smaller pupil size. Our study reveal that pupil deficits are correlated with altered brain activity and abnormal behavior. Since pupillometry can be performed easily in clinical settings we propose pupil monitoring as a biomarker for CDD.

NNP41 | Neuroadaptive changes in the nucleus accumbens following induction of activity-based anorexia in adolescent female rats

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Anorexia Nervosa (AN) is a psychiatric disorder characterized by restricted diet, strenuous exercise regimens and psychosocial disturbances, that result in a dramatic weight loss. Recently, it has been proposed that dieting and intense exercise, initially, are goal-directed behaviors driven by a strong weight-loss desire, that then shift to compulsiveness. This shift involves changes in the Nucleus Accumbens (NAc) synaptic physiology.
Using the well-established activity-based anorexia (ABA) rat model we sought to investigate the putative role of the glutamatergic synapse in the NAc in mediating the hallmarks of the anorexic phenotype.
Following exposure of female adolescent rats to the combination of food restriction and physical activity (i.e. the ABA protocol), we examined NMDA and AMPA receptor composition in the NAc at two time points: immediately after the achievement of the anorexic phenotype (P42) and after 7-days of body weight recovery (P49).
ABA rats show reduced body weight and increased wheel activity over days. Notably, ABA rats show increased mean and maximum speed during wheel activity at P40 and P41, a readout of their motivation to engage in intense physical activity. At molecular level, we found increased membrane AMPA subunits GluA1/GluA2 ratio in the NAc of P42-ABA rats. NMDA receptor levels, measured as GluN2A/GluN2B ratio, were increased at P42, while reduced at P49. Interestingly, the AMPA-related scaffolding protein SAP97 is increased at P42 and markedly reduced at P49, suggesting that the AMPA receptors might be locally unstable in the membrane reducing the synapse strength. PSD95, an index of postsynaptic integrity, is significantly reduced only at P49, suggesting that the ABA-induced synaptic vulnerability might cause long-lasting changes.
This maladaptive plasticity could represent a signal of altered processing of food reward and that might be, in turn, the trigger for the motivational and compulsive mechanisms underlying AN.

NNP42 | Detailed cellular-level modeling uncovers spatial adaptive filtering properties of the cerebellum granular layer

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The cerebellar granular layer is the input stage for signals carried by mossy fibers from sensory systems and from other parts of the brain. This layer gathers information that is recoded and transmitted to the molecular layer and to Purkinje cells, which generate the final output of the cerebellar cortex. Understanding these operations is crucial to understand the subsequent signal processing. In this regard, several theories have been developed; one of the main ones (i.e. the adaptive filter theory) predicts that the granular layer operates as a spatially organized adaptive filter able to process and to modify input patterns. To decipher the microcircuit activity and address these questions, experimental data acquired from multiple cells, along with realistic computational models, are critical. Here, we used a scanless two-photon microscope equipped with a spatial light modulator to investigate the spatial organization of granular layer activity in acute rat cerebellar slices. This system allowed to record the activity of hundreds of granule cells simultaneously, while maintaining single-cell resolution, and to monitor its changes after the induction of long-term synaptic plasticity. The experimental results validated outcomes of a realistic large-scale network model of the granular layer. The model provided mechanistic explanations to the experimental observations, revealing differential activation of glutamatergic and gabaergic receptors in regulating signals integration. These data show that the cerebellar granular layer activity is organized in multi-neuronal units that create spatial filters tunable by synaptic plasticity, thus supporting the adaptive filter theory. The model allows to decipher the microcircuit activity patterns and gives insight into disease conditions involving cerebellar microcircuit alterations (e.g. in autism-related mutations in mice).

NNP43 | A degradable GCaMP variant to significantly reduce the detrimental effects of high and prolonged expression in neurons

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The GCaMP family of proteins are genetically encoded calcium sensors which have been widely adopted across the neuroscience community, but which show some significant drawbacks, such as intracellular calcium buffering and competition of the calmodulin moiety of GCaMP with endogenous calmodulin. When the sensors are expressed chronically, these effects can be detrimental to neurons, where calcium is a critical intracellular signal, potentially leading to severely compromised cortical networks. These issues may be helped by reducing expression levels, but this creates other problems, decreasing the fluorescence signal and may limit its utility for widefield imaging of cortical function.

We therefore generated a novel, conditional variant, dGCaMP, which is continually produced, but constitutively degraded unless a commercial drug is administered. Thus, GCaMP expression is finely regulated by administering the activator only hours before the experiment.

An adeno-associated viral vector (AAV) carrying dGCaMP was produced and used to induce widespread expression, but without the previously reported side-effects associated with such levels of expression. We compared activity in adult mice transfected either with AAV-GCaMP or AAV-dGCaMP (intraventricular injection at P1). GCaMP expression was associated with markedly abnormal calcium activity and electrophysiology, indicative of spontaneous epileptiform discharges, which was not seen following dGCaMP transfection. dGCaMP is particularly useful for *in vivo* studies that require spatially unrestricted expression of GCaMP, but without compromising the level of expression.

NNP44 | Accurate in-vivo mapping of human brain connections: a new hope

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Diffusion magnetic resonance imaging is a noninvasive imaging modality that has been extensively used in the literature to study the neuronal architecture of the brain in a wide range of neurological conditions using tractography. However, recent studies challenged its appropriateness highlighting that the anatomical accuracy of the reconstructions is inherently limited. In particular, it was shown that the presence of false-positive connections in the reconstructions can significantly bias the topological properties of the estimated structural networks raising serious concerns for its use in mapping the brain structure. Several solutions have been proposed to tackle this issue, but none of them proved effective to overcome this fundamental limitation. Some authors prescribed the need for a revolution of tractography techniques to reliably reconstruct the known anatomy while controlling for false positives and, particularly, that the notion of both anatomy and microstructure is essential to progress. With this concept in mind, we developed COMMIT2 (Convex Optimization Modeling for Microstructure Informed Tractography 2) which naturally incorporates both these characteristics. This new framework shares the same convex optimization procedure with the original COMMIT adding the possibility to inject priors about brain anatomy and its organization, and not only about microstructural properties. We evaluated its effectiveness using both simulated and real human brain data. Our results indicate that COMMIT2 dramatically increases the accuracy of the estimated brain networks and, thus, represents a major step forward for the study of connectivity. Our novel processing framework has the potential of changing the landscape of structural network analyses and, most importantly, improves our confidence in the interpretation of group differences or disease differences of certain connections, which now are characterized with anatomical and quantitative microstructural properties.

NNP46 | Activation of 5-HT7 receptors rescues hippocampal synaptic plasticity in a murine model of Fragile X Syndrome by stimulation of adenylate cyclase,protein kinase A and Cyclin-Dependent Kinase 5

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Fragile X Syndrome is a genetic form of intellectual disability associated to epilepsy and autistic behaviour. *Fmr1* knock-out (KO) mice, an animal model of Fragile X Syndrome, exhibit alterations in synaptic plasticity with exaggerated metabotropic glutamate receptor-mediated long term depression (mGluR-LTD) in the hippocampus. We have previously shown that activation of serotonin 5-HT7 receptors (5-HT7R) reverts mGluR-LTD in wild-type (wt) and *Fmr1* KO mouse hippocampus, thus correcting abnormal mGluR-LTD in the murine model of Fragile X Syndrome. Using patch clamp, we have investigated the intracellular pathways involved in 5-HT7R-mediated effect. 5-HT7R-mediated reversal of mGluR-LTD in wt and *Fmr1* KO hippocampal neurons was abolished by SQ22536, an adenylate cyclase (AC) inhibitor, and was mimicked by forskolin (an AC activator) and by Pituitary Adenylate Cyclase Activating Peptide (PACAP). 5-HT7R-mediated reversal of mGluR-LTD in wt and *Fmr1* KO was also abolished by PKI, a protein kinase A (PKA) inhibitor, and by roscovitine, a selective inhibitor of Cyclin-Dependent Kinase 5 (Cdk5). Moreover, the amount of mGluR-LTD in wt neurons was enhanced after Cdk5 blockade, becoming comparable to exaggerated mGluR-LTD of *Fmr1* KO neurons: this result suggests that Cdk5, a kinase involved in several neurodegenerative diseases, might be downregulated in Fragile X Syndrome. In conclusion, our results identify the AC/cAMP/PKA pathway and Cdk5 as novel targets for a possible therapy of Fragile X Syndrome, for which no cure is currently available.

NNP47 | Use of computational tools to evaluate the glycogen distribution in the somatosensory cortex of aged mice

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Astrocytes are the most abundant type of glial cell in the brain, required to ensure optimal neuronal functioning, neurogenesis, and brain vascular tone. Moreover, they play a crucial role in support of neuronal metabolism. The human brain utilizes around 20% of the energy consumed to ensure its proper function. Glucose, an important energy source for the brain, access the neuropil across the blood-brain barrier (BBB), and then is transported into astrocytes through their perivascular end-feet, where it can be stored as glycogen. Furthermore, lactate can be synthesized through glycogenolysis and then shuttled via monocarboxylate transporters (MCTs) to neurons to fuel their tricarboxylic acid (TCA) cycle. This mechanism is known as astrocyte-neuron lactate shuttle (ANLS) and is involved in learning and memory formation. Aging is associated with a decline of faculties such as memory, motor skills, and sensory perception. These deficits are not thought to be due to a substantial loss of neurons but rather changes at the level of connectivity, morphological changes, and white matter structure. In the present study, we aim to compare the glycogen distribution in layer I of the somatosensory cortex between adult (4 months old) and geriatric mice (24 months old). We carried out the visual analysis using Connectome Explorer, which allows us to explore, in real-time, brain reconstructions at the nanometric-level. Using the computational tool GLAM (Glycogen-derived Lactate Absorption Map), we can infer a probability map of the locations where astrocytic glycogen-derived lactate is most likely accessing the surrounding neurites. We analyzed and compared the probability maps on axons, dendrites, boutons, and spines to make a functional hypothesis about single compartments' energy consumption. Our results indicate that aging brains have a more glycolytic metabolism, with fewer peaks facing mitochondria, and smaller glycogen granules.

NNP48 | The role of TrkAR649W mutation in the first animal model of Hereditary Sensory and Autonomic Neuropathy type IV

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Nerve growth factor (NGF) and its high-affinity receptor TrkA play an essential role in the genesis and maintenance of pain states in mammals. Loss-of-function mutations in the NTRK1 gene encoding the TrkA receptor result in an inherited form of congenital painless syndrome, named Hereditary Sensory and Autonomic Neuropathy type IV (HSAN IV). HSAN IV patients are affected by a complete insensitivity to painful stimuli that lead to oral injuries as well as multiple burns, falls and fractures. Further key features of HSAN IV are inability to sweat (anhidrosis) leading to severe episodes of fever and variable degree of mental retardation. Many different mutations have been identified in HSAN IV patients, that mainly lead a diminished kinase activity and absent auto-phosphorylation of the TrkA receptor. We generated a knock-in mouse line for HSAN IV carrying the R649W mutation in human TrkA gene. Our data show that heterozygous human-TrkA^{R649W} mice have a reduced response to thermal and chemical noxious stimuli such as cold or heat hypersensitivity induced by acetone or capsaicin injection. All these evidences are supported by histological analysis of dorsal root ganglia (DRG) where we found a reduced expression of some nociceptive markers and loss of nerve fibers in both glabrous and hairy skin. To evaluate the ability to sweat, we performed a sweat assay pointing out that hTrkA^{R649W} mice are affected by anhidrosis. Moreover, we found an impairment in spatial memory when animals perform spontaneous alternation test (y-maze). To the best our knowledge, hTrkA^{R649W} is the first animal model replicating by and large the main hallmarks of HSAN IV. Our results strongly suggest that the R649W mutation alters pain transduction in both mice and patients. Summarising the most important features of the disease, we provide insights into the mechanisms leading to HSAN IV.

PNPN04 | Sleep-related epilepsies: insights from a putative rodent model of epileptic encephalopathies

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Encephalopathies with *status epilepticus* during sleep (ESES) are insidious pathologies of the childhood, characterized by spike-wave discharges (SWDs) during sleep and by cognitive impairments. To establish a viable experimental model of ESES, we set out to characterize the *in-vivo* electrophysiological and behavioural phenotype of the AJ JAX mouse strain, previously reported to exhibit SWDs during sleep. Video-EEG/EMG signals were continuously acquired for 7 days in adult AJ JAX and matched control mice (C57BL/6J and AJ OlaHsd). Data were analysed to score vigilance states and to detect SWDs. The behavioural evaluation of JAX and C57 mice included an anxiety-trait assessment and tests aimed at measuring the animal's proficiency in spatial as well as Pavlovian learning and memory. Additionally, AJ JAX mice were subjected to 6 hours of total sleep deprivation (TSD), followed by 18 hours of undisturbed rest. Unlike controls, AJ JAX mice consistently displayed SWDs, occurring mainly during slow-wave sleep, and following a specific circadian distribution. AJ JAX mice also showed learning and memory impairments. Interestingly, compared to baseline, the sleep debt (characterized by a massive presence of delta power) induced by 6 hours of TSD substantially reduced the frequency of SWD events during the 6 remaining hours of the rest period. Overall, the electrophysiological and behavioural phenotype of AJ JAX mice shares significant features with human ESES. Moreover, altering the sleep-wake cycle modulated the occurrence of SWD. Further investigations are required to fully assess the viability of the AJ JAX strain as a source of insight into the mechanisms underlying sleep-associated epilepsies.

PNPN06 | An extremely rare case of prenatal brain asphyxia in a preterm infant

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Perinatal asphyxia and hypoxic ischemic encephalopathy are very well known in neonatal clinical practice: in this work we describe an extremely rare case of prenatal asphyxia.
The neonate was born via urgent cesarean section due to cardiotochography alterations, IUGR and severe oligohydramnios at 31 weeks of gestational age (birthweight: 1800 g).
The APGAR score was 3 at the first minute of life requiring intubation, external cardiac massage and Intermittent Positive-Pressure Ventilation (IPPV) and FiO_2. It was 5 at 5 minutes.
The patient was admitted in Neonatal Intensive Care Unit (NICU) with invasive ventilation treated with endotracheal surfactant, extubated and supported with non-invasive ventilation.
The neurological picture showed, severe diffuse hypotonia, poor responsiveness to external stimuli, tremors and clonus of the mandible.
At day 3 the patient had generalized clonic seizures and severe respiratory instability that required intubation and invasive respiratory support. Then barbituric therapy was initiated. After 24 hours the baby is extubated and supported with non-invasive ventilation for about 3 months.
Brain sonography was normal at birth. In the next days, it showed crescent hyperechogenicity of both the thalamic nuclei that became more and more evident in the 2nd week together with an irregular widening of the lateral ventricles and periencephalic liquoral spaces, suggesting a profound prenatal asphyxia.
The baby developed severe brain atrophy with diffuse widening of the ventricles.
Magnetic Resonance Imaging (MRI) confirmed hyperintensity of the thalami and thinning of the corpus callosum.
At 6 months, the patient displays severe axial hypotonia, scarce mobility and is fed with formula via nasogastric tube.
This case is still very difficult to diagnose in pregnancy and extremely challenging for clinicians.

ND25 | Pathogenic FUS promotes the expression of aggregation-prone splicing isoforms of hnRNP A2/B1 in amyotrophic lateral sclerosis

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Several genetic and experimental findings point to a crucial role of RNA dysfunction in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS). In particular, evidence suggests that mutations in FUS, that are associated with genetic ALS, affect the regulation of alternative splicing (AS) of a selected number of target genes. Recently we have demonstrated that a major target of FUS activity is hnRNP A2/B1, an RNA binding protein with key roles in RNA metabolism, including AS regulation, which is mutated in familial ALS. We have now evidence that FUS can regulate AS process both by directly binding A2/B1 RNA through its RGG1 domain, and by interfering with the importin-b1/snurportin-mediated nuclear import of UsnRNPs, the core constituent of the spliceosome. Overall, these data suggest that FUS-mediated alterations in AS regulation of A2/B1 might impair its functions and cause a pathogenic cascade of AS changes, eventually promoting motor neuron degeneration. Indeed, using isoform-specific antibodies we have observed that the expression and distribution of different splice variants of A2/B1 is modified in the lumbar spinal cord of a mouse model of FUS-ALS. Further, by using isoform-specific A2/B1 expression constructs, we have pinpointed distinct abilities of A2/B1 splicing variants to accumulate into cytoplasmic stress granules, thus identifying an isoform-specific feature that might be relevant for the pathogenic mechanism. Finally, we have identified by bioinformatics analysis a restricted number of common splicing targets of FUS and A2/B1 and verified that their AS patterns and protein expression are affected in diseased FUS mice. Altogether, these data support the existence of a pathological connection between FUS and A2/B1 in ALS and suggest the feasibility of a therapeutic strategy using splicing switching oligonucleotides.

ND26|Effectof3Dmicro-scaffoldNichoidonthetranscriptome of Neural Stem Cells

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3D scaffolds are becoming more and more important in the field of regenerative medicine due to their ability to mimic the physiological microenvironment. In this field, Raimondi and colleagues applied two-photon polymerization (2PP) to fabricate the "Nichoid", an engineered stem niche able to mimic the physiological environment where stem cells reside. To investigate the Nichoid's influence on cellular response in terms of global gene expression, murine neural stem cells (NSCs) where expanded inside the micro-scaffold and analysed through a whole transcriptomic approach. Nichoids were fabricated by femtosecond laser 2PP using an organic-inorganic photoresist. 10^4 NSCs were expanded inside the engineered niches and in control conditions for 7 days and then analysed through RNA-seq to evaluate how the NSCs' expansion in the bioengineered 3D micro-scaffold may influence the cellular response. The outcomes of RNA-Seq were confirmed through Real Time PCR and immunofluorescence analysis. Here we demonstrate that the Nichoid impacts the biological and genetic response of stem cells, as genes strongly connected to mechanobiological functions emerged. We fully dissected this mechanism highlighting how the changes start at a membrane level, with subsequent alterations in the cytoskeleton, signaling pathways, and metabolism, all leading to a final alteration in gene expression. Moreover, NSCs grown inside the Nichoid show also significant increase in the expression of pluripotency genes, as results of the changes in membrane, cytoskeleton and nucleus. Thus, RNA-Seq highlights the Nichoid's ability to induce changes in cultured stem cells at any molecular level without any other chemical agents, allowing recreating the stem cells adhesion, migration, differentiation, proliferation and cell signaling that best mimics physiological conditions. Here we provide evidences that this capacity is essential for regenerative medicine based on the development of stem cell therapies.

ND27 | Look over the correlation between serum neurofilament light polypeptide and global structural connectivity in multiple sclerosis patients

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The *neurofilament light polypeptide* (NfL) protein is highly expressed in myelinated axons. The serum levels of NfL (sNfL) increase with increasing in axonal damage, so sNfL is considered a biomarker for multiple sclerosis (MS). So far, the effects of this axonal damages on structural connectivity of MS patients have never been investigated. We evaluated if sNfL is related to changes detectable by structural network metrics estimated with diffusion MRI. The sNfL levels were measured from blood samples of 74 patients (44F, 44.9±14.6yrs, 50 relapsing-remitting and 24 progressive). The white-matter lesions *volume* was computed with an automatic in-house tool on MR images. The networks were built using deterministic tractography; the grey matter was segmented in 85 regions using T1 images, and the connections strength was computed by counting the streamlines between each pair of regions. From each network 5 *global metrics* were extracted: *Density* (ratio between actual and possible connections); *Efficiency* (capability to transfer and process information); *Modularity* (network segregation); *Clustering coefficient* (degree on which nodes tend to cluster together); *Mean strength* (average of the edge weights sum connected to a node). Since discrepancies in density may affect the analyses, we tested its relation with sNfL, then we conducted partial correlations between sNfL and the 4 remaining metrics with *age*, *sex* and *density* as covariates. *Density* was negatively related to sNfL (R=-0.252 p=0.05) suggesting that axonal damage is associated to reduced number of connections. *Efficiency* and *mean strength* were negatively related with sNfL (R=0.325 p=0.011; R=-0.475 p<0.001). *Modularity* and *clustering coefficient* seemed not related to sNfL. The *lesion volume* was positively related with sNfL (R=0.323 p=0.011) confirming previous results. In conclusion, we found that reduced *number of connections*, *efficiency* and *mean strength* are associated with high sNfL values.

ND28 | Decreased mitochondrial DNA methylation levels in sporadic amyotrophic lateral sclerosis patients

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease that occurs in sporadic (sALS, 90%) and familial forms (fALS, 10%). Increasing evidence points to a role of mitochondrial dysregulation and aberrant epigenetic mechanisms in the aetiology of ALS, and it has been suggested that epigenetic dysregulation in mitochondrial DNA (mtDNA) could contribute to the disease. We recently screened families with mutations in the major ALS causative genes, namely *C9orf72*, *SOD1*, *FUS* and *TARDBP*, observing reduced methylation levels of the mtDNA D-loop region, which regulate mtDNA copy number, only in carriers of *SOD1* mutations. However, until now no studies investigated mtDNA methylation levels in the sporadic form of ALS.

The aim of the current study was to investigate the D-loop methylation levels and the mtDNA content in sALS patients and compare them to those observed in healthy controls and in ALS patients with mutations in *SOD1* or *C9orf72*, that represent the major fALS forms.

Pyrosequencing analysis of D-loop methylation levels and quantitative analysis of mtDNA copy number were performed in peripheral blood from 36 sALS patients, 51 age and sex matched controls, and 27 fALS patients.

In the total sample D-loop methylation levels were lower in ALS patients compared to controls, and a significant inverse correlation between D-loop methylation levels and the mtDNA copy number was observed. Both *SOD1*-mutant and sALS patients had lower D-loop methylation levels compared to controls, whilst *C9orf72*-ALS patients had similar D-loop methylation levels than controls.

Present data reveal for the first time altered mtDNA methylation levels in sALS and confirm previous evidence of an inverse correlation between D-loop methylation levels and the mtDNA copy number, as well as mtDNA methylation differences among the major fALS subtypes. Results of the current study suggest that D-loop methylation and mitochondrial replication are strictly related to each other and could represent compensatory mechanisms to counteract mitochondrial impairment in sporadic and *SOD1*-related ALS forms.

ND29 | Estrogen effects on miRNA-218 and Tau alterations in Alzheimer's disease.

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Alzheimer's disease (AD) is the most common form of dementia and it's characterized by amyloid plaques, neurofibrillary tangles and neuroinflammation in the brain. Sex differences in the prevalence, risk, and severity in AD patients have been demonstrated in numerous studies, which revealed that women are more susceptible to developing AD than men. This could be due to estradiol levels, which seem to be inversely correlated with miRNA218 expression. The overexpression of ERα increases miR-218 expression and Tau phosphorylation. On these bases, we observed miR-218 expression in AD patient's liquor analysing the differences between male and female. The data revealed that miR-218 is more expressed in mild cognitive impairment and AD patients in both sexes, but miRNA levels are double in AD women patients than their gender control. In order to investigate the role of estrogen in miR-218 expression we used 2 months-old hTau mice, focusing on the pathological changes of Tau mediated by amyloid-β (Aβ42). Two groups of female mice (OVX) underwent to ovariectomy, one of these groups and a group of male mice were treated with 17β-estradiol. In order to observe the effect of estradiol treatment, we performed western blot analysis to detect the pathological changes of Tau. We also studied the estradiol effect on total antioxidant capability and on miR-218 expression. Our data revealed that Aβ42 monomers produce the pathological alterations of Tau in male or OVX female mice but not in control female. We also observed that estradiol treatment protects against the pathological conformation of Tau and the mechanism mediated by estradiol could be due both by its antioxidant activity and its ability to modulate the expression of miR-218 linked to Tau alterations. This study indicates that factors as age, reproductive stage, hormone levels and the interplay with other risk factors should be considered in women, in order to identify the best therapeutically approach in prevention of AD.

ND30 | Establishment of a neurogenic niche with dentate gyrus identity from human iPSCs

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Zinc finger and BTB/POZ binding domain protein 20, ZBTB20, is a critical transcription factor in hippocampal formation as its loss of function *in vivo* ablates the dentate gyrus and cornu ammonis (Rosenthal et al. 2012, *Hippocampus*). Originally proposed as a hippocampal marker, ZBTB20 is uniformly expressed across all hippocampal layers and is sustained throughout adulthood (Nielsen et al. 2007, *Development*). We hypothesize that ZBTB20 is lateral to neurogenesis and that its expression is conserved in human cells during neurogenesis considering its constitutive, translaminar expression.. To investigate ZBTB20's role in developmental neurogenesis, we established a human induced pluripotent stem cell model whose transcriptomic profile mimics dentate gyrus (DG) progenitors and mature neural markers at discreet developmental stages. We then established a progenitor niche in which DG-like progenitors were expanded over 200 days *in vitro*, expressing Nestin and Ki67. Since longitudinal maintenance is known to compromise stemness, we assessed differentiation *in vitro* and *in vivo*. To elucidate upstream ZBTB20 actuators and its potential role in human neurogenesis, we induced differentiation by NOTCH inhibition in naïve DG-like progenitors and compared the same treatment against 160-day old DG-like progenitors. We found that $NeuN^+$ and $ZBTB20^+$ cell proportions increase under spontaneous differentiation conditions. We observed that cells treated with NOTCH inhibition resulted in a ~20% increase in $ZBTB20^+$ cell proportion in both the naïve group and 160-day old group. This increase in $ZBTB20^+$ cell proportion is linked with an increase in $NeuN^+$ cell proportion in both groups. We then transplanted DG-like progenitors into the healthy DG of wild-type B6 mice and assessed DG-CA1 projections and synaptic markers, observing both maturation and integration in the host DG. With development, we anticipate this may serve as an avenue for stem cell based therapy in neurodegenerations.

ND31 | Effects of exosomes derived from IFNγ-primed mesenchymal stem cells on the phenotype of astrocytes cultured from late symptomatic SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that affects upper and lower motor neurons (MNs) leading to muscle atrophy and paralysis.
ALS is a non-cell autonomous disease, in which astrocytes play a central role in clinical progression. We previously demonstrated that the intravenous administration of mesenchymal stem cells (MSCs) in the $SOD1^{G93A}$ mouse model of ALS prolonged survival, improved motor skills and reduced reactive gliosis. These beneficial effects were not associated with MSC differentiation, being possibly mediated through paracrine mechanisms. We postulated that MSC-derived exosomes can be a mode to sustain the paracrine effects of MSCs. We studied here the effects of MSC-derived exosomes on the phenotype of astrocyte from $SOD1^{G93A}$ mice.
GFAP, S100β and vimentin expression was increased in 120 days-old $SOD1^{G93A}$ astrocytes compared to age-matched WT astrocytes. In-vitro exposure to MSC-derived exosomes significantly reduced the overexpression of the three astrocyte activation markers. The expression of the inflammation complex NLRP3 was increased in $SOD1^{G93A}$ astrocytes and the increase was reversed after exposure to exosomes. Accordingly, the pro-inflammatory cytokines IL-1β, TNF-α and IL-6 were significantly more expressed and more efficiently released in $SOD1^{G93A}$ astrocytes and exosomes significantly decreased their overexpression and release. Conversely, the expression of the anti-inflammatory cytokine IL-10 was decreased in $SOD1^{G93A}$ astrocytes and normalized after exposure to exosomes. The viability of embryonic $SOD1^{G93A}$ mouse-derived MNs was significantly increased when seeded on exosome-treated adult $SOD1^{G93A}$ astrocytes, compared to non-treated astrocytes.
Our results suggest that the reactive phenotype and the inflammation state of $SOD1^{G93A}$ mouse astrocytes are ameliorated by exosomes derived from IFNγ-primed-MSCs. Spinal MN viability was also improved. These results open to the possibility to in-vivo preclinical trials with exosomes in $SOD1^{G93A}$ mice.

ND32 | Effects of the Pharmacological Block of Metabotropic Glutamate Receptor 5 in SOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease leading to motor neuron (MN) death. Among the different pathological mechanisms, glutamate(Glu)-mediated excitotoxicity plays a major role in MN degeneration. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in Glu-mediated excitotoxicity. We previously reported that mGluR1 and mGluR5 activation produced abnormal Glu release in the spinal cord of $SOD1^{G93A}$ mice and that genetically reducing mGluR1 or mGluR5 expression in $SOD1^{G93A}$ mice significantly prolonged survival, slowed down disease symptom progression and ameliorated a number of biochemical, cellular and functional parameters altered in ALS. On this basis, we investigated here the effects of the in-vivo pharmacological blockade of mGluR5. At this purpose, we treated $SOD1^{G93A}$ mice with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy) phenyl)-1H-imidazol-4-yl)ethynyl)-pyridine (CTEP), an orally available mGluR5 negative allosteric modulator, at the doses of 2 mg/kg/48h or 4 mg/kg/24h, starting from 90 day of life, representing an early symptomatic stage of the disease, until mouse euthanasia. CTEP dose-dependently ameliorated clinical features in $SOD1^{G93A}$ mice. The lower dose increased survival and improved motor skills in female mice, while it barely produced positive effects in male mice. The higher dose significantly ameliorated disease symptoms and survival in both males and females being, however, females always more responsive. CTEP treatment also reduced motor neurons death, astrocyte and microglia activation and normalized the abnormal Glu release in the spinal cord. Our previous and present results suggest that mGluR5 represents a promising target to counteract ALS and highlights mGluR5 inhibitors as CTEP or structural analogues as favorable new pharmacological tools with possible translational perspectives.

ND33 | HDAC6 interacts with TDP-43 contributing to ALS pathogenesis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative and still incurable disease characterized by the loss of motor neurons. Recent studies suggested that HDAC6 plays a key role in the regulation of autophagy pathway, which is known to be impaired in ALS. Moreover, TDP43 was found to bind the mRNA of HDAC6, inhibiting its RNA translation. Thus, aim of our work was to evaluate the binding between TDP43 and HDAC6 in ALS model and the effect of the inhibition of HDAC6 in SH-SY5Y cell line. We firstly evaluated the binding of TDP43 to HDAC6 by RNA immunoprecipitation technique in peripheral blood mononuclear cells (PBMCs) of sporadic ALS (sALS) patients and healthy subjects. In a second time, we over-expressed TDP43 to investigate the effect on our cellular model, analysing the level of HDAC6 protein and the effect on the autophagy pathway. Finally, we studied autophagy by both western blot and immunofluorescence after HDAC6 silencing. We found that TDP43 binds the mRNA of HDAC6 in sALS patients, while this binding was not found in healthy subjects. We suppose that the increase of TDP43 in cytoplasmic compartment, which is usually found in ALS, leads to a decreased RNA translation of HDAC6. Moreover, we found that an over-expression of TDP43 leads to the decrease of HDAC6 protein level. These data confirmed the regulating role of cytoplasmic TDP43 in HDAC6 translation. Finally, we found that a decreased level of HDAC6 leads to a stop of autophagy pathway, very similar to the inhibition we found on sALS patients' cells. Our work provides new insight in the pathogenesis of ALS, by investigating a new pivotal role of TDP43 in autophagy impairment.

ND34 | Curcumin formulation in ferritin nanocages as potential therapy against dementia

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Curcumin is a polyphenolic compound derived from the dried rhizome of the plant *Curcuma Longa* that has been intensively studies for its nutraceutical potential. One of the most appealing potential of curcumin is its use as therapeutic agent against dementia. This possibility was studied in different clinical trials with mixing results. Some studies suggest that curcumin could be use of as a preventive treatment for dementia allowing a reduction of the natural predisposition of beta amyloids (Aβ) to accumulate in brain. Other studies highlighted the fact that curcumin is almost insoluble in water, poorly adsorbed by the organism and rapidly degraded and thus its pharmacological effects are limited.

Staring from these premises, we optimized H-Ferritin nanocages (HFn) as nano drug delivery system to enhance the effectiveness of curcumin.

Thanks to the use of HFn as nanocarrier is was possible to obtain formulation of curcumin (HFn-Cur) with an increment of the apparent solubility of about 700-fold and stable for several days at room temperature. The toxicity of HFn-Cur was studied by zombie violet assay for cell viability with excellent results. HFn-Cur was also proven as being able to deliver the drug to the central nervous system in an in vitro model of blood brain barrier (BBB).

As preliminary results on the pharmacological activity HFn-Cur was tested as preventive treatment able to improve the ability of PBMC to metabolize Aβ.

Overall, the results obtained suggest that HFn-Cur could be an effective nanoformulation able to improve the effect of curcumin as treatment for dementia.

ND35 | Nanoparticle based Enzyme Replacement Therapy for the treatment of Krabbe disease

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Krabbe disease (KD) is a rare genetic lysosomal storage disorder (LSD). It is an autosomal recessive neurodegenerative lipidosis caused by the lack of the lysosomal enzyme β-galactosylceramidase (GALC). Many mutations in the GALC gene are known to lead to the enzymatic deficiency, which is the cause of the accumulation of the cytotoxic galactosyl sphingosine (or psychosine, PSY). This accumulation induces the onset of pathogenic signs both in the central (CNS) and in the peripheral nervous system (PNS), principally owing to the death of oligodendrocytes and Schwann cells. Currently, there is no effective cure for KD, and the available treatments are supportive or symptomatic only.

We here develop a new enzyme delivery system based on the encapsulation of cross-linked enzyme aggregates (CLEAs) into PLGA nanoparticles (NPs) functionalized with brain targeting peptides for the treatment of Krabbe disease. Firstly, we verify the capability of our vector to successfully vehiculate the active enzyme inside cells and accumulate in the lysosomes, where GALC is supposed to perform its physiological activity. Subsequently, we proceed with the testing of our NP formulations in vivo in the Twitcher (TWI) mouse, the murine model of KD, through a single intraperitoneal injection. 4 hours after the injection, we find substantial GALC activity in the accumulation organs examined and GALC activity that was approximately equal to the activity of the heterozygous mice for GALC mutation, which are characterized by a completely healthy phenotype.

Together these studies allow us to demonstrate for the first time the capability of targeted polymeric NPs to perform delivery of a protein into the brain of the TWI mouse, overcoming the blood-brain barrier and delivering functional enzyme in therapeutically relevant amount.

ND36|Developingahumanspidersilkscaffold-basedplatform to generate functional and reproducible bioengineered forebrain organoids

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The inaccessibility of functional human brain tissue and the inability of two-dimensional *in vitro* cultures to recapitulate the complexity and role of neuronal circuitries have made the study of brain functions and dysfunctions challenging. Three-dimensional (3D) human brain organoids have rapidly become a widely used system to study brain development in a dish. The organoid cultures relied on the intrinsic self-organization property of human pluripotent stem cells (hPSCs) to mimic multiple structural regions of developing brain in a physiologically relevant cellular context. Cultured over long periods of time, 3D organoids provide a unique opportunity to model in a dish human neural tissue features such as cytoarchitecture and cell-cell interactions reminiscent of human brain complexity, generating precise patterns of synaptic connectivity including regulatory crosstalk between specific cell types. 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 forebrain organoid differentiation by using silk scaffold, sustain the homogeneous and functional generation of mature neurons throughout all compartments of the organoid in a highly efficient manner. The capacity of silk scaffolds to form porous microarchitectures with naturally formed cavities after foam reabsorption facilitates the delivery of oxygen, nutrients, and extrinsic patterning cues, thus creating more favorable growth and differentiation conditions. Overall these findings pointed out silk technology as an easily accessible *in vitro* methodological platform that allows the generation of reproducible and homogeneous forebrain organoids.

ND37 | Two related cases affected by slow and fast dementia reveals epigenetics impairments in specific brain areas

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Objectives: Genetics, transcriptomic and epigenetics play an important interplay in the clinical diagnosis of patients affected by neurodegenerative diseases is often complex and requires neuropathological examinations. For this reason, it is necessary to strengthen molecular efforts to sustain clinical diagnosis. Here, we present the molecular characterization of two kindred cases (mother and son) diagnosed as slow and fast dementia and classified as Alzheimer's Disease (AD) and Dementia with Lewy Body (DLB) respectively after neuropathological examination.
Methods: NGS was done using a panel of over 6000 genes (Agilent Technologies). Total RNA from hippocampus, parietal lobe, *Substantia Nigra* and basal ganglia was extracted and RNA-Seq analysis was run (Lexogen). Differentially Expressed Genes (DEGs) were identified via R package DESeq2. AlphaLisa was performed in the same four brain regions, to investigate the dimethylation status and the acetylation status of the Histone 3 Lysine 9 (H3-K9) residue.
Results: By NGS, no dementia-causative variants were found in the two patients, however the missense mutation c.2990T>C (p.Leu997Pro) was found in the HDAC4 gene only in the son. DEGs analysis revealed a dysregulation in the son's substantia nigra, with a 10-foldhigher number of DEGs. Moreover, the *Substantia Nigra* of the son resulted hypo-methylated and hyper-acetylated at the H3-K9 residue, while the mother resulted globally hyper-methylated in all the brain areas analyzed at the H3-K9 residue, except in the basal ganglia where resulted hyper-methylated.
Conclusions: Our investigation highlighted that beyond the common genetic background shared by the two individuals which may confer genetic susceptibility, the two different forms of dementia may have been influenced by a different epigenetic landscape, opening to an intriguing investigation on the role of the epigenetics in the modulation of neurodegenerative disorders.

ND38 | Study of mechanotransduction and migration behavior in a Krabbe disease cell model.

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Krabbe disease (KD) is a genetic rare lysosomal storage disorder causing a progressive nervous system degeneration both in the central nervous system (CNS) and in the peripheral nervous system (PNS). Accumulation of toxic lipids, devastating myelin loss, neuron death and gliosis are the major features of this pathology. The involved gene codifies for the galactosylceramidase (GALC) enzyme, fundamental for the catabolism of ceramide derivatives. GALC is widely expressed through all the body and the most important symptoms are directed through CNS and PNS. Nowadays, potential treatments (like gene therapy and enzyme replacement) are still under investigation for KD. Some of them have promising results in the short-term, unluckily the involvement of several molecular pathways (oxidative stress, inflammation and more generally in cellular homeostasis) does not assure a total remission of the disease. Our research focuses on the identification of new molecular players in the KD pathogenesis. We are studying the connections between the lysosomal pathway and mechanosensing. Here, some of the more important molecular players are focal adhesion protein clusters (FAs), which act as sensors of the extracellular environment and cytoskeleton fibers. We have set up primary fibroblast cell cultures from the Twitcher mouse, the most widespread mouse model of the Krabbe disease, and we have analyzed cell migration *in vitro* in galactosylceramidase-deficient and healthy cells. Then, we have investigated focal adhesions (FAs) and cytoskeleton, to better characterize their involvement in this pathology. The aim is to identify new molecular targets (tested in combination with other therapeutical approaches, i.e. enzyme replacement therapy) to improve the treatment for the Krabbe disease. We found deregulated mechanosensing responses in the GALC-deficient cells, suggesting an involvement of this process in Krabbe disease.

ND39 | Optimization of AAV9 gene therapy for Spinal Muscular Atrophy with respiratory distress Type 1 using in vivo disease models

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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. Recently, we demonstrated that 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 at post natal day 1. Expression analysis demonstrated a significant increase in the IGHMBP2 protein expression level compared to control. Treatments resulted in an extended survival time, higher body weight and improvement of the motor behaviours, with an amelioration of the performance in the hindlimb splay and rotarod tests. Histopathological analysis, performed on mice muscle, showed an increased number of innervated neuromuscular junctions, usually morphologically altered and reduced in *nmd* mice. Finally, to support the translatability of the therapy, we assessed the lack of a significative alteration of the toxicity biomarkers after the treatment. Although further experiments are needed these results provided a promising starting point for the development of an effective treatment for SMARD1 patients.

ND40 | Reactive features and neurogenic potential of striatal astrocytes upon excitotoxic lesion: role of the transcription factor Sox2

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Astrocytes show a heterogeneous repertoire of responses to diverse brain insults including the transition toward a neurogenic state leading to the production of a huge number of neuroblasts. However, such transition takes place only in striatal astrocytes after quinolinic acid (QA)-mediated excitotoxic lesion or stroke. The neurogenic transition of striatal astrocytes appears a complex multi-stage process as testified by the long time (2-3weeks) it takes for astrocytes to start neuron production after QA-mediated lesion. However, the stage-specific events occurring in astrocytes are not fully determined. With the aim to get insight into such process, I examined astrocyte changes during the post lesion course. Besides typical reactivity markers and proliferation, I analysed the regulation of SOX2, a transcription factor known to be expressed in astrocytes and capable, upon overexpression, to convert striatal astrocytes into neuroblasts *in vivo*. Results showed that at early time points astrocytes underwent a severe astrogliosis with proliferation, which was accompanied by SOX2 upregulation and the acquisition of neurospherogenic capacity. Thus, we hypothesised a role for SOX2 in both early astrocyte reactivity and later neurogenic activity. This was confirmed by studying the effects of SOX2 abrogation in astrocytes before lesion. Specifically, I focused on changes occurring in astrogliosis and tissue response and found that astrocyte reactivity was altered in term of marker expression, loss of proliferative activity and neurospherogenic capacity. Interestingly also the inflammatory environment was influenced by SOX2 deletion. This study shows that early astrocytic changes induced by QA and in turn their impact on the environment are regulated by astrocytic SOX2 and suggest that they are linked to the subsequent acquisition of the capacity to generate neurons.

ND41 | Neurofilament light chain as a possible biomarker in adult SMA type 2 and 3 patients undergoing Nusinersen treatment

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Neurofilaments light chain (NfL) are intermediate filaments exclusively expressed in neurons. As NfL levels raise following axonal damage, they might be promising diagnostic and prognostic biomarkers in motor neuron diseases (Gaetani, et al., 2019). This study aims to investigate the role of NfL as disease and treatment-response biomarker in adult Spinal Muscular Atrophy (SMA) type 2 and 3 patients treated with nusinersen, the only disease modifying therapy approved for SMA patients.

33 SMA type 2 and 3 patients received nusinersen in a loading phase (L1 baseline, L2 day 14, L3 day 28, and L4 day 63) and a maintenance phase (M1, M2 and M3, every four months). Cerebrospinal fluid (CSF) NfL were tested at each time point with an enzyme-linked immunosorbent assay (ELISA) kit (Uman-Diagnostics, Sweden); additional neurodegeneration biomarkers total tau (t-Tau) and phosphorylated tau (p-Tau) proteins were tested at time points L1 and L3 with an automated Chemiluminescent Enzyme Immunoassay (CLEIA) analyzer (LUMIPULSE G600 II by Fujirebio, Japan). Human Profilin-1 (PFN-1) was tested at each time point as an exploratory biomarker with an ELISA kit (Cusabio, China).

Baseline CSF NfL, t-Tau and p-Tau levels were overall included in the reference ranges for healthy donors. Correlation was found between baseline log[NfL] and age. Log[t-Tau] and log[p-Tau] correlated with log[NfL] at L1, but not at L3. NfL significantly increased in loading phase until L3. No significant difference was found with baseline at M1, M2 and M3. PFN-1 at baseline was higher in SMA than in healthy controls. PFN-1 showed a complex dynamic during loading phase, with a significant reduction at L4.

Our study partially reinforces recently published results in similar patients (Wurster, et al., 2019) (Wurster, et al., 2020) (Faravelli, et al., 2020), adding insights on NfL dynamic during the first month of treatment. Neurodegenerative biomarkers might inadequately relate to long disease duration.

ND42 | MINCR: a Long non-coding RNA between cancer and neurodegenerative pathways

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The long non-coding RNA (lncRNA) system includes thousands of molecules involved in fundamental regulatory processes. Many lncRNAs participate to more than one process and interact with multiple targets, leading to different outcomes and making extremely challenging to clarify their mechanism of action. Understanding the multiple functions of non-coding transcripts has become more and more important in the past years due to the numerous roles exerted by lncRNAs in physiological or pathological conditions. Therefore, the aim of this work was to investigate the potential role of the MYC-Induced Long Non-Coding RNA MINCR depending on its expression level in SH-SY5Y cells through RNA-seq approach. Since it has been demonstrated that MINCR is deregulated in different tumours and in Amyotrophic Lateral Sclerosis, we focused our attention on MINCR potential role in cancer and neurodegeneration. Our results showed that MINCR overexpression caused great alteration in the expression level of cancer-related genes leading to disruption in many fundamental processes, such as cell cycle and growth factor signaling. Whereas MINCR downregulation highlighted a small number of deregulated genes involved in different neurodegenerative disorders, mostly concerning RNA metabolism and inflammation. Taken together, our data showed, firstly, a new prospect to the mechanisms through which lncRNAs act depending on their expression level and, secondly, shed light on the interplay between cancer and neurodegeneration, supporting the idea that these diseases can share key genes and pathways conversely regulated.

ND43 | Potential biomedical applications and structural characterization of New Sophisticated Neuromelanin Models

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Neuromelanins (NMs) are dark pigments made of melanic, lipid, and peptide moieties, linked together by covalent bonds. NMs are found inside neurons in cytoplasmic organelles and mostly derived from the oxidation of two catecholamines: dopamine (DA) and norepinephrine (NE), present in *substantia nigra pars compacta* (SNc) and *locus coeruleus* (LC), respectively. Loss of NM-containing neurons in SN is a primary macroscopic neuropathological characteristic of Parkinson's Disease (PD). The current understanding of the structure and biosynthesis of this protective pigment is very limited, especially at the molecular level. This is due to the insoluble nature of the substance, its heterogeneity, and the very small amount that can be isolated from human brains (typically 1.0 mg NM from SN requires 4-5 brains of suitable subjects, which reduces to about 1/10 for NM from LC). However, important information about the structure, properties, and reactivity of the NMs can be obtained through the investigation of synthetic NM models. In addition, an important functional feature of the synthetic NMs is their ability to induce microglia activation in cell culture lines, reproducing the chronic neuroinflammation process by NM occurring in a PD brain. A main objective of this work was to obtain water-soluble synthetic conjugates starting from NE, iron and copper with the aim of mimic the NMs present in LC, starting from DA metabolites in order to elucidate their role in the formation of NMs or using a neuronal protein (α-synuclein, amyloid-β) to better simulate the natural pigment. The results obtained in the present study will have an impact on the biomedical research on neurodegenerative diseases, and in particular PD, providing new insights, at the molecular level, on the structural and functional core of NMs in neurons. Moreover Magnetic resonance imaging (MRI) of NM could be used to confirm diagnosis of PD, monitor the progression of disease and response to drug treatment.

ND44 | Clinical features and disease evolution in Italian Lafora disease patients

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Lafora disease (LD) is an autosomal recessive progressive myoclonus epilepsy, usually occurring in early adolescence in otherwise neurologically normal individuals. Initial symptoms turn rapidly into progressive dementia, refractory status epilepticus (RSE), language and motor impairments, and lead to respiratory failure and death within one decade. Two genes located in chromosome 6, *EPM2A* and *EPM2B*, seem equal to contribute to the disease, causing deficiency of protein laforin and malin which are involved in glycogen metabolism. A prototype of glycogen storage disease, LD is characterized by the presence of Lafora bodies (LBs), periodic-acid-Schiff positive intracellular inclusions localizing in perikaryal and dendrites but not in axons. We report the clinical features and genetic findings of 26 Italian patients. Disease progression, motor and mental functions were assessed by a simplified disability scale; spontaneous and action myoclonus severity was scored by the Magaudda Simplified Myoclonus Rate Scale. Mutation analysis was performed by Sanger or targeted re-sequencing of the *EPM2A/EPM2B* coding regions. Age at disease onset ranged from 10 to 22 years (mean, 14.04±2.62). The mean follow-up duration was 11.48±7.8 years. Twelve out of the 26 (46%) patients preserved walking ability and 13 (50%) retained speech. Seventeen (65%) patients showed *EPM2B* mutations and 9 (35%) carried a *EPM2A* mutation. Slower disease progression, confirmed by walk and speech retention after 4 or more years of follow-up, was confirmed in 6 (35%) out of the 17 *EPM2B*-mutated patients. These findings suggest that the clinical course of the disease may differ within different mutations and patients with *EPM2A* mutations may show major walk and speech deterioration.

ND45 | Role of the alpha-synuclein antisense transcript SNCA-AS1 in Parkinson's Disease: implications in synapses- and aging-related pathways

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SNCA protein product, alpha-synuclein, is widely renowned for its role in synaptogenesis and implication in both aging and Parkinson's Disease, but research efforts are still needed to elucidate its physiological functions and regulation. In this work, we aim to characterize the long non-coding RNA SNCA-AS1, antisense transcript to the SNCA gene, and its implications in cellular processes. SH-SY5Y cells were stably transfected with either SNCA-AS1 or SNCA and their transcriptional signature was investigated via RNA-sequencing. Real Time-PCR and western blot were used to verify SNCA-AS1's effect on SNCA's expression, and neurite extension was assessed via immunofluorescence analysis. The overexpression of SNCA-AS1 upregulates SNCA mRNA and protein, and both genes appear to strongly impact neurite extension and synapses' biology, through specific molecular signatures. We report a reduced expression of markers associated with synaptic plasticity, and we specifically focus on GABAergic and dopaminergic synapses, for their relevance in aging processes and PD, respectively. As part of this signature is co-regulated by the two genes, we discriminate between functions elicited by genes specifically altered by SNCA-AS1 or SNCA's overexpression, and we observed a highly relevant role for solely SNCA-AS1. We also highlight how numerous deregulated pathways are implicated in aging-related processes, suggesting that SNCA-AS1 could be a key player in cellular senescence, with implications for aging-related diseases. Indeed, the upregulation of SNCA-AS1 leads to alterations in numerous PD specific genes, with an impact highly comparable to that of SNCA. SNCA/SNCA-AS1 ratio is also significantly altered in PBMCs from PD patients versus healthy controls. Our results show that SNCA-AS1 elicits its cellular functions not only through the regulation of SNCA, but also through a selective and specific modulation of synaptogenesis and senescence, with significant implications for PD.

ND46 | Study of the oncogenic lncRNA ZEB1-AS1 in sporadic ALS: implication for neuronal differentiation and identification of a novel disease pathway

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Alterations in the expression levels of RNAs in the pathogenesis of sporadic ALS (sALS) are becoming increasingly relevant, with RNA-seq data highlighting numerous deregulated long non-coding RNAs (lncRNAs) in tissues derived from sALS patients. The oncogenic lncRNA ZEB1-AS1 emerged as strongly downregulated in peripheral blood mononuclear cells (PBMCs) of sALS patients. In cancer-derived cell lines, ZEB1-AS1 has been shown to act in a feedback negative loop with mir200c, acting as a molecular sponge for this miRNA. Furthermore, ZEB1-AS1's interaction with mir200c results in the upregulation of the downstream molecule BMI1. In PBMCs and spinal cords of sALS patients versus healthy controls we observed a downregulation of ZEB1-AS1's expression but not of its sense gene ZEB1. We observed an increase of mir200c and a decrease of BMI1, in an opposite pattern to what is observed in cancer, suggesting a possible sALS involved pathway. Furthermore, we observed an upregulation of BMI1's downstream mediators p53 and GSK3b, both involved in neuronal death in ALS. We created an in vitro model silencing ZEB1-AS1 in SH-SY5Y, both undifferentiated and differentiated. This downregulation does not influence ZEB1's levels, mimicking what observed in sALS. Concordantly, we found that the investigated pathway presents the same deregulations as in PBMCs of sALS patients. Interestingly, we found that ZEB1 and ZEB1-AS1's levels change during neuronal differentiation, suggesting an implication for the lncRNA in this process. We demonstrated that ZEB1-AS1 can bind the ALS-implicated RNA binding protein FUS, both in SH-SY5Y cells and in PBMCs, and in this last tissue we found a reduction in the amount of ZEB1-AS1 bound to FUS in sALS patients. In conclusion, our results show an implication for ZEB1-AS1's pathway in ALS and neuronal differentiation, and we report an interaction of ZEB1-AS1 with FUS, impaired in sALS patients, suggesting the mechanism connecting ZEB1-AS1 to sALS pathology.

ND47 | Characterization of the first steps of neuromelanin synthesis: a chemometrics approach

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Neuromelanins (NMs) are the melanins mainly found in human's pigmented neurons of Substantia Nigra (SN), where they exert a protecting role sequestering unstored dopamine (DA) and toxic metal ions, such as iron and copper. Depletion of dopaminergic neurons of SN alongside with microglia activation are common features of PD affected brains. This latter aspect causes the disruption of NM and the consequent release of toxic metals and compounds bound to NM, leading to a vicious cycle of chronic inflammation.
Since NM is originated from metal-catalyzed oxidation of catecholamines, like DA, our goal is to understand how this process is affected by chemical-physical parameters such as temperature, type of metal ion (iron, copper or both), pH, ionic strength and the presence of neuronal peptides. With the aim of having further insights regarding NM synthesis, the reactivity studies have been performed following the DA oxidation by UV-Vis spectroscopy, changing the parameters listed above. In order to overcome the difficulties related to DA chemistry, the spectra and their first derivative were analysed by a multivariate approach, based on different chemometric tools. The results clearly indicate that copper is more reactive than iron in melanin formation. Furthermore, when both metal ions are present, the oxidation rate and the type of product are in between of the situation when the two metals are taken alone and no cooperation is observed. Of particular importance is that, following the absorbance changes with time, it is possible to asses whether the reaction is driven by iron, copper or the two metal ions together in the medium. As for the effect of ionic strength and buffer type, the rate of DA oxidation decreases with the increase of the ionic strength and by switching from inorganic to organic type of buffer. Future perspectives are directed toward the study of the effects of neuronal related peptides on DA oxidation and melanin synthesis.