2nd BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

NOVEMBER 14th-15th-16th 20019
MILAN - ITALY

Congress Venue:
«Mario Negri» Institute Auditorium
Via Mario Negri 2, Milan, Italy

www.braynconference.com

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Chiara Gabbi Humanitas Medical Care, Milan (Italy)
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Dear Young Neuroscientists,

We are delighted to introduce you to the 2nd 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. We believe that the first BraYn conference will boost the number of connections between laboratories across Italy and Europe, thus improving the chance for potential collaborations. At the same time, by hosting and involving neuroscientists from abroad, our goal is to make the BraYn conference a flagship event for young European researchers. More than 400 delegates have registered to the BraYn conference 2019. They include experienced senior leaders, attending as mentors and discussants, and four invited speakers. We have scientists attending from different disciplines of neuroscience including neurodegeneration, perinatal neurology, neuroimmunology, neuronal plasticity and neuro-oncology who will show the most recent advances in these fields. We are looking forward to welcoming you to the 2nd BraYn conference. We hope that you will enjoy the meeting at Mario Negri institute and the beautiful city of Milan, Italy!

The BraYn Staff

NOVEMBER 14th

13:30 Registration

14:30 Opening Ceremony | **GIOVANNI FERRARA**

14:40 Lecture | **JEAN-MICHEL CIONI** *Axonal protein synthesis in health and disease: a new role for endosomes.* (Chairwoman: B. Bettegazzi)

15:10 Lecture | **DANIEL JAQUE GARCIA** *Near infrared (NIR)-II imaging for brain studies.* (Chairman: J.L. Cañavate)

SESSION 1 - NEURAL PLASTICITY • ORAL COMMUNICATIONS
(Supported by European Society of Neurochemistry, ESN)
Chairwomen: E. Boda, G. Calabrese, R.C. Paolicelli, E. Conti

15:40 **Roberta Schellino** *Human medium spiny neuron progenitors grafted into an HD rat model early integrate into the host circuits, express striatal markers and support functional recovery.*

15:55 **Martina Lorenzati** *Axo-glial interplay in oligodendrocyte specification and myelination: role of JNK1.*

16:10 **Giulia D'Arrigo** *Astrocytes-derived Extracellular Vesicles in motion at the neuron surface.*

BraYn Educational Symposia 1
Chairpersons: S. Amoretti, J.L. Cañavate, M. Velasco

16:25 **Luca Mazzitelli** (Carlo Erba Reagents) | *Biology at true resolution with 10X Genomics.*

16:45 **Francesca Galbiati** (Charles River Laboratories) | *Essential Tips for new Mouse Researchers.*

17:05 **Cristina Spalletti** *Robotic Rehabilitation and neuromodulation after stroke: novel approaches in a mouse model.*

17:20 **Valentina Cerrato** *The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning.*

17:35 **Matteo Pedrazzoli** *Glucocorticoid receptor modulation alters dendritic spine density and microglia activation in the hippocampus of 3xTg-AD mice.*

17:50-20:00: Poster Session 1 • Coffee break mini 1
BraYn Educational Workshop Session 1 (Conference Hall B)

17:50 **Stefano Fiorina** (AB Sciex) | *Metabolomics & Lipidomics.*

18:40 **Marilisa Marinelli, Claudio Comunian** (Bio-Rad) | *Extracellular Vesicles in Neurological Disease.*

19:15 **Francesco Biancardi** (Carl Zeiss) | *Beyond the Computed Tomography: X-Ray Microscopy in Life Science.*

NOVEMBER 15th

9:00	Lecture | **MARCO PRINZ** *Myeloid cell diversity in the central nervous system.* (Chairwoman: R.C. Paolicelli)
	SESSION 2 - NEUROINFLAMMATION • ORAL COMMUNICATIONS *Chairpersons*: S. Angiari, E. Volpe, A. Musella, I. Prada, S. Raffaele
9:30	**Placido Illiano** *Astroglial TNFR2 regulates learning, memory and anxiety.*
9:45	**Laura Brambilla** *Real Time Quaking Induced Conversion assay as innovative tool to investigate neurodegeneration in Multiple Sclerosis.*
10:00	**Alessandro Leuti** *Resolution of inflammation is impaired in multiple sclerosis and entails a loss of pro-resolving features of monocytes/macrophages.*
	BraYn Educational Symposia 2 *Chairpersons*: S. Amoretti, J.L. Cañavate, M. Velasco
10:15	**Angela Guerra Alvarez** (Biogen) | *Pioneering the future of Neuroscience.*
10:35	**Zsolt Iván** (Femtonics) | *Outstanding benefits of breakthrough innovations of 3D Acousto-Optic technology in Neuroscience*.
10:50	**Valentina Serpieri** *Agenesis of the putamen and globus pallidus caused by recessive mutations in the homeobox gene GSX2.*
11:05	**Matteo Carlo Kaleva Ciccarese** *Role of lipid mediators in the aging process.*
11:20	**Morris Losurdo** *Preconditioned Bone Marrow Mesenchymal Stem Cell-derived Extracellular Vesicles Exert Immunomodulatory Effects in a model of Alzheimer's Disease.*
11:35	Workshop | **CHIARA GABBI** *How to write a succesful grant and/or a fellowship application.* (Chairwoman: M. Romeo)
	12:00-14:00: Lunch box with **Poster Session 2** **BraYn Educational Workshop Session 2 (Conference Hall B)**
12:00	**Elisa Zuffi** (Miltenyi Biotec) | *Novel approach to study adult neural cells in vitro.*
13:00	**Pietro Veglianese** (Beckman Coulter) | *Modulation of microglial cells by drug-nanovectors in spinal cord injury.*
13:30	**Paola Marcon** (Biogen) | *The long journey: from the bench to the bedside.*
14:00	Lecture | **ROSSELLA GALLI** *Leveraging brain tumor stem cells for disease modeling and therapy improvement.* (Chairwoman: B. Bettegazzi)

SESSION 3 - NEURO-ONCOLOGY • ORAL COMMUNICATIONS
Chairpersons: G. D'Alessandro, M. Tamborini, R. Azzarelli

14:30 **Massimiliano Del Bene** *Clinical Significance of Extracellular Vesicles in Plasma from Glioblastoma Patients.*

14:45 **Paola Infante** *Oncogenic role of the aminopeptidase ERAP1 in Hedgehog-dependent cancer.*

15:00 **Silvia Valtorta** *Combined Positron Emission Tomography imaging approach for identification of new potential biomarkers for treatment response in glioma models.*

BraYn Educational Symposium 3
Chairpersons: S. Amoretti, J.L. Cañavate, M. Velasco

15:15 **Philippe Trochet** (Fujifilm VisualSonics) | *See the Whole Mouse Brain in vivo and in Real-time*

15:30 **Prospero Civita** *Towards a 3D all human in vitro model of glioblastoma multiforme for drug screening: glioblastoma and astrocyte interactions?*

15:45 **Carmela Serpe** *Microglia-Derived Microvesicles Affect Microglia Phenotype in Glioma.*

16:00 **Elena Parmigiani** *Notch signaling controls glioma proliferation and shapes the tumor microenvironment.*

16:15-18:30: Coffee break with **Poster Session 3**
"Lost in the Protocol" session

18:30 Lecture | **PIERRE GRESSENS** *Neuroinflammation and encephalopathy of prematurity.* (Chairman: L. Ramenghi)

SESSION 4 - PERINATAL NEUROLOGY • ORAL COMMUNICATIONS
(Supported by EuBrain)
Chairpersons: G. Ferrara, A. Carta, L. Ramenghi, M.S. Paladini

19:00 **Eridan Rocha-Ferreira** *Repurpose of exendin-4 for the treatment of neonatal brain injury.*

19:15 **Sara De Crescenzo** *Growth parameters and minor brain lesions in very low birth weight (VLBW) newborns as prognostic factors of negative neurodevelopmental outcome at two and three years of age.*

19:30 **Cristiana Pelorosso** *A novel double-hit mechanism involving different genes of the MTOR pathway in hemimegalencephaly with intractable childhood epilepsy.*

20:30 BraYn Social Dinner

NOVEMBER 16th

9:00 Lecture | **SONIA GAREL** *Microglia at the crossroads of cortical wiring and environmental signals.* (Chairwoman: R.C. Paolicelli)

SESSION 5 - NEURODEGENERATION • ORAL COMMUNICATIONS
Chairpersons: G. Nardo, B. Bettegazzi, M. Medelin, G. Zanetti

9:30 **Bianca Barzaghini** *Therapeutic effect of neural progenitor cells expanded in the 3D nano-engineered Nichoid substrate in a Parkinson's disease preclinical model.*

9:45 **Morgane Rouault** *Incorporation of spatial mapping and confirmation of gene signatures by a multiplex in situ hybridization technology into single cell RNA sequencing workflows.*

10:00 **Federica Campanelli** *Effects of chronic continuous theta-burst stimulation (cTBS) on bidirectional striatal synaptic plasticity in a model of Parkinson's disease and L-Dopa- induced dyskinesia.*

10:15 **Loredana Leggio** *Astrocyte-derived extracellular vesicles and cell-to-cell communication in the nigrostriatal regions: implications for dopaminergic neuroprotection.*

BraYn Educational Symposia 4
Chairpersons: S. Amoretti, J.L. Cañavate, M. Velasco

10:30 **Gennaro Pagano** (Roche) | *Translational medicine journey in neuro-degeneration: focus on alpha-synuclein.*

10:50 **Gianluca Rotta** (BD Biosciences Italia) | *Three intersections between flow cytometry and multiple sclerosis.*

11:10 **Maria Velasco-Estevez** *Mechanosensation: a new horizon in the regulation of myelination?*

11:25 **Cristiano Carlomagno** *Raman spectroscopy as biochemical "fingerprint" of biofluids for the investigation of neurodegenerative diseases.*

11:40 **Silvia Picciolini** *SPRi-based biosensor for the detection of circulating extracellular vesicles as biomarkers of neurological diseases.*

11:55 **Ludovico Cantuti-Castelvetri** *Defective cholesterol clearance limits remyelination in the aged central nervous system.*

12:10 Lecture | **GIACOMO RIZZOLATTI** *The mirror mechanism: a neural mechanism for understanding others.* (Chairman: G. Nardo)

12:40 Closing Remarks • BraYn Awards (Best Oral and Poster Presentation and BraYn Starting Grant)

ORAL COMMUNICATIONS

Human medium spiny neuron progenitors grafted into an HD rat model early integrate into the host circuits, express striatal markers and support functional recovery

Roberta Schellino[1] - Marina Boido[1] - Dario Besusso[2] - Roberta Parolisi[1] - Sara Belloli[3] - Valentina Murtaj[3] - Rosa Maria Moresco[3] - Annalisa Buffo[1] - Elena Cattaneo[2] - Alessandro Vercelli[1]

Neuroscience Institute Cavalieri Ottolenghi _ Dip. di Neuroscienze, Università degli Studi di Torino, Torino, Italy[1] - Laboratory of Stem Cell Biology and Pharmacology of Neurodegenerative Diseases, Department of Biosciences _ Università degli Studi di Milano, Milano, Italy[2] - Experimental Imaging Center, IRCCS San Raffaele Scientific Institute, MIlano, Italy[3]

Huntington's Disease (HD) is a neurodegenerative disorder characterized by the prominent loss of medium spiny neurons (MSNs). To replace the affected cells, we transplanted H9 embryonic stem cells with a MSN character into the striatum of a quinolinic acid (QA) lesioned rat model of HD, and we examined their maturation and organization until 2 months after graft. We found that transplanted neurons survive in the host and express striatal markers (e.g. Ctip2, Darpp32 and Ebf1), with a limited amount of proliferation. Viral vector-based tracing experiments revealed that grafted cells start to integrate into the host circuits since the first month after transplantation and extend neurites at long distance (thalamus and substantia nigra). Moreover, behavioural tests performed at different time points showed that QA lesion induces a remarkable decrease in the use of the contralateral forelimb, but MSN engraftments can early improve the striatum-dependent motor performances. Taken together our data suggest that human striatal progenitors by surviving, differentiating and connecting into the lesioned striatum may support some functional recovery already at early phases post-lesion.

Axo-glial interplay in oligodendrocyte specification and myelination: role of JNK1

Martina Lorenzati[1] - Enrica Boda[1] - Tiziana Borsello[2] - Annalisa Buffo[1] - Alessandro Vercelli[1]

Neuroscience Institute Cavalieri Ottolenghi, Department of Neuroscience Rita Levi Montalcini, Torino, Italy[1] - University of Milan, Department of Pharmacological and Biomolecular Sciences, Milano, Italy[2]

The C-Jun N-terminal kinase (JNK) pathway participates in several physiological and pathological mechanisms. JNK is expressed in three isoforms and, in particular, JNK1 exerts pleiotropic roles during brain development. JNK1 KO mice show alterations of the corpus callosum suggestive of myelin defects. Therefore, we investigated the role of JNK1 in the development of myelinated tracts. In particular, we focused on oligodendrocyte (OL) development. The somatosensory cortex of JNK1 KO mice was stained with anti-PDGFRalpha antibodies to label oligodendrocyte precursor cells (OPCs), and with anti-APC and anti-myelin basic protein (MBP) antibodies to label the mature, myelinating OLs. Immunohistochemical analyses revealed a significant increase in the density of OPCs at P7 and P15 in KO mice, with no changes in their distribution. Furthermore, JNK1 KO mice at both early postnatal and adult ages showed a lower extent of MBP expression, indicative of abnormal myelin deposition. MBP expression was also altered in the corpus callosum. Based on these data, we analysed the structure of myelinated axons and examined the nodes of Ranvier by labelling for contactin associated protein 1, one of the proteins of the adhesion complex that mediates their assembly. We found that JNK1 KO mice display a higher density of nodes and that the nodes are longer. With the aim to assess cell autonomous defects of JNK1 KO OLs, we performed in vitro cultures of rat OPCs treated with DJNKi (a specific inhibitor of the three isoforms of JNK that partly mimics JNK1 KO). Results suggest alterations in proliferation rate and cell morphology of OL treated with the inhibitor. Our findings suggest for the first time that JNK1 takes part in OL development and in the axo-glial interplay. Further experiments will be devoted to examine the ultrastructural alteration of JNK1 KO myelin and nodes and to disentangle the relative contribution of JNK1 in OLs or neurons to the observed phenotype.

Astrocytes-derived Extracellular Vesicles in motion at the neuron surface

Giulia D'Arrigo[1] - Martina Gabrielli[1] - Federica Scaroni[1] - Dan Cojoc[2] - Giuseppe Legname[3] - Claudia Verderio[1]

National Research Council of Italy, Institute of Neuroscience, Milano, Italy[1] - National Research Council of Italy, Institute of Materials, Area Science Park - Basovizza, Trieste, Italy[2] - International School for Advanced Studies of Trieste, Neuroscience Area, Trieste, Italy[3]

Extracellular Vesicles (EVs) shed from the plasma membrane of astrocytes are key players in glia-neuron communication in healthy and diseased brain. However, almost nothing is known about how large EVs can interact with neurons and reach preferential sites. To investigate this issue, astrocytic EVs were added to the medium of cultured hippocampal neurons and, using optical manipulation, trapped and delivered to neuron surface. After contact, EVs efficiently adhered to the neuronal cell body, dendrites and axons. Surprisingly, after adhesion a large fraction of EVs moved on the surface of neurites in both retrograde and anterograde directions. Interestingly, the EV velocity is in the same range of retrograde actin flow, which regulates membrane diffusion of receptors linked to actin. Accordingly, we found that EV movement is highly dependent on neuron energy metabolism. Moreover, inhibition of neuron actin filaments rearrangements with cytochalasin D or blebbistatin, but not depolymerization of microtubules with nocodazole, reduced EVs in motion, revealing that neuronal actin cytoskeleton is implicated in EV-neuron dynamics. Interestingly, the delivery of EVs from prion protein knock out ($PrP^{-/-}$) astrocytes on $PrP^{-/-}$ neurons shown that EV motion is driven by the binding of vesicular PrP to a PrP receptor surfing on the plasma membrane of neurons. Unexpectedly, we found that EVs can contain actin filaments and ATP and have an independent capacity to actively move at the neuron surface in an actin-dependent manner. Our data support two different way of EV motion. First, EV displacement could be driven by the binding with neuronal receptors linked to the actin cytoskeleton. Second, EVs could possess motile ability like that produced by actin in cells and move along a gradient of neuronal receptors. Moreover, for the first time, we show that astrocytic EVs exploit vesicular PrP and its neuronal receptors to passively/actively reach their target sites on neurons.

Robotic Rehabilitation and neuromodulation after stroke: novel approaches in a mouse model

Cristina Spalletti[1] - Claudia Alia[1] - Stefano Lai[2] - Alessandro Panarese[2] - Maria Pasquini[2] - Sara Conti[2] - Silvestro Micera[3] - Matteo Caleo[4]

National Research Council, Neuroscience Institute, Pisa, Italy[1] - Scuola Superiore Sant'Anna, The Biorobotic Institute, Pisa, Italy[2] - Bertarelli Foundation, Ecole Polytechnique Federale de Lausanne, Lausanne, Switzerland[3] - Università di Padova, Dipartimento di Scienze Biomediche, Padova, Italy[4]

Stroke is a major cause of chronic motor disabilities and re-acquisition of motor skills is crucial for stroke survivors. Innovative techniques combining physical rehabilitation and neuromodulation represent a promising approach but solid clinical results are missing and appropriate animal models of these novel strategies are needed. We use optogenetics, electrophysiology, behavioral tests and a novel kinematic analysis of reaching movement to test the effectiveness of different therapeutic strategies to improve forelimb motor function after stroke forelimb primary motor cortex in mice. We first assessed the role of the healthy hemisphere on post-stroke electrophysiological alterations in spared perilesional tissue by measuring Field Potentials and Multi Unit Activity following optogenetic stimulation in the homotopic contralesional cortex. We found significant, GABAergic-mediated, increased inhibition exerted by the healthy hemisphere over the injured one. Accordingly, we coupled robotic rehabilitation with transient inhibition of the healthy hemisphere. We found that this combined approach results in a functional improvement in general motor tasks and in kinematics of grasping, with re-establishment of pre-lesion interhemispheric balance (Spalletti et al., 2017). These data demonstrated the effectiveness of combined therapy in promoting true motor recovery. We have now improved the rehabilitative treatment on the robotic platform with a real-time control of friction and isometric measure of forces (Pasquini et al., 2018). We're coupling the treatment with other neuroplastic treatments: (i) enhancement of endogenous serotonin release in a chemogenetic model for controlled serotonin release; (ii) induction of gamma oscillation during rehabilitative treatment in transgenic animals expressing ChR2 in Parvalbuminergic interneurons.

The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning

Valentina Cerrato[1] - Elena Parmigiani[2] - Sara Mercurio[3] - Roberta Bardini[4] - Laura López-mascaraque[5] - Silvia K. Nicolis[3] - Annalisa Buffo[1]

Neuroscience Institute Cavalieri Ottolenghi, University of Turin, Dept. of Neuroscience Rita Levi Montalcini, Torino, Italy[1] - Department of Biomedicine, University of Basel, Basel, Switzerland[2] - Department of Biotechnology and Biosciences, University of Milano-Bicocca, Milan, Italy[3] - Department of Control and Computer Engineering, Polytechnic of Turin, Turin, Italy[4] - Cajal Institute, Department of Molecular, Cellular, and Developmental Neurobiology, Madrid, Spain[5]

In the cerebellum, astrocytes are characterized by a peculiar heterogeneity, closely related to specific functional features fundamental for the correct development and functioning of this brain area. However, the ontogenesis of such astroglial diversity remains poorly explored. By combining in vivo clonal analyses with both proliferation/birthdating studies and meta-analyses of multiple publicly available single cell (sc)RNA-seq datasets, we investigated cerebellar astrogliogenesis at the single progenitor and molecular level. We demonstrated that a tightly regulated developmental program drives cerebellar astrogliogenesis and comprises (i) a time-dependent decline in both clone size and progenitor multipotency, associated with a specific spatial pattern of clone allocation; (ii) diverse lineage potentials of embryonic and postnatal progenitors, leading to distinct clonal relationships among astrocyte types; and (iii) stereotyped clone architectures, correlated to layer-specific dynamics of postnatal proliferation/differentiation. Furthermore, meta-analytical explorations of scRNA-seq data allowed to unveil an inedited molecular heterogeneity among the distinct cell types and across diverse maturational stages. Interestingly, Cre-mediated Sox2 deletion selectively in postnatal astrocytes led to a progressive mis-localization of Bergmann glia (BG) postnatally, correlated with ataxic features. In this study, we demonstrate that cerebellar astrocyte heterogeneity emerges according to an unprecedented and remarkably orderly developmental program. Moreover, we define a functional requirement of Sox2 for BG phenotype maintenance, of potential relevance for ataxia in mouse mutants, and in human patients.

Glucocorticoid receptor modulation alters dendritic spine density and microglia activation in the hippocampus of 3xTg-AD mice

Matteo Pedrazzoli[1] - *Morris Losurdo*[2] - *Giovanna Paolone*[3] - *Manuela Medelin*[1] - *Lejdi Jaupaj*[1] - *Barbara Cisterna*[1] - *Anna Slanzi*[4] - *Manuela Malatesta*[1] - *Silvia Coco*[2] - *Mario Buffelli*[1]

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Chronic exposure to high dose of glucocorticoids (GC) represents a key risk factor for the development and progression of Alzheimer's Disease (AD). In 3xTg-AD mouse, a model of AD, the hyperactivation of Glucocorticoid Receptors (GR) increases the production of the typical hallmarks of this dementia. Considering the ability of GC to regulate, also, the dendritic spine turnover and the inflammation, in that project we focus our attention on the effects of GR agonists and antagonists on spine density and microglia activity in the CA1 region of hippocampus of 3xTg-AD mice. Thus, through an innovative combined Golgi Cox and immunofluorescence technique, we found that 5 days of treatment with 8mg/kg of dexamethasone (DEXA), an agonist of GR, vigorously reduced the dendritic spine density in CA1 region of 3xTg-AD mice, both at 6 and 10 months of age. Contextually, the same treatment strongly enhanced the density of microglia and their levels of activation in the same region and increased the portion of microglia filaments in contact with neuron dendrites. On the contrary, the treatment with 20mg/kg of mifepristone (MIFE), an antagonist of GR, strongly enhanced dendritic spine density in CA1 region, at both ages, as verified also by electron microscopy analyses. The antagonist improved, also, the 3xTg-AD mice performance in Y-maze task and reduced microglia density, without, however, affecting their state of activation. Additionally, in vitro experiments confirmed the ability of DEXA to increase the activation of microglia, a result never described before. On the contrary, MIFE apparently promoted both activation and inhibition of microglia inflammatory state, suggesting the existence of a biphasic behavior of GC also on inflammation regulation. In conclusion, stress can exacerbate AD and promote a more rapid progression of the pathology; consequently, the use of GR antagonist, like MIFE, could represent a promising therapeutic strategy to slow down the progression of AD.

Astroglial TNFR2 regulates learning, memory and anxiety

Placido Illiano[1] - Haritha Desu[1] - Shwetha Mudalegundi[1] - Melanie Plastini[1] - Mohammed M. Moosa[1] - Minna Yli-karjanmaa[2] - David J. Titus[1] - Stephen A Tapanes[1] - Coleen M. Atkins[1] - Daniel J. Liebl[1] - Roberta Brambilla[1]

University of Miami, The Miami Project to Cure Paralysis, Miami, United States[1] - University of Southern Denmark, Department of Clinical research, Odense, Denmark[2]

Tumor necrosis factor (TNF) is a pleiotropic cytokine implicated in key physiologic and pathologic processes in the central nervous system (CNS). These range from modulating synaptic plasticity, thereby regulating memory and cognitive function, to participating in the pathophysiology of neurologic disorders such as multiple sclerosis, Alzheimer's, stroke. TNF exists in two forms, transmembrane (tmTNF) and soluble (solTNF), whose functions are mediated by TNFR1 and TNFR2. The signals activated by the two receptors are often opposite: TNFR1 mediates apoptosis and inflammation, while TNFR2 mediates cell survival, immunity and myelination. Studies with knockout mice have implicated TNFR2 in the regulation of cognitive function in physiological conditions. However, the cell type that contributes to this effect is still unknown. Given that astrocytes are key players in synaptic function and express TNFR2, we sought to investigate whether astroglial TNFR2 could play a role in regulating cognition and memory. To do so, we generated inducible conditional knockout mice to selectively ablate TNFR2 in GFAP expressing astrocytes of adult mice ($GFAPcre^{ERT2}$:$Tnfrsf1b^{fl/fl}$ mice). $GFAPcre^{ERT2}$:$Tnfrsf1b^{fl/fl}$ mice showed deficits in learning and memory functions, measured with the novel object recognition and Morris water maze tests, respectively. Additionally, they displayed anxiety-like behaviors assessed with the light-dark transition test. In parallel, $GFAPcre^{ERT2}$:$Tnfrsf1b^{fl/fl}$ mice showed increased numbers of $GFAP^{+}$ (and $ACSA2^{+}$) and $Iba1^{+}$ cells in the hippocampus, indicative of astroglial and microglial activation. Finally, ablation of astroglial TNFR2 in $GFAPcre^{ERT2}$:$Tnfrsf1b^{fl/fl}$ mice resulted in reduced expression of SNARE complex synaptic proteins and glutamate receptors in the hippocampus, which may explain, at least in part, the cognitive and memory impairments observed in these mice. Taken together our data point at a role for astroglial TNFR2 in hippocampal homeostasis, cognition, memory and anxiety. Further studies are warranted to better understand the mechanisms of these effects, and whether they are maintained under CNS disease conditions.

Real Time Quaking Induced Conversion assay as innovative tool to investigate neurodegeneration in Multiple Sclerosis

Laura Brambilla[1] - Fabio Moda[2] - Edoardo Dalmato Schilke[1] - Olga Carletta[1] - Elena De Cecco[3] - Giuseppe Legname[3] - Renato Mantegazza[1] - Paolo Confalonieri[1]

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by a main relapsing-remitting (RR) course, followed after years by secondary progressive phase (SP) with accumulation of clinical disability. In MS grey matter (GM) atrophy correlates with and predicts clinical progression.
Tau and amyloid-b are proteins involved in neurodegenerative disease such as Alzheimer and have been studied also in MS.
Real Time Quaking Induced Conversion (RT-QuIC) is an innovative technology able to detect small concentrations of misfolded proteins including tau and amyloid-b in cerebrospinal fluid (CSF) of demented patients. We extended RT-QuIC using recombinant tau as substrate for a preliminary analysis of CSF collected at diagnosis from 6 RR MS patients (pMS). At the time of analysis 3/6 have evolved in SP. Results demonstrated that patients who remained RR induced tau aggregation (seeding), while the evolved SP did not (no seeding). According to these results, we conducted additional RT-QuIC studies on 40 CSF samples collected from newly diagnosed RR pMS, to evaluate the ability to promote or not tau aggregation and to correlate RT-QuIC results with clinical and radiological data.
At the time of diagnosis mean disease duration was 24.9 months ±27.2 and median EDSS was 2.0, 0-3.5. Brain MRI was performed to calculate total and GM volume. At time of CSF analysis patients had mean clinical follow-up of 38.6 months ±19.1, mean disease duration of 4.8 years (±2.5) and median EDSS of 1.0 (0-5.5); no patients evolved in SP and 3/40 get to EDSS≥3.0. According to RT-QuIC we identified no seeding and seeding patients, as previously observed. Comparing the two groups, the total and GM brain volume was statistically significant lower in no seeding patients ($p<0.05$).
Combined together and if confirmed, results suggest that RT-QuIC could be an innovative tool useful for an early identification of pMS at a high risk to develop disease progression.

Resolution of inflammation is impaired in multiple sclerosis and entails a loss of pro-resolving features of monocytes/macrophages

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Multiple Sclerosis (MS) is an autoimmune neurodegenerative disease in which an aberrant immune response conveys an attack towards the neurons' myelin sheath in the brain, leading to disability. Chronic and unresolved inflammation displayed in MS is starting to be linked to an impairment of the resolution of inflammation, which is the spontaneous process that confines inflammation and avoids its chronicization. Innate immune cells, in particular monocytes/macrophages, are emerging as important gears in MS pathogenesis, due both to their ability to produce and being among the main cellular targets of the specialized pro-resolving mediators (SPMs) –the endogenous lipids that mediate the resolution of inflammation, which include lipoxins (LX), resolvins (Rv), neuroprotectins (NPD) and maresins (MaR)– as well as to their ability to infiltrate the brain and exacerbate neuroinflammation during MS. Targeted-metabololipidomics showed altered, disease-specific patterns in the SPM levels of MS patients; also, peripheral blood mononuclear cells (PBMCs) expressed dysfunctional levels of the main enzymes (5- 12- and 15-LOX) and receptors (GPR32, GPR18, ALX) of the SPM system. Furthermore, we found that several SPMs, such as LXA4 and LXB4 as well as RvD1 and NPD1, significantly reduced pro-inflammatory cytokines production (TNF-α, IL-1β, IL-6 and IL-12) in monocytes of MS patients, although with less potency that observed in monocytes of healthy subjects. Interestingly, when polarized towards pro-inflammatory M1 or pro-resolving M2 phenotypes, MS macrophages exhibited altered production of RvD1 and RvD2 and expression of their receptors and biosynthetic enzymes. These findings show the first evidence of a deep change in the role played in MS by monocytes/macrophages during resolution of neuroinflammation, which might provide new insights on the pathogenesis of this disease, as well as the basis for potential novel diagnostic and therapeutic approaches.

Agenesis of the putamen and globus pallidus caused by recessive mutations in the homeobox gene GSX2

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Basal ganglia are subcortical grey nuclei which play essential roles in controlling voluntary movements, cognition and emotion. While basal ganglia dysfunction is observed in many neurodegenerative or metabolic disorders, congenital malformations are rare. In particular, neurodevelopmental syndromes characterized by basal ganglia agenesis are not known to date. We ascertained two unrelated girls presenting with spastic tetraparesis, severe generalized dystonia and intellectual impairment, sharing a unique brain malformation characterized by agenesis of putamen and globus pallidus, dysgenesis of the caudate nuclei, olfactory bulbs hypoplasia, and anomaly of the diencephalic-mesencephalic junction with abnormal corticospinal tract course. Whole Exome Sequencing identified two novel homozygous variants, c.26C>A; p.(S9*) and c.752A>G; p.(Q251R) in the GSX2 gene, a member of the family of homeobox transcription factors highly expressed in the lateral and median ganglionic eminences, which are key regulators of embryonic development. The truncating variant resulted in complete loss of protein expression, while the missense variant affected a highly conserved residue of the homeobox domain, resulted in reduced protein expression and caused impaired structural stability of the homeobox domain and weaker interaction with DNA according to molecular dynamic simulations. Moreover, the nuclear localization of the mutant protein in transfected cells was significantly reduced compared to the wild type protein. Expression studies on both patients' fibroblasts demonstrated reduced expression of GSX2 itself, as well as significant expression changes of related genes such as ASCL1 and PAX6. Moreover, whole transcriptome analysis revealed a global deregulation in genes implicated in apoptosis and immunity, two broad pathways known to be involved in brain development. This is the first report of the clinical phenotype and molecular basis associated to basal ganglia agenesis in humans.

Role of lipid mediators in the aging process

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The "inflammaging" theory attributes the aging not only to free radical damage, but also to a chronic low-level inflammation, which develops over time. A crucial role in the process of chronic inflammation has been shown to be played by specialized pro-resolving lipid mediators (SPMs). The inbred LOU/C/jall (LOU) rat has been described as a model of successful aging with a prolonged healthy lifespan associated with maintained motor and cognitive functions. To evaluate the role of SPMs in the delayed cognitive decline of LOU rats, we submitted young (5 months) and aged (30 months) LOU rats and young (4 months) and aged (24 months) Wistar rats (control group) to a battery of behavioral tests, and then we analyzed the plasmatic levels of the SPM resolvins D1 (RvD1) and resolvins D2 (RvD2). The behavioral performances of LOU rats and respective controls significantly differed. In particular, locomotion data indicated more activity of the LOU group compared to controls. In mnestic and visuospatial tests the aged LOU rats showed an intact new object recognition memory, contrary to their respective controls. As for the levels of investigated SPMs, while in the Wistar group we found a tendential decrease in the basal levels of RvD1 and RvD2 during aging, in the LOU rats we found opposite results. Namely, in the young LOU rats the levels of RvD1 were significantly superior to those of young controls. In the aged LOU rats we found a substantial increase in both lipid mediators in comparison to aged Wistar rats. These results suggest an age-related change in inflammation resolution pathways. The behavioral and biological results indicate that within the factors associated with the aging-related behavioral and biological alterations there is the production of SPMs, in particular of RvD1 and RvD2.

Preconditioned Bone Marrow Mesenchymal Stem Cell-derived Extracellular Vesicles Exert Immunomodulatory Effects in a model of Alzheimer's Disease

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Aims | Analysis of the role of preconditioned human Bone Marrow Mesenchymal Stem Cells-derived Extracellular Vesicles (MSC-EVs) as a therapeutic strategy to modulate the inflammatory response in in vitro and in vivo AD models.
Methods | The immunosuppressive hMSC phenotype was induced by preconditioning cells with pro-inflammatory cytokines (TNFa+IFNg). Preconditioned hMSC-EVs were then isolated from the cell culture medium by ultracentrifugation and their immunomodulatory ability was investigated following the next steps:
1) In an in vitro model of inflammation, we assessed the ability of MSC-EVs to affect the polarization of microglia - previously challenged with an inflammatory insult (TNFa+IFNg) - by evaluating the microglia cytokine release (IL-6, IL-1b, IL-10, IL-4) and the expression of different phenotype-labeling markers (Iba-1, iNOS, CD68, CD206) by ELISA and Western Blot, respectively;
2) In in vivo AD model (7-month-old 3xTg mice), upon two intranasal injection (IN) of EVs (total amount »15x10^9), we investigated - by immunofluorescent analysis - their efficacy in counteracting microglia activation (cell density, morphological changes, expression of phenotypic markers) and dendritic spine degeneration (Golgi-Cox staining), 3 weeks after EV treatment.
Results | *In vitro*, hMSC-EVs seemed to foster microglia protective phenotype as evidenced by the negative modulation on the pro-inflammatory cytokines IL-6 and IL-1b and the increased release of the anti-inflammatory IL-10. *In vivo*, EV treatment was able to reduce microglial activation and to increase dendritic spine density in 3xTg mice when compared to the controls.
Conclusion | Our study indicates that IN injection of EVs derived from cytokine-preconditioned MSCs is a feasible approach to reduce microglia activation and to counteract dendritic spine degeneration in 7-month-old 3xTg mice, thus supporting the recently emerged therapeutic potential of MSC-EVs in AD.

Clinical Significance of Extracellular Vesicles in Plasma from Glioblastoma Patients

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INTRODUCTION | Glioblastoma (GBM) is the most common primary brain tumor. The identification of blood biomarkers reflecting the tumor status represents a major unmet need for optimal clinical management of patients with GBM. Their high number in body fluids, their stability, and the presence of many tumor associated proteins and RNAs make extracellular vesicles potentially optimal biomarkers

MATERIALS AND METHODS | Plasma from healthy controls (n = 33), patients with GBM (n = 43), and patients with different central nervous system malignancies (n = 25) were collected. Extracellular vesicles were isolated by ultracentrifugation and characterized in terms of morphology by transmission electron microscopy, concentration, and size by nanoparticle tracking analysis, and protein composition by mass spectrometry. An orthotopic mouse model of human GBM confirmed human plasma extracellular vesicle quantifications. Associations between plasma extracellular vesicle concentration and clinicopathologic features of patients with GBM were analyzed. All statistical tests were two sided

PURPOSE | We investigated the potential role of plasma extracellular vesicles from patients with GBM for diagnosis and follow up after treatment and as a prognostic tool

RESULTS | GBM releases heterogeneous extracellular vesicles detectable in plasma. Plasma extracellular vesicle concentration was higher in GBM compared with healthy controls ($P < 0.001$), brain metastases ($P < 0.001$), and extra-axial brain tumors ($P < 0.001$). After surgery, a significant drop in plasma extracellular vesicle concentration was measured ($P < 0.001$). Plasma extracellular vesicle concentration was also increased in GBM-bearing mice ($P < 0.001$). Proteomic profiling revealed a GBM distinctive signature

CONCLUSIONS | Higher extracellular vesicle plasma levels may assist in GBM management: their reduction after GBM resection, their rise at recurrence, and their protein cargo might provide indications about tumor, therapy response, and monitoring.

Oncogenic role of the aminopeptidase ERAP1 in Hedgehog-dependent cancer

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The Hedgehog (Hh) pathway is essential for embryonic development and tissue homeostasis. Aberrant Hh signaling may occur in a wide range of human cancers, such as medulloblastoma (MB), the most common brain malignancy in childhood. Here, we identify endoplasmic reticulum aminopeptidase 1 (ERAP1), a key regulator of innate and adaptive antitumor immune responses, as a previously unknown player in the Hh signaling pathway. We demonstrate that ERAP1 binds the deubiquitylase enzyme USP47, displaces the USP47-associated βTrCP, the substrate-receptor subunit of the $SCF^{\beta TrCP}$ ubiquitin ligase, and promotes βTrCP degradation. These events result in the modulation of Gli transcription factors, the final effectors of the Hh pathway, and the enhancement of Hh activity. Remarkably, genetic or pharmacological inhibition of ERAP1 suppresses Hh-dependent tumor growth *in vitro* and *in vivo*. In particular, we show that the inhibition of ERAP1 strongly reduces MB in vivo cell growth in orthotopic allograft animal models and in HH-MB Patient-Derived Xenograft (PDX). Of note, the pharmacological inhibition of ERAP1 leads to a significant increase of survival in Math1-cre/$Ptc^{C/C}$ mice, which spontaneously develop MB. Our findings unveil an unexpected role for ERAP1 in cancer and indicate ERAP1 as a promising therapeutic target for Hh-driven tumors.

Combined Positron Emission Tomography imaging approach for identification of new potential biomarkers for treatment response in glioma models

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Glioblastoma multiforme (GBM) carries a poor prognosis with a median survival of 10–11 months with standard treatment (cytoreduction associated to radiotherapy, RT, plus chemotherapy with Temozolomide, TMZ). Despite the low rate of survival, alternative treatment doesn't exist. The combined use of chemotherapeutic agents with drugs targeting cell metabolism is becoming an interesting therapeutic option for cancer. Metformin (MET), a biguanide currently used for diabetes II patients, displays anticancer effects. Here, we evaluated the response of GBM mouse models to metformin and TMZ using combined Positron Emission Tomography (PET) imaging. To this aim, human TMZ-sensitive and –resistant glioma cells carrying an Epidermal Growth Factor Receptor (EGFR) mutation were treated both *in vitro* and *in vivo* with TMZ and MET alone or in combination. In vivo response to therapy was monitored using Magnetic Resonance Imaging (MRI) and PET with [^{18}F]FLT (cell proliferation) and [^{18}F]VC701 (TSPO receptor, inflammation). At sacrifice, brains were collected and processed for immunohistochemistry (IHC) analysis. *In vitro*, MET treatment both improved sensitivity of TMZ and overcame resistance to TMZ. *In vivo*, combined treatment with TMZ and MET influenced tumour growth and survival in a cell-dependent manner. MET alone didn't increase survival independently from TMZ sensitivity. Treated TMZ-sensitive tumour displayed a significant decrease in [^{18}F]FLT uptake compared to control. In TMZ-sensitive group, the addition of metformin significantly increased survival and decreased [^{18}F]VC701 uptake. Markers for ki67 and Iba1 showed variable changes in different treatment groups. In conclusion, MET is able to enhance the efficacy of TMZ both *in vitro* and *in vivo*. The use of combined PET radiotracers allows to evaluate changes of several cancer-related features induced by treatment.
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Towards a 3D all human in vitro model of glioblastoma multiforme for drug screening: glioblastoma and astrocyte interactions?

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Modelling the brain tumour microenvironment is one of the most challenging areas of research due to the difficulties in reproducibility and the inherent complexity of the cellular nature of the neoplasm and the organ in which it grows. The glioblastoma tumour microenvironment is characterized by complex networks between cancer cells and stromal cells such as astrocytes, microglia, and endothelial cells and release of soluble factors[1]. In addition, the presence of extracellular matrix (ECM) can affect cell plasticity and tumour behaviour making it challenging to pre-clinically test drugs[2]. Recently, a number of studies have demonstrated that the tumour microenvironment, particularly the stromal cells, contributes to malignant behaviour in human glioma [3,4] as well as to also chemo-resistance. The role of astrocytes, the most abundant glial cells in the glioma microenvironment, in the development of this disease is poorly understood, particularly with regard to invasion and drug resistance[3]. To assess the role of astrocytes in GBM growth, invasion and drug resistance, we established a 2D co-culture model and a 3D hyaluronic acid-gelatin hydrogel model (HyStemTM HP) with different ratios of GBM cells to astrocytes. A contact co-culture of fluorescently labelled glioblastoma cells and astrocytes showed that the latter promote tumour growth and migration of glioblastoma cells. Notably, the presence of astrocytes, even in low amounts in co-culture, elicited drug resistance in glioblastoma cells.

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Microglia-Derived Microvesicles Affect Microglia Phenotype in Glioma

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Extracellular-released vesicles (EVs), such as microvesicles (MV) and exosomes (Exo) provide a new type of inter-cellular communication, directly transferring a ready to use box of information, consisting of proteins, lipids and nucleic acids. In the nervous system, EVs participate to neuron-glial cross-talk, a bidirectional communication important to preserve brain homeostasis and, when dysfunctional, involved in several CNS diseases. We investigated whether microglia-derived EVs could be used to transfer a protective phenotype to dysfunctional microglia in the context of a brain tumor. When MV, isolated from microglia stimulated with LPS/IFNγ were brain injected in glioma-bearing mice, we observed a phenotype switch of tumor associated myeloid cells (TAMs) and a reduction of tumor size. Our findings indicate that the MV cargo, which contains upregulated transcripts for several inflammation-related genes, can transfer information in the brain of glioma bearing mice modifying microglial gene expression, reducing neuronal death and glioma invasion, thus promoting the recovery of brain homeostasis.

Notch signaling controls glioma proliferation and shapes the tumor microenvironment

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The Notch signaling pathway plays a crucial role in regulating brain development and neural progenitor cell homeostasis. Aberrant Notch activity is linked to several neuropathological conditions including glioma, one of the most aggressive forms of brain tumor. In line with the identification of Notch inactivating mutations in patients with glioma subtypes, we have recently found that Notch signaling can behave as a tumor suppressor in glioma. To identify the mechanisms underlying Notch tumor suppressive function, we combined conditional genetics in mouse models of glioma with expression profile analyses of Notch-mutant tumors. We found that Notch inhibition in tumor cells releases expression of genes involved in neural stem cell activation and cell cycle progression, while downregulating expression of genes associated with quiescence, thereby promoting an active proliferative state. Surprisingly, blocking Notch also decreases the expression of genes involved in communication between tumor cells and immune cells. We confirmed that immune cell recruitment is impaired after Notch inhibition in tumor cells both in vitro and in vivo. Moreover, in vivo manipulation of the tumor immune microenvironment strongly suppresses the formation of Notch-intact tumors, while Notch-inhibited tumors were more refractory to the treatment. Interestingly, individual Notch receptors have distinct functions during glioma development, and only specific Notch receptors or receptor combinations can activate a tumor suppressive signal. Our data indicate that Notch suppresses glioma formation by impacting on both intrinsic and extrinsic regulators of tumor cell growth. We also identified a novel and therapeutically attractive role of Notch signaling in controlling immune evasion in glioma.

Repurpose of exendin-4 for the treatment of neonatal brain injury

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Neonatal mortality accounts globally for 46% of total deaths in children under the age of 5. The most common risk factors are prematurity and hypoxia-ischemia. Survivors are at a high risk of developing lifelong neurological neurodisabilities, including cerebral palsy. There are no treatments available for preterm birth and subsequent brain haemorrhage. In late preterm and term infants, hypothermia is only partially protective against hypoxia-ischemia. Exendin-4 is a drug currently used for treating Type 2 diabetes mellitus and has shown neuroprotective aspects with current ongoing clinical trials for Alzheimer's and Parkinson's diseases. Using a 4-dose regimen (12h interval) intraperitoneal administration of exendin-4 (0.5µg/g), we have treated late preterm (postnatal day 7, P7) or term mice (P10), starting either immediately or within 2h after hypoxia-ischemia. P5 preterm rats, receive a single dose of collagenase (0.3U) into the medial striatum to induce germinal matrix haemorrhage (GMH) before treatment with exendin-4. Our results show that intraperitoneal administration of exendin-4 started either immediately or within 2h after hypoxia-ischemia significantly reduces brain damage. Furthermore, exendin-4 enhanced clinically used hypothermia treatment. In the GMH model, exendin-4 treated rats show within 48h a significant decrease in brain tissue loss. This neuroprotection is maintained over time, as shown by the significantly improved motor coordination of exendin-4-treated rats detected in the negative geotaxis test, as well as histopathological measurements at P16. Overall, exendin-4 treatment was well tolerated in both species and neonatal brain injury models. The demonstrated safety and tolerability of high-dose exendin-4 administrations, combined with its significant neuroprotective effects alone or in conjunction with TH make the repurposing of exendin-4 for the treatment of neonatal brain injury is very promising.

Growth parameters and minor brain lesions in very low birth weight (VLBW) newborns as prognostic factors of negative neurodevelopmental outcome at two and three years of age

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VLBW newborns (Very Low Birth Weight, <1500 g at birth) are at high risk for negative neurodevelopmental outcome. Severe cerebral lesions are known to interfere with neurodevelopmental outcome, however the role of other potential risk factors, such as minor brain injury and auxological data (weight gain, head circumference growth), is unclear. Aim of the study: to investigate the correlation between auxological data, minor brain lesions and neurodevelopmental outcome. 176 VLBW patients, without evidence of major cerebral lesions on brain magnetic resonance imaging, were included in the study. We identified patients with minor hemorrhagic lesions: germinal matrix hemorrhage – intraventricular hemorrhage (GMH-IVH) without ventricular dilation and micro-cerebellar hemorrhage (CBH). Patients were divided into SGA (Small for Gestational Age, birth weight <10th centile) and non-SGA, Z-scores for head circumference and weight age were calculated. Z-scores ≤-2 SD for age and sex were considered pathologic. The Griffiths Mental Development Scale II (GMDS) was performed by operators blinded to patient's medical history and auxological data at two and three years of corrected age. We did not find significant differences in GMDS scores at 2 and 3 years between SGA and non-SGA patients at birth. Patients with pathological weight at 6 and 12 months of corrected age had significantly lower GMDS scores than normal weight population. We did not find significant differences in the neurocognitive outcome up to 3 years between patients with pathological head circumference and controls. Patients with GMH-IVH or micro-CBH had statistically significant lower GMDS scores than patients without lesions. Our results confirm that a deficient growth in weight, as well as minimal brain lesions, are factors correlated to negative neurodevelopmental outcome up to 3 years of age. We observed greater impact on neurodevelopmental outcome of pathologic weight at 6 and 12 months than being born SGA.

A novel double-hit mechanism involving different genes of the MTOR pathway in hemimegalencephaly with intractable childhood epilepsy

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Single germline or somatic activating mutations of mTOR pathway genes are emerging as a major cause of Type II Focal Cortical Dysplasia (FCD), hemimegalencephaly (HME), and Tuberous Sclerosis Complex (TSC). These neurodevelopmental disorders are all characterized by abnormal lamination of the cerebral cortex, ectopic subcortical neurons, giant dysmorphic neurons and, in some cases, balloon cells, and are often associated with intractable epilepsy and intellectual disability. In addition to the autosomal dominant mechanism, a double hit mechanism based on a primary germline mutation in one allele and a secondary somatic hit affecting the other allele of the same gene in a small number of cells has been documented in a limited number of patients with TSC or FCD. Using different next generation sequencing approaches in paired DNA samples extracted both from blood and dysplastic brain tissue, we identified two somatic variants affecting two different genes of the mTOR pathway (RPS6 p.R232H and MTOR p.S2215F) in a patient with HME, severe intellectual disability and intractable seizures, who underwent hemispherectomy. To date, variants in RPS6 had not been associated with human disease, while MTOR p.S2215F had previously been related to FCD and, in a single patient, to HME. Overexpressing the two variants independently in animal models, we demonstrated that the MTOR p.S2215F variant caused neuronal migration delay and cytomegaly, while the RPS6 p.R232H variant prompted increased cell proliferation. Double mutants exhibited a more severe phenotype, pointing to a synergistic effect of the two variants. This study indicates that, in addition to single activating mutations and double-hit inactivating mutations in mTOR pathway genes, HME can result from activating mutations affecting different genes of this pathway. Our data also suggest that RPS6 is a potential novel disease-related gene.

Therapeutic effect of neural progenitor cells expanded in the 3D nano-engineered Nichoid substrate in a Parkinson's disease preclinical model

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Recently biomaterials have been used to create 3D micro scaffolds, such as the one named "Nichoid", which mimics the biomechanical characteristics of stem cell niches. The aim of this study was to investigate the proliferation, differentiation and stemness properties of neural precursor cells (NPCs) and the therapeutic effect and safety in vivo of NPCs grown inside the niches in preclinical experimental model of Parkinson's Disease (PD). Nichoids were fabricated by two photon laser polymerization using a photosensitive resin. NPCs were grown for different periods inside the Nichoid and cells features were characterized by Real Time PCR analysis, immunofluorescence and Western Blot. Parkinsonism was induced by the intraperitoneal administration of MPTP in C57/black mice by using an acute protocol. NPCs grown inside the Nichoid create a 3D carpet and, 7 days after plating, cells show a significantly higher proliferation than in normal floating culture conditions. NPCs expanded inside the Nichoid maintain their biological features and show an increase in stemness potential, as demonstrated by Real Time-PCR, Western Blot, immunofluorescence and methylation assay. The therapeutic effect and safety of Nichoid-grown NPCs was evaluated by their intrastriatal infusion in PD affected mice. Behavioral performances were evaluated with two different tests showing that Nichoid-grown NPCs promoted the recovery of PD symptoms and favor the expression of tyrosine hydroxylase in the pathology affected brain areas. Stem cells show an increase in stemness potential when grown inside the Nichoid, demonstrating great promise and strong application in the field of regenerative medicine applied to neurodegenerative disease.

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Incorporation of spatial mapping and confirmation of gene signatures by a multiplex in situ hybridization technology into single cell RNA sequencing workflows

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Complex and highly heterogenous tissues such as the brain are comprised of multiple cell types and states with exquisite spatial organization. Single-cell RNA sequencing (scRNA-seq) is now being widely used as a universal tool for classifying and characterizing known and novel cell populations within these heterogenous tissues, ushering in a new era of single cell biology. However, the use of scRNA-seq presents some limitations due to the use of dissociated cells which results in the loss of spatial context of the cell populations being analyzed. Incorporating a multiplexed spatial approach that can interrogate gene expression with single cell resolution in the tissue context is a powerful addition to the scRNA-seq workflow. In this study, we used the RNAscope Multiplex Fluorescent and RNAscope HiPlex *in situ* hybridization (ISH) assays to confirm and spatially map the diverse striatal neurons that have been previously identified by scRNA-seq in the mouse brain (Gokce *et al*, *Cell Rep*, 16(4):1126-1137, 2016). We confirmed the gene signatures of two discrete D1 and D2 subtypes of medium spiny neurons (MSN): *Drd1a/Foxp1, Drd1a/Pcdh8, Drd2/Htr7*, and *Drd2/Synpr*. The heterogenous MSN subpopulations were marked by a transcriptional gradient, which we could spatially resolve with RNA ISH. Numerous striatal non-neuronal cell populations identified by scRNA-seq, including vascular cells, immune cells, and oligodendrocytes, were also confirmed with the multiplex ISH assay. Finally, the spatial relationship between the D1 and D2 MSN subtypes identified by Gokce *et al.* was visualized using the RNAscope HiPlex assay, which allows for detection of up to 12 RNA targets simultaneously in intact tissues. In conclusion, we have demonstrated the utility of two multiplexed RNAscope ISH assays for the confirmation and spatial mapping of scRNA-seq transcriptomic results in the highly complex and heterogenous mouse striatum at the single cell level. Incorporating spatial mapping by the RNAscope technology into single cell transcriptomic workflows complements scRNA-seq results and provides additional biological insights into the cellular organization and functional states of diverse cell types in healthy and disease tissues.

Keywords: Transcriptome, Single cell, RNA, Brain/Nervous system, RNA-seq

Effects of chronic continuous theta-burst stimulation (cTBS) on bidirectional striatal synaptic plasticity in a model of Parkinson's disease and L-Dopa- induced dyskinesia

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Parkinson's disease (PD), a neurodegenerative disease, is characterized by the progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. The most effective drug for the treatment of PD is 3,4-dihydroxy-l-phenylalanine (L-Dopa), though the long-term use of this dopamine precursor leads to the development of L-Dopa-induced dyskinesias (LIDs), non-motor symptoms and induces an impairment in corticostriatal bidirectional synaptic plasticity. In the last decade, Transcranial Magnetic Stimulation (TMS) has been widely used as a possible treatment for both PD and hyperkinetic movement disorders, such as LIDs, based on the ability to shape cortical activity with different stimulation protocols. Although studies on the efficacy of TMS in treating movement disorders have produced variable results, many reports support the idea that TMS has a therapeutic potential. In this context we assume that TMS used during L-Dopa chronic treatment, with an stimulation protocol known to inhibit motor activity such as continuous Theta Burst Stimulation (cTBS), could lead to a reduction of LIDs and a correlated recovery of synaptic down-scaling (Long Term Potentiation, LTP, and depotentiation), usually lost in model of PD. To test this hypothesis, 6-OHDA-fully lesioned animals were subjected to this co-treatment and synaptic plasticity was studied ex vivo with patch clamp and intracellular recordings techniques and Abnormal Involuntary Movements (AIMs) were scored to test the antidyskinetic effect of cTBS. Electrophysiological recordings from cortical striatal slices in these animals showed that this co-treatment was able to induce a recovery of bidirectional synaptic plasticity. The behavioral data associated with these results exhibited a significantly reduction of AIMs severity and a reduced frequency of dyskinetic behaviors. Finally, our results provide an experimental support for the clinical use of TMS with bursting stimulation in PD therapy.

Astrocyte-derived extracellular vesicles and cell-to-cell communication in the nigrostriatal regions: implications for dopaminergic neuroprotection

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Astrocytes (AS) are key players in the regulation of dopaminergic (DA) neuron homeostasis both in health and disease, such as Parkinson's disease (PD), a neurodegenerative disorder affecting cell bodies of DA neurons in subtantia nigra pars compacta of the ventral midbrain (VM) and their terminals in the striatum (Str). Our previous work demonstrated that AS activated by chemokines, such as Ccl3, exert a robust DA neuroprotection/regeneration against the PD neurotoxin MPTP, both *in vitro* and *in vivo*, but the mechanism(s) underlying the complex cross-talk between AS, neurons and neural stem cells is still unknown. We hypothesize a possible role for AS-derived extracellular vesicles (AS-EVs) in this intercellular signaling. EVs are a heterogeneous class of vesicles continuously released outside by cells both in physio- and pathological conditions. We herein characterized AS from both the VM and Str and addressed the effect of the Ccl3 on cellular physiology and secretive activity. We found that AS morphology changes after the Ccl3 exposure – with no effect on proliferation – displaying more membrane protrusions and suggesting an increase of EV secretion. We therefore characterized AS-EVs from both brain regions, before and after the Ccl3 treatment, via electron microscopy, nanoparticle tracking analysis and western blot. We found a major population of EVs in the size range of ~100 nm, enriched in exosomal markers (Cd63/9, Alix1), confirming the presence of exosomes in AS-EVs. Also, we demonstrated the presence of RNAs by qPCR, finding both mRNAs and miRNAs associated with EVs. The whole exosomal RNAome has been characterized via RNA-seq, identifying secreted vs. retained sequences. All these findings suggest a possible role for AS-EVs in the glial-neuron communication. This in-depth characterization was preliminary to our next steps of investigation that aim to evaluate, *in vitro* and *in vivo*, the impact of AS-EVs on PD target cells.

Mechanosensation: a new horizon in the regulation of myelination?

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Demyelinating diseases of the central nervous system (CNS) are often characterised by both a breakdown of the myelin sheath and by secondary neuronal damage. Demyelination can cause excessive calcium influx into neurons leading to excitotoxicity which, in turn, accelerates oligodendrocyte degeneration. Current therapies for demyelinating disorders such as Multiple Sclerosis, act predominantly as immune-modulators and are ineffective at protecting neurons against damage in the latter stages of disease. However, research in the past few years have strongly suggested a physical contribution to myelin initiation and repair. In this study, we investigated whether the mechanosensitive channel Piezo1, a stretch-activated mechanosensitive cation channel, plays a role in the regulation of myelination. We showed that the stretch-activated cation channel blocker, GsMTx4 enhanced developmental myelination and, more interestingly, prevented psychosine-induced demyelination in the *ex vivo* model of cerebellar slices; while activation of Piezo1 by the activator Yoda-1 led to demyelination. These results were corroborated *in vivo*, where GsMTx4 prevented LPC-induced demyelination, neuronal and astrocytic toxicity, and microglial activation, in a model of LPC focal demyelination using stereotaxic surgery in young adult mice. Thus, our data suggest that targeting Piezo1 channels and attenuating excessive calcium influx blockage of stretch-activated cation channels, may help to prevent secondary progressive neurodegeneration in latter stages of demyelinating diseases, placing Piezo1 as a potential future target for new therapies.

Raman spectroscopy as biochemical "fingerprint" of biofluids for the investigation of neurodegenerative diseases

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Raman Spectroscopy (RS) is a label-free investigative technique, able to detect in a fast and sensitive process, all the biochemical variations of a considered biological sample. In the case of biofluid, RS can analyze the biochemical profile of proteins, lipids, nucleic acids and metabolites identifying possible modifications due to a specific pathological state[1]. For this reason we tried to perform RS analysis on biofluids collected with a minimal- or not-invasive process from patients affected by neurodegenerative disorders, fine tuning the methodology for highly reproducible results. The aim of the studies was to develop a diagnostic, predictive and monitoring tool for the pathological state evaluation. The analyzed biofluids were serum collected from patients affected by Alzheimer's Disease (AD) and saliva collected from patients affected by Amyothrophic Lateral Sclerosis (ALS).

- **AD**: Serum collected from AD patients was analyzed taking advantage of the amplified Raman effect obtained after the contact of serum protein with silver nanoparticles (SERS effect)[2]. Using a simple protocol and a minimal invasive procedure for biofluid collection, we analyzed serum from 10 patients affected by AD comparing the signal with the one obtained from 11 Healthy Controls (HC).
- **ALS**: Saliva represents a complex mixture of molecules transported from different biological districts collectable with a fast and minimal invasive procedure[3]. Using RS, a fast characterization of the fluid biochemical composition was performed on saliva collected from 12 ALS patients and 7 HC.

The data obtained from the RS analysis were processed using statistical multivariate analysis showing, in both the pathological cases, statistically significant differences between the two groups when compared with the respective HC. These results confirmed the potential applications of RS in clinical field, being able to be used as a fast and highly sensitive diagnostic, predictive and monitoring tool.

SPRi-based biosensor for the detection of circulating extracellular vesicles as biomarkers of neurological diseases

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One of the main hurdle in the rehabilitation and treatment of neurological diseases is the lack of easily accessible and sensitive biomarkers for the prediction of the disease progression rate and the evaluation of therapy efficacy. Extracellular vesicles (EVs) are nanoscaled vesicles released by body cells studied as promising biomarkers of neurological diseases as they are involved in the onset and progression of Alzheimer's disease (AD), and in the regenerative and repair processes occurring after ischemic stroke. In the strive for a reliable and sensitive method to analyze EVs, we propose a biosensor based on Surface Plasmon Resonance imaging (SPRi). We applied our recently optimized SPRi biosensor for the detection and characterization of EVs isolated from the blood of stroke and AD patients. The SPRi-array was designed to separate simultaneously EVs released by neurons, astrocytes, microglia, oligodendrocytes, endothelial cells and apoptotic bodies, and to evaluate the presence and the relative amount of specific surface molecules related to pathological or recovery processes. Our results showed differences in the relative amount of specific cell-derived EV populations, and also in their cargo during the disease progression or resolution. In particular, variations in the amount of specific receptors related to neuroinflammation and neuroregeneration were observed in the serum EVs of stroke patients before and after rehabilitation. Similarly, the characterization of EVs from AD patients demonstrated the presence of EVs loaded with altered cargoes compared to healthy subjects: differences in the lipid moieties present on neuronal EVs and variations in the activation phenotype of microglia EVs were observed. Our results provide support for using the SPRi-based biosensor for the detection and characterization of circulating EVs to evaluate their potential as peripheral biomarkers for the prediction of the recovery after stroke and for the monitoring of AD patients.

Defective cholesterol clearance limits remyelination in the aged central nervous system

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Age-associated decline in regeneration capacity limits the restoration of nervous system functionality after injury. In a model for demyelination, we found that old mice fail to resolve the inflammatory response initiated after myelin damage. Aged phagocytes accumulated excessive amounts of myelin debris, which triggered cholesterol crystal formation and phagolysosomal membrane rupture and stimulated inflammasomes. Myelin debris clearance required cholesterol transporters, including apolipoprotein E. Stimulation of reverse cholesterol transport was sufficient to restore the capacity of old mice to remyelinate lesioned tissue. Thus, cholesterol-rich myelin debris can overwhelm the efflux capacity of phagocytes, resulting in a phase transition of cholesterol into crystals and thereby inducing a maladaptive immune response that impedes tissue regeneration.

POSTER SESSIONS

NP01 | Generalized recovery of motor functionality after stroke by combined motor training and ipsi-lesional optogenetic stimulation

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Neuro-rehabilitative research is developing novel strategies to enhance the effectiveness of therapies after stroke by using a combination of physical and plasticizing treatments. Previous studies have shown that optogenetic stimulation of neurons in the peri-lesioned area induces a significant improvement in cerebral blood flow and neurovascular coupling response. Nevertheless, the mechanisms underneath the reshaping of cortical functionality induced by rehabilitation after stroke are widely unknown. Here, we investigated how cortical activity is differentially modulated by neuronal stimulation alone and in combination with robotic motor training. Both approaches are supposed to promote forelimb functional recovery by fostering the stabilization of regions of the cortex linked to the stroke core and stimulating the remodelling of peri-infarct areas. In our study we induced a photothrombotic stroke in the primary motor cortex and the expression of Channelrhodopsin 2 (ChR2) in the somatosensory area on Thy1-GCaMP6f mice. We took advantage of a robotic platform (M-Platform) to perform the rehabilitation of mouse forelimb. The motor training consists of fifteen retraction movements: after the forelimb is passively extended, the animal has to pull back up to the resting position. Then, to promote the remodelling in the peri-infarct area, we performed daily stimulation of the region of the cortex surrounding the damage expressing ChR2 with a blue laser. Through behavioural experiments, i.e. Schallert test, we evaluate changes of forelimb functionality during rehabilitation. After one month of combined treatment we observed a generalized recovery of forelimb functionality in terms of manual dexterity and cortical profiles of activation. By analysing the temporal calcium dynamics, we found that the functional recovery of the injured forelimb is associated with the rescue of essential features of cortical activation profiles evoked during the forelimb retraction.

NP02 | Direct analysis of stem cell-derived extracellular vesicles with super resolution microscopy for live imaging in neural progenitor cultures

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Extracellular vesicles (EVs) have prevalent roles in cell differentiation, cancer biology and regenerative medicine. Conventional techniques available to characterise EVs isolated from cultures include electron microscopy (EM), nanoparticle tracking analysis (NTA) and tuneable resistive pulse sensing (TRPS), however these have been reported to show high variability in particle count (EM) and poor sensitivity in detecting EVs below 50 nm in size (NTA and TRPS), making accurate and unbiased EV analysis technically challenging. In the present investigation, an approach based on direct stochastic optical reconstruction microscopy (d-STORM) is presented as an efficient and reliable characterisation alternative for stem cell-derived EVs. Using a photo-switchable lipid dye, d-STORM imaging enabled rapid detection of EVs down to 20-30 nm in size, providing higher sensitivity and lower variability compared to standard EM, NTA and TRPS techniques. Imaging of EV uptake by live neural stem cells in culture was further demonstrated to confirm the potential of this approach for downstream neural cell biology applications, and for the analysis of vesicle-based cell-cell communication pathways.

NP03 | 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.

NP04 | Enhancement of activity rescues the early establishment of Mecp2 null neuronal features

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The X-linked Methyl-CpG-Binding Protein 2 (MeCP2) gene encodes for a multifunctional protein ubiquitously expressed from developmental stages to adulthood. Mutations in *MECP2* are linked to Rett syndrome (RTT), the most common genetic cause of severe intellectual disability in females. Although MECP2 plays a crucial role in the maintenance of proper neuronal functionalities, several evidences now suggest that early signs of the pathology can be observed (in both humans and animal models) long before the typical RTT symptoms become overt. Focusing on the development of neuronal networks, our data demonstrate that the dynamics of differentiation (both *in vitro* and in pre- and early postnatal cortical tissues) are affected by lack of Mecp2 from a transcriptional, functional and morphological point of view. In fact, we show *in vitro* that the reduced expression of genes encoding for mediators of neuronal activity diminishes the magnitude of Ca^{2+} transients induced by exposure to stimuli (such as glutamate or NMDA) and alters the electrophysiological properties of the maturing neuronal network. As a consequence of such defects, and in line with the role played by neuronal activity in driving structural maturity, null neurons display poor morphological complexity, as dendritic arborization and length are reduced. Intriguingly, we demonstrate that strategies aiming at transiently enhancing neuronal activity during critical stages of neuronal network establishment rescue part of the typical defects displayed later by Mecp2 null neurons. Together, our data demonstrate that the impairments affecting adult RTT animal models can be considered the worsening of a condition that is already generated during early development.

NP05 | The transcriptional regulator COUP-TFI/Nr2f1 exerts an anti-astrogliogenic function in adult mouse hippocampal NSCs/progenitors enabling adult neurogenesis

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In the adult hippocampal dentate gyrus (DG), radial glial-like neural stem cells (NSCs) are multipotent (generating both neurons and astrocytes) while progenitors are fate-restricted to the neuronal lineage. Despite the importance of a tight control of neurogenic versus astrogliogenic potential, the underlying transcriptional program is still largely unknown. In this study, we found that a large subset of NSCs/progenitors co-expressed the chicken ovalbumine upstream promoter-transcription factor I (COUP-TFI, also known as Nr2f1) in the healthy DG, whereas neuroinflammation led to its downregulation. By combining inducible knockouts to fate mapping approaches we showed that COUP-TFI deletion from adult DG NSCs reduced neurogenesis and increased astrocyte production likely by inducing the pro-astrogliogenic factor NFIA. Remarkably, this shift also occurred upon COUP-TFI loss by retroviral targeting of mitotic progenitors, indicating that these cells might still be bipotent and need COUP-TFI to limit their potential to the neuronal fate. Moreover, complementary experiments clearly demonstrated that COUP-TFI overexpression abolished the production of new astrocytes under physiological conditions and was sufficient to abate the inflammation-induced gain in astrogliogenesis and to restore proper neurogenesis levels, thus revealing a crucial function for COUP-TFI in protecting the DG niche from inflammatory insults. Finally, downregulation of COUP-TFI takes place within the mouse hippocampal niche early during physiological aging concomitantly with the drop in neurogenesis, further supporting COUP-TFI as a central regulator of the adult DG neurogenic niche.

NP06 | SNARE Complex Polymorphisms Associate with Alterations of Visual Selective Attention and mRNA expression in Alzheimer's Disease

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The SNARE complex plays a crucial role in the synaptic exocytosis of neurotransmitters, a process involved in Alzheimer's Disease (AD). SNAP-25 and STX1a are the core proteins of the SNARE complex, and single nucleotide polymorphisms (SNPs) in SNARE complex genes, as well as altered gene expression, in particular of SNAP-25, were shown to associate with neurological diseases and cognitive impairments. We evaluated the possible involvement of *STX1a* and *SNAP-25* SNPs in disease risk. SNPs distribution was analyzed in 192 AD, 187 mild cognitive impairment (MCI) and 200 healthy controls (HC), and it was correlated with cognitive impairment in a subgroup of 90 AD and 70 MCI patients. Correlations between *STX1a* SNPs and gene expression, measured by qRT-PCR platform QuantStudio® 12K OpenArray in PBMCs, were evaluated as well in 71 AD and 70 HC individuals. *SNAP-25* rs363050 genotype distribution was statistically different in AD and MCI compared to HC ($p=1.5x10^{-4}$ and $p=8.7x10^{-3}$ respectively). Similarly, *STX1a* rs4717806 genotype distribution was significantly skewed in AD *vs.* HC (p=0.032). Distribution of the SNARE complex SNPs combination (*SNAP-25/STX1a* rs363050/rs1747806) significantly differed in MCI compared to HC (p=0.018). A major impairment of visual selective attention was observed in *STX1a* rs4717806 AA ($p_c=0.027$) genotype carriers as well as in MCI subjects carrying *SNAP-25/STX1a* rs363050/rs1747806 AA/A combination ($p_c=0.022$). STX1a gene expression was decreased in AD compared to HC (p=0.055), and in AD patients carrying the STX1a rs1747806 TT genotype compared to HC carrying the same genotype (p=0.028). Finally, AD patients carrying rs4717806 AA genotype were characterized by a higher STX1a expression compared to those carrying AT (p=0.003) and those carrying TT (p=0.007). These results suggest that rs363050 and rs1747806 SNPs may influence the activity of the SNARE complex gene expression, resulting in impairments of attention brain areas.

NP07 | Changes in neuronal activity affect vesicular positioning at cortical synapses

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Networks' hyperexcitability is often caused by an unbalance between excitatory and inhibitory neurotransmission, manifested in patients as a propensity for epileptic seizures. While much is known about the causes of some forms of epilepsy, plastic rearrangements that maintain the epileptic focus and their effects are only partly understood. Here we focus on functional and structural changes in vesicular array at cortical synapses of mice injected with tetanus neurotoxin (TeNT) in the visual cortex. Using an ultrastructural readout of in vivo activity, we investigated the positioning of synaptic vesicles released in the visual cortex in response to visual stimulation at two different stages (10 and 45 days) after toxin injection. The nanoscale analyses show that the proportion of activated synaptic vesicles at excitatory synapses is unchanged in TeNT-injected mice, but the positioning of such vesicles is no longer biased towards the active zone as happens in control animals. Moreover, in TeNT-injected mice we observe a longer active zones at inhibitory synapses. Proteomic analysis also revealed an up-regulation of specific proteins (i.e. Carboxypeptidase E, Synaptotagmin V, Dickkopf 3 Protein 3 and Secretogranin I) involved in synaptic vesicles' availability and biosynthesis of neurotransmitter dense-core secretory vesicles. The data suggest that the presynaptic remodelling could represent the base of hyperexcitability maintenance.

NP08 | DAT atypical inhibitors as novel antipsychotic drugs

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Despite its classification as a psychiatric disease, schizophrenia is both a behavioral and a biological disorder resulting in neurocognitive dysfunction. Social and economic costs of schizophrenia are extremely high compared to its incidence and prevalence, however, due to a heterogeneous pattern of brain pathology and symptoms and to an unknown etiology, developing an effective treatment has been really challenging. Among the many neurochemical hypothesis, the dysregulation of dopaminergic neurotransmission has been considered as a central dogma of schizophrenia over the last few decades. In fact, patients with this pathology exhibit increased dopamine (DA) synthesis and release in the striatum which seems to correlate with positive symptoms and moreover, most of the effective antipsychotic drugs (APDs) are D2-receptor antagonists. Unfortunately, chronic treatment with APDs is associated with the induction of extrapyramidal side effects (EPS). In order to identify new possible APDs with a novel mechanism of action and potentially less EPS we tested 3 different compounds generated from the structural modification of vanoxerine (or GBR12909), a known atypical inhibitor of the presynaptic DA transporter (DAT) with cocaine-like activity but cardiotoxic properties that have precluded its clinical use. Preliminary *in vitro* studies showed that DAhLIs (DAT atypical inhibitors) are able to bind to DAT and inhibit DA reuptake. Additionally, our *in vivo* results showed that DAhLI i) have putative central effects, ii), unlike vanoxerine, reduce novelty-induced locomotor activity, and iii) counteract cocaine stimulating effects, suggesting that DAhLI may potentiate DA reuptake via DAT. These compounds may provide a way to reduce DA extracellular levels and DA neurotransmission with a selective action on active DA synapses, thus with reduced EPS typical of D2 antagonists, representing a new promising class of presynaptic APDs.

NP09 | Metabolic changes influence brain plasticity in mice

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It is becoming clear that diet and lifestyle can affect brain physiology. In spite of the well-known effects of specific diets' on the outcome of several neurological diseases, little is known about how metabolism modulates neural function. For instance, fasting and ketogenic diet (KD) control seizures in epileptic kids, although how fasting and ketone bodies' affect brain physiology, and the molecular mechanisms involved, are still enigmatic. Here, we investigate how a specific metabolic challenge: i.e. fasting, could influence neural physiology and plasticity in mouse models. Since the visual system is probably the deepest understood system of the human brain and a classic model to study experience-dependent plasticity in rodents, we focused on the visual system to assess 48 hours (h) fasting impact on neural function and plasticity in mice, and to analyze the molecular/epigenetic adaptation to this metabolic challenges. Using intrinsic optical signal imaging, we found that ocular dominance(OD) plasticity was enhanced in adult mice after 48h fasting simultaneously to 2 days of monocular deprivation. In critical period (CP) mice undergoing the same protocol, OD plasticity was not affected by 48h fasting. However, following a deeper analysis of visual responses to different spatial frequencies, we observed specific changes correlating with alterations in blood glucose concentration. To further investigate the effect of 48h fasting on mouse general activity along the 12h light:12h dark cycle, locomotor activity was assessed both in juvenile and in adult mice. As expected, we observed increase in general activity in both young and adult mice during the fasting period. To look inside the molecular mechanisms underlying fasting-driven plasticity we performed a RNA-seq on the visual cortex of CP mice. Strikingly, a large set of genes was differentially expressed in the fasting group compared to ad libitum fed control animals, Moreover, significant alterations in gene expression was also detected in adult mice subjected to fasting. In particular, plasticity related genes, like *Npas4, Bdnf, Arc,* were increased after fasting. Finally, since fasting is able to increase beta-hydroxyl-butyrate (BHB) plasma concentration, and BHB is a new epigenetic factor, we analysed the new post-translational modification K9-beta-hydroxyl-butyrylation (bhb) on histone H3. ChIP-seq revealed a significant enrichment of H3K9-bhb in promoter and enhancer regions of genes upregulated in the fasting group compared to control animals. In summary, our data suggest that fasting is able to affect brain physiology and, particularly, to modify plasticity level in the visual cortex through different mechanisms still under-investigation and probably involving BHB-driven molecular changes.

NP10 | Greater occurrence of “immature” neurons in mammals with expanded neocortex

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Cortical immature neurons (INs) were discovered in the rodent piriform cortex. They are generated pre-natally, then continuing to express markers of immaturity (e.g., doublecortin – DCX - and PSA-NCAM), thus representing a possible reservoir of young cells in the adult brain. Since they are restricted to the paleocortex of rodents but extend into neocortex in some mammals, the hypothesis has been made that INs might be more important in large-brained animals characterized by a decline of adult neurogenesis. We collected brains from 12 mammals belonging to 8 orders, endowed with different neuroanatomy and lifespan. Occurrence of INs in paleo- and neo-cortex, their morphology (type 1, small-bipolar; type 2 cells, large-ramified), and amount (linear density: cells/mm of cortical layer II) were evaluated at 4 comparable brain levels in 4 individuals/species. Cell proliferation and immaturity/maturity markers were employed. While morphology and phenotypic features were rather constant, the topographical extension and relative amount of INs highly varied among animal species. Their occurrence increased in the neocortex of non-rodent species, particularly in gyrencephalic brains. Especially their amount (linear densities) appeared higher in neocortex of mammals with a large, gyrencephalic brain (an order of magnitude from mice to chimpanzee). In addition, the StereoInvestigator software was used to estimate the number of granule cells, DCX+ and Ki-67+ cells in 3 adjacent sections (of corresponding levels) of the dentate gyrus (rabbit, cat, sheep, and mouse). Preliminary data indicate that the number of DCX+ cells is quite higher with respect to proliferating cells (from 40- to 140-fold) in sheep, cat and rabbit, while this ratio is around 3 in mouse, which is typically endowed with high rates of adult neurogenesis. Hence, INs can have higher relevance in mammals with expanded neocortex but they might also accumulate in neurogenic niches due to slow, protracted maturation.

NP11 | Post-trascriptional control of gene expression by Foxg1

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Foxg1 is a transcription factor gene that plays pleiotropic roles in telencephalic development ranging from neural precursor proliferation to neuronal and astroglial differentiation. In addition to its canonical function of transcriptional regulator, recent experimental evidence suggests that Foxg1 might also exert post-transcriptional control of gene expression, to date unknown. It has been showed that Foxg1 is localized in the cytoplasm, in mitochondria, and it interacts with non-nuclear factors, mainly involved in mRNA translation (e.g. EEF1G, EEF1D, and PUM1). For these reasons, we investigated a possible Foxg1 involvement in translation control. This subject was studied on primary cultures of cortico-cerebral neurons engineered by lentiviral transgenesis and Tet-ON technologies. We selected a small set of genes likely to undergo translation regulation, and we measured the ribosomal recruitment of their mRNAs under Foxg1 overexpression. For this purpose, we run Translating Ribosome Affinity Purification (TRAP) assay, comparing the ribosome-associated mRNA fraction (IP) with the "free" cellular remaining fraction (SN) of candidate mRNAs. TRAP results showed that the IP/SN ratio of a subset of these genes is upregulated, suggesting that Foxg1 specifically enhanced ribosomal recruitment of their mRNAs. To cast light on molecular mechanisms underlying Foxg1 impact on translation, we evaluated physical interaction of Foxg1 with the initiation translation factor eIF4E, via Co-Immunoprecipitation assay, and with candidate mRNAs, by RNA-immunoprecipitation (RIP). Mutations of *FOXG1* human gene lead to severe mental retardation, pointing to a key role of Foxg1 in human cognitive functions. In this respect, we are currently assaying the biological impact of Foxg1 on translation in the context of neural activity, by molecular and live-imaging approaches.

NP12 | Gas7 is a direct target of miR-125a-3p and a new player in oligodendrocyte maturation

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Multiple sclerosis (MS) is a chronic immune-mediated demyelinating disease in which immune system attacks myelin, a fatty substance produced by oligodendrocytes, leading to abnormal transmission of nerve impulses. To become myelin-producing cells, oligodendrocyte precursors (OPCs) follow a very precise maturation process, finely regulated by intrinsic and extrinsic mechanisms. In this respect, we recently identified miR-125a-3p as a new regulator of OPC maturation, showing that its over-expression impairs, whereas its inhibition stimulates this process. Here, by using a combined transcriptomic and bioinformatic approach, we identified Gas7, a cytosolic factor known to be involved in neuron morphological differentiation, as new miR-125a-3p direct target and demonstrated that its silencing contributes to the reduction of MBP expression, suggesting a new regulatory mechanism in OPC maturation and the involvement of GAS7 in oligodendrocyte terminal maturation. We also showed that the expression of Gas7 increases during oligodendrocyte maturation and decreases in corpus callosum after lysolecithin-induced demyelination, a mouse model of MS. Interestingly, a significant correlation between Gas7 and miR-125a-3p expression levels was found in the normal appearing white matter of MS patients, but this correlation was not observed in the active lesions, suggesting that the pathological environment may interfere with this homeostatic regulation. The identification of miR-125a-3p direct target will contribute to shed light on the complex molecular mechanisms underlying de/re-myelination processes. Moreover, our results suggest that alteration in Gas7 expression may contribute to impair remyelination in demyelinating diseases. Sponsored by Fondazione Cariplo, grant n° 2014-1207 to DL.

NP13 | The effect of ageing on the spatial distribution of glycogen in Layer I somatosensory cortex of mice

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Astrocytes are the most abundant type of glial cell in the brain. Their most characterized role is the support of neuronal metabolism, to maintain the proper conditions for efficient neuronal function. Glucose, an important source of energy for the brain, access the neuropil across the blood brain barrier (BBB) and then is transported into astrocytes through their perivascular endfeet, where it can be stored as glycogen. Lactate can be synthetized through glycogenolysis, and then shuttled via monocarboxylate transporters (MCTs) to neurons to fuel their TCA cycle. This mechanism is known as astrocyte – neuron lactate shuttle (ANLS), and is involved in learning and memory formation. With the use of the computational tool GLAM (Glycogen-derived Lactate Absorption Map) on 3D dense reconstructions from serial EM micrographs, we are able to infer a probability map of the locations where astrocytic glycogen-derived lactate is most likely accessing the surrounding neurites. Hence, in the present study we compare the glycogen distribution between adult (4 months old) and geriatric mice (24 months old). In order to understand whether ageing might affect such distribution, we analyzed and compared the probability maps on axons, dendrites boutons and spines, to make functional hypothesis about single compartments energy consumption. Preliminary observations points to the fact that aging brains have a more glycolytic metabolism, with less peaks facing mitochondria, and smaller glycogen granules.

NP14 | Neural Plasticity in First-Time Mothers: a neuroimaging perspective

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FUNDACION PARA LA INVESTIGACION BIOMEDICA DEL HOSPITAL GREGORIO MARAÑÓN, Laboratorio de Imagen Médica, Madrid, Spain[1]

Neuroimaging researchers commonly assume that the brain of a mother is comparable to that of a nulliparous women. However, motherhood is a life-changing event that implies dramatic adaptations in the structure and function of all physiological systems, including the brain. Studies in animal models demonstrate that pregnancy and peripartum hormonal fluctuations facilitate the onset of maternal care, and trigger structural and functional changes in the so-called maternal brain. Results from neuroimaging studies in humans also indicate that pregnancy and motherhood modifies the function and structure of reward and social cognition circuits and that these changes are associated with maternal care. Specifically, results suggest that global brain volume decreases during pregnancy with an increase in brain volume after delivery. A study leadered by our group observed gray matter volume reductions in primiparous women scanned before and after pregnancy. Reductions affected a network of regions that support social cognition, and predicted levels of mother-to-child attachment, suggesting this is an adaptive process that facilitates the transition into motherhood. The authors proposed that pregnancy and peripartum periods represent sensitive periods during which hormonal priming triggers an augmented state of neuroplasticity. We recently demonstrated that the magnitude of the morphological change in these mothers were on par with the changes observed in female adolescents during the pubertal transition, suggesting the brain morphometric changes associated with these two periods reflect similar hormonally primed biological processes. However, the effect of human pregnancy on brain function and connectivity, the biological mediating factors and its implication for the onset of mental postpartum disorders, are still unknown. Our research group's objective is to set up multimodal neuroimaging and hormonal studies that are aimed to understand the neural basis underlying pregnancy and parturition.

NP15 | Neuromorphological and hormonal correlates of paternal behavior

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The set of features characterizing paternal behavior is retained to play a crucial role in the offspring development, although the biological and anatomical correlates underlying paternal experience are still unclear. The information on the effects that just the pregnancy (and not the successive direct contact with pups) has on fathers' brains is even more fleeting. This state of art induced us to investigate the presence of neuromorphological and hormonal changes related to becoming fathers. Male mice (Thy1-YFP) were divided in 4 experimental groups: subjects housed with a pregnant female and living with the pups until the 6th post-natal day; subjects housed with a pregnant female until delivery and having no direct contact with pups; subjects paired with an ovariectomized female unable thus to get pregnant; subjects paired with another male. All animals were submitted to Sociability Test with pups (STp), to evaluate the response to an unfamiliar pup. In the same animals we analyzed the dendritic morphology of pyramidal neurons of hippocampus and prelimbic (PrL) cortex. Furthermore, the concentrations of vasopressin in the males' serum were measured. At behavioral level, the animals that lived with pups or were exposed only to the pregnancy showed enhanced approach behaviors toward the pup in STp. Surprisingly, compared to the other groups, mice exposed only to the pregnancy displayed more complex dendritic arborizations of pyramidal neurons in PrL cortex. Furthermore, the levels of vasopressin were higher in the subjects that lived with pups, compared to the other groups. The results suggest that the experience of living with pups induced approach behaviors toward pup. This approach behaviors were showed also by mice have been living just the pregnancy, without having ever seen a pup. This behavioral pattern was paralleled by increased processes of neural plasticity in the PrL pyramidal neurons, correlate that supports and prepares the subjects to become fathers.

NP16 | Role of GPR83 in stress response, cocaine addiction and motivation for food

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The G protein receptor 83 (GPR83), a recently deorphanized receptor for the abundant neuropeptide PEN, is highly expressed in brain regions involved in control of metabolism, learning, stress, anxiety and reward. Recently it was showed high GPR83 expression on cholinergic interneurons in the nucleus accumbens and moderate expression on ventral tegmental area dopamine neurons. To investigate its role in the brain we characterized mice lacking the GPR83, performing behavioral experiments. The behavioral results in GPR83 knockout mice suggest an involvement of the GPR83 in the modulation of behavioral effects related to stress response in a novel environment, cocaine addiction and motivation for food. Additionally, experiments on the central effect of the specific GPR83 ligand PEN suggest an involvement of GPR83 in anxiety-related behaviors. Previous histological and present behavioral data suggest a major influence of GPR83 transmission on the mesostriatal dopaminergic system. Future experiments are required to define the involvement of specific GPR83 expressing neuronal populations in specific behaviors.

NP17 | Characterization of a new conditional PCDH19 KO mouse model to understand the pathophysiology of PCDH19-related epilepsy

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PCDH19-related epilepsy is a debilitating neurological condition characterized by early-onset seizures, intellectual disability and autism. It is due to mutations in the X-chromosome gene *PCDH19* that encodes for protocadherin-19 (PCDH19). PCDH19 is a calcium-dependent cell-cell adhesion molecule of the cadherin superfamily, which is highly expressed in the cortex and in the hippocampus. PCDH19-related epilepsy is considered a particular X-linked disorder due to its mechanism of inheritance, as it affects females while sparing males, with the exception of somatic mosaic males. A cellular interference mechanism has been hypothesized: the coexistence of two neuronal populations (PCDH19-negative cells and PCDH19-positive cells), which are unable to communicate properly. By exploiting the Cre-LoxP technology, our laboratory has generated a conditional knock out (KO) model for PCDH19 (PCDH19 floxed) to study the pathophysiological mechanisms behind the disorder and validate the cellular interference model. The breeding of PCDH19 floxed mice with hSyn1-Cre mice generated PCDH19 KO mice with a mosaic expression of PCDH19 in their brains. Interestingly, female KO mice displayed a delayed growth compared to their wild-type (WT) littermates during postnatal day (P) 21 and P35. Considering that PCDH19-FE patients suffer from epilepsy during infancy while seizure tend to decrease or even disappear during adolescence, this time window might represent a critical period in which to investigate hyperexcitability and higher susceptibility to seizures in mice. No alteration in the expression of synaptic markers were noticed in mosaic KO mice compared to WT controls, with the exception of a reduction of GABA(A)R a1 subunit expression in the forebrain, confirming the interplay between PCDH19 and GABA(A)R recently reported in Bassani et al., 2018. Ongoing experiments aim at characterizing PCDH19 mosaic mice from a morphological, functional and behavioural point of view.

NP18 | Alteration of cognitive function and cortical glutamatergic mechanisms in an experimental model of anorexia nervosa

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Anorexia nervosa (AN) is a complex mental illness characterized by restricted eating and strenuous exercise regimens. Despite the high incidence and mortality rate in adolescent female the etiopathogenesis remains unclear. Our hypothesis is that the AN-induced imbalance in energy intake through maladaptive plasticity in the medial prefrontal cortex (mPFC) might impair cognitive abilities and alter incentive motivational system driving weight loss seeking. Female adolescent rats at postnatal day (P) 35 were individually housed and divided in 2 groups: controls (CTRL, food ad libitum–sedentary) and ABA (food restricted-exercise). On P38, ABA rats were food-restricted (2h/d) till P42. A group of animals were sacrificed when the anorexic phenotype was reached (P42) and another group after 7-days of body weight recovery (P49). After 24 hours of AN induction, ABA rats reduced body weight and constantly increased wheel activity over days in the mPFC of ABA rats at P42, as expected. At structural level, we found a reduction in the density of dendritic spines, while no changes were observed in critical determinants of glutamate homeostasis, measured as expression levels of NMDA and AMPA receptor subunits. Seven days of recovery restored body weight of ABA rats and, in the mPFC, it reduced filopodia and increased mushroom-shaped spines, i.e. the mature dendritic spines. This effect was coupled with an overall reduction of the glutamatergic signaling in the cortical post-synaptic density. Compared to CTRL, ABA rats exhibited a cognitive deficit in the temporal order object recognition test test at both time points. The herein shown AN-induced dysregulation of the excitatory signaling coupled with an altered structural plasticity lead, in turn, to cognitive dysfunction, consistently observed in AN patients, that might be the trigger for the motivational mechanisms underlying AN.

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NP19 | The neuroanatomo-functional correlates of paternal behavior

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Experimental and human studies on attachment have been mainly focused on maternal bond, identifying changes in the mothers' brain and body, especially starting from gestation. And yet, recently human researches showed the significant role that the fathers can play in the child development. The involvement of several brain regions that modulate paternal behavior has been demonstrated, thus confirming that the experience of paternity has effect on the fathers' brains. The aim of the present study was to verify the behavioral and anatomo-functional effects of becoming fathers to prove if these effects were mediated by the paternal experience with the offspring or by the only experience of living the pregnancy. We used a transgenic mouse model expressing Thy1-YFP. We compared males housed with another male (*No F* group), or mated with a female with which they remained until before (*F* group) or six days after the birth of the litter (*F+P* group) or with an ovariectomized female (*Partner Ov* group). We analyzed behavioral responses towards an unfamiliar pup through the Sociability test and subsequently we evaluated c-Fos gene activation and expression in amygdala, medial prefrontal cortex and hippocampus by using immunofluorescence techniques. Compared to *No F* and *Partner Ov* groups, *F+P* and *F* groups showed greater interest towards the pup, spending significantly more time in pup compartment, more contact times and frequencies with pup in respect to empty compartment. Interestingly, while *F+P* group showed activation of c-Fos$^+$ cells only in the amygdala, *F* group showed significantly increased c-Fos$^+$ cells activation in the amygdala, medial prefrontal cortex and hippocampus. As for *F* group, the pup has become a salient stimulus although *F* fathers have never come into direct contact with pups. These results suggest that is the experience of living pregnancy the crucial factor that predisposes to becoming father.

NP20 | Electrophysiological and biochemical characterization of Tph2 transgenic mouse model

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In Parkinson's Disease (PD) animal models, serotonin terminals establish a compensative mechanism by releasing dopamine to counteract the nigrostriatal dopaminergic degeneration. Serotoninergic neurons can convert Levodopa (L-DOPA) into dopamine but they cannot manage its reuptake and degradation, bringing to abnormal dopaminergic receptors stimulation and altered glutamatergic transmission. In our study we used a transgenic mouse model displaying lack of 5-HT synthesis (Tph2-/-), while retaining an intact serotonergic innervation. We analyzed the role of serotonergic raphe-striatal innervation by studying the alterations of intrinsic membrane properties and synaptic plasticity (long-term depression, LTD; long-term potentiation, LTP) of striatal projection neurons (SPNs) in mice with normal content (Wt), partial (Het) and total absence (KO) of 5-HT synthesis, through whole-cell patch clamp and sharp recordings. From our results, if on one side both gender of the three genotypes expressed LTD, on the other side only SPNs of KO-/- did not display LTP but rather LTD. Another important data has been the different responses of the SPNs to the low-frequency stimulation (LFS) protocol depending on the gender of the animals. The SPNs of the Het-/- female mice did not show the ability to reverse LTP after LFS application compared to those of Het-/- male mice that expressed the depotentiation of the synapses. Furthermore, the amperometric results confirmed a gender diversity: Het-/- female mice showed a higher dopamine release compared to Het-/- male mice, also when exogenous 5-HT was added. The lack of depotentiation and increased dopamine release levels in Het-/- female mice could represent a good indication of synaptic mechanisms underlying the more inclination to develop L-DOPA-induced dyskinesia compared to male gender. These data suggest that the serotonergic system is necessary for intact striatal function, confirming then its role in the corticostriatal plasticity.

NP21 | Anticipated expression of D-aspartate oxidase since embryonic stage drastically reduces D-aspartate levels in the mouse brain and influences spatial memory at adulthood

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Together with D-serine (D-Ser), D-aspartate (D-Asp) is the only free D-amino acid present in substantial amount in the mammalian brain. Several studies revealed that D-Asp has a transient emergence in the brain, being abundant in the embryonic phase and in the first post-natal days before significantly decreasing thereafter. It has long been established that D-aspartate oxidase (DDO) is the enzyme responsible for D-Asp catabolism. Accordingly, the post-natal decrease of D-Asp content is associated with a concomitant, progressive increase in *Ddo* expression and DDO activity in the rodent's brain. D-Asp exists at extracellular level and acts as an agonist at NMDA and mGlu5 receptors (NMDAR and mGluR5, respectively). Adult mice with abnormally high cerebral D-Asp levels, obtained by targeted deletion of the *Ddo* gene (*Ddo*$^{-/-}$) or exogenous administration of D-Asp, showed increased NMDAR-dependent Long-Term Potentiation (LTP), dendritic length and spine density. To clarify the still enigmatic biological meaning of D-Asp during brain development, we have generated a knockin mouse model in which the expression of DDO is anticipated since the zygotic stage. As a proof of the successful achievement of our gene targeting strategy, we found an increased transcription of cerebral *Ddo* in knockin mice during ontogenesis. As a result of this, we observed a selective depletion of cerebral D-Asp content in the embryonic and postnatal brain. Then, we performed a morphological and behavioural characterization of *Ddo* knockin mice. Histological analysis revealed no gross differences in brain size or structural organization. On the other hand, we observed a significant increase of parvalbumin-positive interneurons in the medial prefrontal cortex of mutant mice, which was accompanied by increased spatial memory in the Morris water maze task.

NP22 | HuR's interaction with lincBRN1a and lincBRN1b is implicated in neuronal stem cells differentiation

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LncRNAs play crucial roles in cellular processes but their regulatory effects in the adult brain and neural stem cells (NSCs) remain to be entirely characterized. Interestingly, it has been shown that genetic ablation of specific lncRNAs can cause strong impairments in mouse brain's development. Here, we demonstrate that 10 lncRNAs (LincENC1, FABL, lincp21, HAUNT, PERIL, lincBRN1a, lincBRN1b, HOTTIP, TUG1 and FENDRR) are deregulated during murine NSCs differentiation. By RNA immunoprecipitation assay we show that they interact with the RNA binding protein ELAVL1/HuR. Furthermore, we characterized the function of two of the deregulated lncRNAs, lincBRN1a and lincBRN1b, during NSCs' differentiation. Their inhibition obtained by siRNA approach leads to the induction of differentiation, with a concomitant decrease in stemness and increase in neuronal markers, indicating that they exert key functions in neuronal cells differentiation. The treatment with the transcriptional inhibitor actinomycin D allowed the evaluation of the half-lives of these RNA molecules. Moreover, we found that the HuR's inhibition, by siRNA or by using the specific pharmacological inhibitor dihydrotanshinone I, leads to the modification of lincBRN1a and lincBRN1b's decay rates both in NSCs and in differentiated cells. We also identified six human homologs of the ten lncRNAs studied in mice and we report their deregulation during human iPSCs differentiation into neurons. Our results show that lincBRN1a and lincBRN1b play a role in NSCs biology influencing their differentiation capabilities, as the alteration of their levels may have an effect on this process. Moreover, we report that the inhibition of HuR's interaction with the analyzed lncRNAs leads to neural differentiation, suggesting a complementary role for the lncRNAs and HuR in stemness. The study of their expression in human iPSCs differentiation into neurons suggest that our results could also be applied to human neuronal development.

NP23 | Investigation of microglia-mediated synapse remodeling

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Microglia are the immune cells of the brain. Besides their immunity role, microglia also orchestrate several processes, critical for proper neuronal functioning. In the healthy brain, microglia are implicated in a series of physiological tasks, ranging from removal of neuronal debris to precise refinement of synaptic terminals, contributing to maturation of neural circuits. Microglia are very dynamic, constantly engaged in physical contacts with neighboring cells, and, specifically, with synaptic structures. Experimental evidence indicates that such close contacts of microglia with synapses are driven by synaptic activity, and are thought to be essential for microglia-mediated synapse remodeling. Defects in microglial activity have been linked to synaptic impairments associated with neurodevelopmental and neurodegenerative disorders. To date, modulations of microglia-synapse interactions are only partially known, and microglia involvement in brain homeostasis is underestimated. Thus, analyzing such interactions is of major importance to understand how microglia mediate synapse remodeling. To this end, this problem will be tackled from three angles. Firstly, we will use light sheet microscopy on organotypic slices prepared from microglial and neuronal specific reporter mouse lines to decipher their physical interactions, helping to unveil microglia specific behavior toward synapses. Secondly, electrophysiological recordings will assess the outcome of microglial fine-tuning on the synaptic network. Finally, synaptosomes and synaptic materials engulfed by microglia will be analyzed and measured to characterize their molecular synaptic composition. The comprehension of this major synaptic-remodeling process will open new perspectives in the understanding of brain development and its proper functioning. Moreover, in a close future, these discoveries could help to better understand synaptic impairments, as found in the autistic spectrum disorder or in Alzheimer's disease.

NP24 | Audiogenic kindling alters functional activity of the hypothalamic-pituitary-adrenal axis in Krushinsky-Molodkina rats

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Epilepsy is one of the most common neurological disorders. In the past years, numerous neuropeptides including hormones of the hypothalamic-pituitary-adrenal (HPA) axis have been linked to epilepsy. Aberrant levels of stress hormones were observed in blood plasma from epilepsy patients. However, the mechanisms contributing to the dysregulation of the HPA axis associated with epilepsy remain unknown. Object of this study was Krushinsky-Molodkina (KM) rats, which demonstrate seizure in response to acoustic stimuli. Multiple audiogenic seizures in KM rats result in overspreading of epileptiform activity from brain stem structures to limbic structures and neocortex and formation of epileptic network. The phenomenon of spread of epileptic activity to forebrain is called audiogenic kindling and represents a model of limbic epilepsy. The aim of present work was to analyze changes in functional state and the mechanisms of HPA axis regulation in KM rats caused by audiogenic kindling. Audiogenic kindling resulted in a decrease of GAD67 and CRH immunoreactivity in the median eminence (ME) that was accompanied by a decrease activity of transcription factor CREB, level of POMC and ACTH in the anterior pituitary gland. ACTH level also was reduced in blood. However, blood level of corticosterone and weight of the adrenal glands were increased after audiogenic kindling. We supposed that audiogenic kindling decreased CRH secretion into the hypothalamic-hypophyseal portal circulation, which can be caused by a decline in the excitatory action of GABA on ME, and/or a decrease in POMC transcription and ACTH secretion in the systemic blood flow, while activity of the adrenal glands were increased.

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NP25 | Novel Role of ATR in the Central Nervous System

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The ATR (ATM and Rad3-related) protein is a serine/threonine protein kinase that belongs to the phosphatidylinositol 3-kinase superfamily and it is largely known for its function as DNA repair protein. Indeed, in undifferentiated cells ATR activates in response to genomic stress and, in particular, upon single strand breaks. More recently, it has been discovered that ATR plays fundamental functions also in postmitotic neurons where it regulates the synaptic vesicles recycling. In line with these findings, we were interested in understanding if ATR may be involved in the correct development of the nervous system and in the control of neuronal plasticity. To address this issue, we treated cultured hippocampal neurons with a selective blocker of the ATR kinase activity. Following the treatment, we first analysed the excitatory-to-inhibitory switch of GABA by Ca^{2+} imaging experiments in developing neurons. Secondly, we measured neuronal properties and plasticity by electrophysiology in mature cells. Ca^{2+} imaging data show that inhibition of ATR activity does not affect the development of the GABAergic system, as demonstrated by a comparable percentage of depolarizing neurons in response to a GABA stimulus in drug- and vehicle-treated cells. Electrophysiological and immunofluorescence experiments indicate that both acute and chronic treatments with the ATR blocker generate an increased GABA-mediated neurotransmission. Moreover, neuronal plasticity processes result affected by the chronic ATR blockade as indicated by analysis of long-term potentiation (LTP) and depression (LTD). Finally, from a molecular point of view, real time PCR data suggest that ATR inhibition is related to a reduced activation of Egr1, a transcription factor known to regulate memory processes. Thus, taken together, these results point to a selective role of ATR in the maintenance of neuronal functional properties, suggesting its possible involvement in psychiatric and/or neurological pathologies.

NP26 | Characterization of a synaptic SUMO2/3-ylome

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SUMOylation is a very dynamic post-translational modification that consists in the covalent but reversible conjugation of the Small Ubiquitin-like MOdifier (SUMO) protein to specific lysine residues of target proteins. SUMOylation regulates the activity, stability, subcellular localization and protein-protein interactions of its target proteins. Originally characterized as a nuclear protein modification, SUMOylation emerged as a key regulator of extranuclear functions. An increasing number of neuronal proteins have been shown to be SUMOylated and evidence for alterations in SUMOylation were reported in many neurological diseases. It's now clear that the SUMOylation is essential for the regulation of several neuronal processes such as neuronal excitability, post-synaptic differentiation as well as in synaptic transmission and plasticity. To understand the role of SUMOylation in health and diseases, it now requires a global screen of SUMOylated proteins. Using a specific SUMO2/3 antibody, we performed immunoprecipitation on synaptosomes to isolate endogenous SUMO2/3 -ylated synaptic proteins. By mass spectrometry approaches, we identified around 800 SUMOylated proteins at synapses. I validate the MS list performing immunoprecipitation using antibodies against 4 different target proteins and SUMO blot. Our data provide invaluable resources to globally assess the regulation of synaptic functions by SUMOylation. Interestingly, some of our top candidate are associated to neuronal diseases. Therefore, identifying SUMOylated proteins known to be involved in neurological disoders open up new perspectives to understand the link between SUMOylation and neuropathologies.

NP28 | Investigating brain development and neuronal circuit assembly in primary immunodeficiency WHIM syndrome models

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Chemokine signalling is essential for coordinated cell migration in multiple tissues and processes, and is known to govern cell positioning in time and space in both physiological and pathological context. In particular, the relevance of CxCl12/CxCr4 axis has been extensively characterized in leukocytes migration and homing as well as tumour growth and invasion. In addition to its role in the immune system, the Cxcl12/Cxcr4 system has been centrally implicated in the development of the central nervous system especially in the regulation of neuronal migration, in particular of GABAergic inhibitory cortical interneurons and cerebellar granule cells (purkinje cell). Numerous somatic and germline mutations (mainly nonsense and frameshift) have been identified to be linked to the onset of pathological conditions, like primary immunodeficiency. The WHIM syndrome represents indeed a rare form of juvenile immunodeficiency characterized by Warts, Hypogammaglobulinemia, recurrent Infections and Myelokathexis, and it is caused by inherited autosomal dominant mutations of the CXCR4 gene, which blocks the receptor internalization after Cxcl12 stimulation in myeloid cells, and determines the persistent activation of Cxcr4 receptor, making it hyperfunctional. Patients affected by WHIM are more susceptible to potentially life-threatening bacterial infections, and to a less extent, to viral infections. Despite the important role of Cxcr4-Cxcl12 signalling in physiological brain development, WHIM syndrome has been primarily considered for its devastating immunological signs, which can be faithfully recapitulated in the well-established WHIM (Cxcr4 mutant) animal models. In this project we aim at characterizing the effect of Cxcr4/Cxcl12 alterations in the pathological context of the disease by integrating cellular, molecular and behavioural characterization of the WHIM mouse model, in order to investigate its impact on the development of the cerebral cortex and the cerebellum. Our preliminary data suggest, indeed, that in WHIM embryos and early postnatal animals the development of the GABAergic cortical and cerebellar systems are impaired, at both molecular and morphological levels, with compromising long lasting effects on neuronal

wiring and animal socio-motor behaviours. It is compelling that very first neurological, morphological and psychological assessment of WHIM patients recently correlates with our experimental data in vivo, providing evidence for neuromotor and neuropsychological dysfunction, associated to cerebellar malformations in these patients. Thus, we aim at investigating the cellular and molecular mechanisms mediated by Cxcr4 that underlie such alterations, to first expand our understanding onto the neuronal phenotype – for long overlooked- of this rare and devastating disorder, and, ultimately exploit the animal model to shed light on novel therapeutic approaches for the treatment of WHIM syndrome in humans.

NP29 | Discovery and Characterization of Novel Selective NKCC1 Inhibitors for Down Syndrome, Autism and Brain Disorders with Depolarizing GABAergic Transmission

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Proper GABAergic transmission through Cl-permeable $GABA_A$ receptors is fundamental for physiological brain development and function. Indeed, defective GABAergic signaling -due to a high ratio of expression of the Cl importer NKCC1 and Cl exporter KCC2- has been implicated in several neurodevelopmental disorders, including Down syndrome (DS). Interestingly, NKCC1 inhibition by the FDA-approved diuretic bumetanide reverts long-term plasticity and cognitive deficits in the TS65Dn mouse models of DS and core symptoms of other brain disorders (e.g., autism) in a number of rodent models and/or clinical trials. However, the required chronic treatment with bumetanide is burdened by its diuretic side effects caused by the antagonism of the kidney Cl^- importer NKCC2, which leads to hypokalemia and jeopardizes drug compliance. Crucially, these issues would be solved by selective NKCC1 inhibitors, thus devoid of the diuretic effect. Starting from bumetanide's structure, we applied a computational ligand-based approach to design new molecular entities that we tested *in vitro* for their capacity to selectively block NKCC1. Among the 3 newly-identified and highly promising NKCC1 inhibitors, one showed excellent solubility and metabolic stability *in vitro.* Moreover, analysis of WT and Ts65Dn mice systemically treated with this NKCC1 inhibitor revealed no diuretic effect. Finally, chronic treatment with our novel, selective NKCC1 inhibitor was able to rescue cognitive deficits in Ts65Dn mice in four different memory tasks, and social impairments and repetitive behaviors in a mouse model of autism, with no major signs of toxicity. Thus, our selective NKCC1 inhibitor devoid of the diuretic effect could represent a suitable and solid therapeutic strategy for the treatment of Down syndrome, autism and all the brain disorders with depolarizing GABAergic transmission.

NP30 | Molecular mechanisms underlying stress resilience and vulnerability: a role for neuroplasticity and redox balance

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Although stress is the main environmental risk factor for psychiatric diseases such as major depressive disorder (MDD), adverse events do not always lead to psychopathologies and a high percentage of stressed-subjects is able to counteract the detrimental stress-impact by an active response. Accordingly, understanding the mechanisms underlying this ability -the resilience- may represent a useful approach to develop therapeutic strategies for stress-related disorders. On this base, the goal of our study was to investigate the molecular systems that may contribute to a resilient phenotype with respect to anhedonia, a core symptom of MDD. To this aim, we used the chronic mild stress paradigm in the rat to induce an anhedonic behavior in the majority of the stressed animals (70-80%, vulnerable group) and a physiological behavior in a lower percentage (20-30%, resilient group). Interestingly, the stress-resilient rats did not develop anhedonia even after an acute injection of lipopolysaccharide, an immune challenge that causes a depressive-like phenotype by activating the immune system. Since resilience should reflect an active process, we first analyzed if the vulnerable and resilient behaviors were associated with changes at translational level within the brain. In line with our hypothesis, we found a significant activation of the mTOR pathway, known to be involved in protein synthesis, in the dorsal hippocampus of resilient rats. This effect was paralleled by increased expression of mediators of neuroplasticity such as the neurotrophin BDNF, proteins crucial for synaptic structure/function i.e. PSD-95 and anti-oxidant mediators of the redox system i.e. the transcription factor NRF2, all systems/mechanisms known to be compromised in MDD. These data indicate an active role for neuroplasticity and antioxidant defense in resilience, supporting the potential of therapeutic strategies able to favor these systems in stress-related disorders.

NP31 | Inter-individual variability of short-term ocular dominance plasticity in human adults

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Recent studies have revealed an unexpected residual plastic potential of the adult visual cortex by demonstrating a form of short-term ocular dominance (OD) plasticity, which has been linked with GABAergic inhibitory signalling in the visual cortex. To quantify this phenomenon and gather insight into its inter-individual variability, we measured OD using binocular rivalry before and after 2-hour monocular deprivation (eye patching) in 35 human adults. All but two subjects showed the expected OD shift in favour of the deprived eye. Nearly 50% of the variance in this OD plasticity effect could be predicted from the dynamics of binocular rivalry before patching. More mixed percepts predict stronger OD plasticity, together with an interaction between the amount of mixed percepts and the rate of switch between eyes. We speculate that switch rate and mixed percepts reflect two types of inhibitory signals: specific inter-ocular inhibition (promoting binocular fusion, hence mixed percepts) and generally related to the stability of perceptual representations (promoting slower switch rates). Switch rate and mixed percepts are relatively stable characteristics of each individual; the unexplained portion of variance in OD plasticity leaves room of intra-individual differences, which has been suggested to arise from factors like physical exercise and metabolism.

NP32 | Optogenetic stimulations to promote the extinction of fear memories

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Only a minority of exposed-to-trauma individuals develops significant fear symptomatology, due to individual differences in regulating fear inhibition. In trauma-related disorders, the basolateral amygdala (BLA) reactivity is not regulated by the altered medial prefrontal cortex (mPFC) reactivity, preventing thus fear extinction. In animal studies, mice showing approach or avoidance behaviors are non-extinguishing phenotypes that exhibit potentiated mPFC and BLA neurotransmission. In this research, the effects of optogenetic stimulation were assessed in extinction-impaired mice. By using spontaneously extinguishing or extinction-impaired mice expressing Thy1-ChannelRhodopsin2 (Thy1-ChR2), behavioral and electrophysiological responses to fear extinction were evaluated in relation to *in vivo* optogenetic manipulations. Firstly, male Thy1-ChR2 mice were classified as spontaneously Non-Extinguishing (NEx) or Extinguishing (Ex) mice. Then, the mice were implanted with a guide cannula on right infralimbic (IL) or prelimbic (PL) mPFC. Next, the mice were tested in a Contextual Fear Conditioning (CFC) task with repetitive extinction sessions, in which they received optogenetic or sham stimulations. Cellular excitability and spontaneous excitatory postsynaptic currents of Thy1-ChR2-expressing mPFC neurons were recorded. When submitted to the optogenetic stimulation on IL mPFC, NEx mice extinguished the fear memories, and a normal excitatory mPFC neurotransmission was rescued. Conversely, Ex mice submitted to the optogenetic stimulation on PL mPFC did not extinguish the fear memories, and the excitatory mPFC neurotransmission was altered. When delivered to NEx mice, the optogenetic stimulation modified the BLA-mPFC loop made dysfunctional by trauma (CFC), promoting the fear extinction. In this framework, the individual differences in approach and avoidance behavior represent predictors of responses to fear, stress and anxiety.

NP33 | Modelling Moyamoya Angiopathy in vitro: from Italian patient's PBMCS to endothelial cells

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Background Moyamoya angiopathy (MA) is a rare and disabling cerebrovascular condition characterized by recurrent ischemic/hemorrhagic strokes. Surgical revascularization is the only available treatment to reduce the follow-up stroke risk. The pathogenesis is still largely unknown and an imbalance of vasculogenic and angiogenic mechanisms has been suggested. Circulating endothelial progenitor cells (cEPCs) were hypothesized to be involved in vascular remodelling of MA. It remains unclear whether cEPCs might be considered as a prognostic MA marker or a disease effect. Our aim is to characterize the level and function of cEPCs from MA patients.

Methods 18 healthy donors (HD), 10 unrelated controls (UNR) and a subgroup of 20 MA patients belonging to the wider MA patient-population of GEN-O-MA project, were included. For each patient, clinical and neuroradiological data and a blood sample were collected. cEPCs were isolated from whole blood as CD-45^{dim} CD34^{+}CD133^{+}mononuclear cells. Gene-expression (GE) analysis, functional assays (Tube Formation assay) and ELISA were also performed to characterize cEPCs.

Results Only MA adult, Caucasian, unoperated patients (85% females, mean age 47,3 ± 11,5) were included in our study. cEPC level was significantly reduced in MA patients as compared to HD (*p=0,038*). At early stage of endothelial differentiation, MA patient-EPCs showed an impaired vasculogenic capacity, an altered release of endothelial growth factors and an increased GE of CD31 and HGF in comparison to HD, without significant differences.

Conclusion Our findings suggest that cEPC level could represent a potential pathogenetic marker of MA. A better characterization of phenotype and function of MA-cEPCs is expected through a careful stratification of MA patients based on their clinical/neuroradiological profile. The validation of our results on a larger population and the correlation with clinical data could help our understanding of EPC function in MA.

NP34 | Active training promotes recovery of visual functions in adult amblyopic rats

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Amblyopia is the most diffused form of visual function impairment affecting one eye, with a prevalence of 1-5% in the world population. Amblyopia derives from an early imbalance between the two eyes, owing to anisometropia, strabismus, or congenital cataract, leading to severe deficits in visual acuity, contrast sensitivity and stereopsis. While amblyopia can be efficiently treated in children, it becomes irreversible in adults, because of the dramatic decline in visual cortex plasticity that occurs at the end of the critical period (CP). Recent evidence in animal models and in human patients have started to challenge this view, revealing the possibility to enhance plasticity in the adult visual cortex and to achieve substantial visual function recovery. We showed that two non-invasive active training procedures based on voluntary physical exercise or visual perceptual learning promote a marked recovery of visual acuity and visual depth perception ability in adult amblyopic rats, acting through a modulation of the GABAergic interneuron circuitry in the primary visual cortex.

NP35 | Pupillometry reveals perceptual differences that are tightly linked to autistic traits

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Autistic spectrum disorders are amongst the neurodevelopmental disorders with higher prevalence, as well as higher heterogeneity. This stresses the necessity for objective and quantitative indices to profile the individual patient along the many dimensions that may be altered. Autistic traits are commonly associated with altered perceptual styles, characterized by local detail-focused perception. We (Turi et al., eLife 2018) recently showed that pupillometry can provide an objective index of local/global perceptual styles when viewing a bistable rotating cylinder, and that the index correlates strongly with autistic traits (estimated by the Autistic Quotient: AQ). Here we combine this approach with dichoptic viewing to simplify the paradigm and eliminate the need for active reports of perceptual states, to make it user-friendly for young children and clinical populations. Participants viewed a structure-from-motion cylinder formed by a front and a rear surface of white and black dots. While in the original study the 3D perception was illusory and bistable, here we used disparity cues to define the front and rear surface, which switched every 5±0.5s. As with bistable motion, typical adults with high AQ scores showed strong pupil dilations or constrictions when the black or white dots were reported as front surface, whereas participants with low AQ showed no such oscillations. Pupil oscillations remained similar when participants watch the stimulus passively, without reporting the perceptual state. This simplified, report-free paradigm marks an important step towards applying pupillometry to measure perceptual styles in young and clinical populations, as suggested by preliminary data in young ASD participants and controls.

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NI01 | Effects of microglia-derived extracellular vesicles on GPR17-expressing oligodendrocyte precursor cells and post-stroke recovery

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Microglia, the resident immune cells of the central nervous system, acquire multiple activated phenotypes following brain injury, contributing to both detrimental and regenerative responses. A dualistic role of microglial cells has been associated with several neuropathological conditions, including ischemic stroke. In particular, during the early phase after brain ischemia 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 obtained from microglia on the subpopulation of GPR17-expressing OPCs. Briefly, middle cerebral artery occlusion has been performed in GPR17-iCreERT2: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 and post-stroke recovery. Results show that EVs derived from pro-regenerative microglia enhanced GFP$^+$-OPC maturation, ameliorated functional outcome and increased the number of microglial cells displaying a regenerative phenotype, unveiling their use as a promising tool to implement tissue repair. Elucidating EV molecular components responsible for these effects will set the basis to design engineered-EVs for therapeutic purposes.

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NI02 | Activation of the MET receptor as therapeutic tool in MS: a new neuroprotective mechanism involving the glutamatergic system

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Excitoxicity, caused by excessive glutamate levels, is one of the pathological mechanisms involved in the neuronal death associated to Multiple Sclerosis and neurodegenerative diseases. Glutamate is the main excitatory neurotransmitter in the brain and NMDA receptor (NMDAR) is one of the ionotropic glutamate receptors: NMDAR hyperactivation leads to an excessive Ca^{2+} influx, responsible of neuronal cell death. Hepatocyte Growth Factor (HGF) is a cytokine with prosurvival functions, which upon binding to its receptor Met protects against neuronal death. In this study we want to assess the role of HGF-Met in the protection from excitotoxicity. Fist, we aim to investigate the physical and functional association between Met and NMDARs. Second, we want to validate a monoclonal antibody activating the Met receptor (MetamAb) in substitution of HGF, as a new neuroprotective drug. Fura-2 imaging experiments and LDH release assay on cortical neurons showed that HGF led to a significant inhibition of NMDA-induced calcium influx and protected from neuronal death. As a mirror experiment, pharmacological inhibition of Met receptor potentiated calcium influx and excitotoxicity. Notably, MetamAb mimicked HGF in neuroprotection against excitotoxicity. Coimmunoprecipitation experiments showed that the Met receptor formed a complex with the GluN1 subunit of NMDAR in HEK293 cells stimulated with HGF. A Proximity Ligation Assay (PLA) will assess whether the Met receptor is close at a distance of a few nanometers to the NMDAR on the surface of HEK293. The dissection of the interaction and crosstalk between Met and NMDARs represents a new therapeutic strategy to target excitotoxicity in CNS.

NI03 | Defective miRNA-223-Mediated Regulation of NLRP3 Inflammasome Activation in Alzheimer's Disease

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IL-18, Caspase-1 and IL-1b release by immune cells is a predominant feature during NLRP3 inflammasome-complex activation. Although these cytokines have a beneficial role in promoting inflammation and eliminating infectious pathogens, in Alzheimer's Disease (AD) the involvement of inflammasome with a consequent constitutive inflammatory status contribute to disease progression and severity. The present study aimed to investigated the role of Stavudine (DT4), a commonly drug used as antiretroviral therapy, as inflammasome-inhibitor in PBMC of 10 AD patients and 10 sex-matched healthy controls (HC) cultured with Lypopolisaccaride (LPS)+Amyloid-beta (Ab_{42}) with/without D4T; in addition D4T effect to the transcriptional control of inflammasome gene expression microRNA-mediated was also evaluated. In particular mir-223 specificity was shown to targets the 3'UTR region of NLRP3 and prevents the accumulation of the protein and inhibits IL-1b production. Gene-expression of inflammasome pathway and mir-223 was evaluated by RT-PCR analysis; consequent cytokines and caspase-1production were detected by ELISA. Comparing AD *vs* HC, results shown increase of NLRP3, ASC, IL-1b, IL-18 gene expression and inflammatory cytokines, IL-18 and Il-1b and caspase-1 in LPS+Ab_{42} stimulated PBMC from AD patients ($p < 0.05$); the addition of D4T to cell cultures effects a down-regulation of both NLRP3 and all the genes related to the inflammasoma-complex and protein in AD and HC as well ($p < 0.005$ LPS+Ab_{42} *vs* LPS+Ab_{42}+D4T*)*. Also, D4T regulates mir-223 expression: in AD patients no significant results were obtained when PBMC were treated with stavudine, otherwise was shown an increase of miR-223 in HC (mean nFold LPS+ Ab_{42}: 0.816; LPS+ Ab_{42}+D4T: 1.022) suggesting a negative feedback that blocks the activation of the NLRP3 only in HC.

NI04 | The role mechanosensation in neuroinflammation and neurodegeneration of Alzheimer's disease

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A defining pathophysiological hallmark of Alzheimer's disease is the amyloid plaque; an extracellular deposit of aggregated fibrillar Aβ1-42 peptides. Amyloid plaques are hard, brittle structures scattered throughout the hippocampus and cerebral cortex. Reactive astrocytes and microglia envelop the exterior of amyloid plaques and infiltrate their inner core. Glia are highly mechanosensitive cells and can almost certainly sense the mismatch between the normally soft mechanical environment of the brain and very stiff amyloid plaques via mechanosensing ion channels. Piezo1, a non-selective cation channel, can translate extracellular mechanical forces to intracellular molecular signalling cascades through a process termed "mechanotransduction". Here, we utilised an ageing transgenic rat model of Alzheimer's disease (TgF344-AD) to study expression of mechanosensing Piezo1 in hippocampus and cortex of both wild-type and transgenic rats and reactive state of glial cells, and we observed that Piezo1 was upregulated in the reactive astrocytes around the amyloid plaques and this upregulation was increased with age. We also studied the effects of peripheral infection and neuroinflammation using a model of repeated urinary tract infections with *E.Coli*, a common comorbidity in elderly people with dementia. Interestingly, this inflammatory state caused further elevations of astrocytic Piezo1. Taken together, we report that ageing, Alzheimer's pathology and peripheral infection led to an increased expression of mechanosensing Piezo1 in the reactive astrocytes. Further research is required to investigate the role of astrocytic Piezo1 in the Alzheimer's brain, whether modulating channel opening will protect or exacerbate the disease state, and most importantly, if Piezo1 could prove to be a novel drug target for age-related dementia.

NI05 | Amniotic mesenchymal stromal cell secretome protects the brain from acute injury by modulating glial activation

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We have demonstrated that human amniotic mesenchymal stromal cells (hAMSC) protect the brain after traumatic injury (TBI) in mice. In an *in vitro* model, we demonstrated that hAMSC or their secretome exert similar protective effects after acute brain injury. Here we evaluate if hAMSC-secretome protects cerebral tissue from acute brain injury, both *in vitro* and *in vivo* models.
In vitro: organotypic brain slices from prefrontal cortex of newborn mice were injured by oxygen and glucose deprivation (OGD) for two hours. One hour after OGD, slices were cultured in culture medium (control), or with 50% hAMSC-secretome. Gene expression analysis at 48h after injury revealed that, compared to control slices, treatment with hAMSC-secretome: 1) rescued OGD-induced downregulation of the neuronal marker MAP2; 2) reduced the OGD-induced oxidative stress (downregulation of HO-1 and NQO1 expression); 3) polarized microglia activation toward a protective phenotype (upregulation of Ym1 expression); 4) reverted OGD-induced astrocyte activation and toxic polarization (downregulation of GFAP and Serping 1 expression).
In vivo: C57BL/6J male mice (8 weeks old) were subjected to sham or TBI followed by daily intraperitoneal injection of 150 µl of saline (control) or hAMSC-secretome starting 3 h after injury. Compared to control, TBI hAMSC-secretome treated mice show an early (from 1 week) and persistent (up to 5 weeks) improvement of sensorimotor function assessed by SNAP and neuroscore tests.
In conclusion, hAMSC-secretome protects the brain after acute injury. *In vitro* it modulates glial activation toward beneficial phenotype, mechanism that possibly contributes to the protective effects observed *in vivo*. These data support hAMSC-secretome as a cell-free therapeutic strategy for acute brain injury.

NI06 | New insights into DMF mechanism of action in experimental MS: miR-142-3p as key molecular target against inflammatory synaptopathy and motor disability

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Inflammation-driven synaptopathy includes excitotoxic dysfunctions of synaptic transmission contributing to motor and cognitive deficits in multiple sclerosis (MS) and its mouse model, experimental autoimmune encephalomyelitis (EAE). Since EAE/MS synaptopathy is precocious and potentially reversible, it represents an attractive therapeutic target for disease-modifying treatments, like the oral drug dimethyl fumarate (DMF). Here, we observed that *in vivo* therapeutic administrations of DMF or its active metabolite monomethyl fumarate (MMF), as well as e*x vivo* acute incubations of cerebellar slices with MMF, can ameliorate cerebellar glutamatergic transmission in EAE mice, exerting a neuroprotective action. At molecular level, we revealed that MMF reduces the expression of miR-142-3p, which we recently demonstrated to be a crucial mediator of the Il-1beta excitotoxic signal by inhibiting the level of the glial glutamate transporter GLAST/EAAT1. Interestingly, miR-142 knock-out mice are fully resistant to EAE induction, highlighting an important role for miR-142-3p also in the immune system. Furthermore, EAE miR-142 heterozygous (HE) mice are susceptible to EAE induction as the wild type (WT) littermates but, most importantly, they do not show any abnormalities in cerebellar glutamatergic currents, similarly to EAE mice receiving a miR-142-3p inhibitor. Such observation prompted us to assess the possible effects of DMF on EAE-miR-142 HE mice. Notably, we discovered a synergistic interaction between a preventive and peripheral DMF treatment and the genetic background of animals, based on a more effective improvement of motor disability in EAE-miR-142 HE mice than in WT mice. Altogether these results reveal for the first time that DMF or MMF exert neuroprotective and therapeutic effects by the inhibition of miR-142-3p in EAE and highlight miR-142-3p as promising molecular target in both the nervous and immune systems with relevant therapeutic implication for MS.

NI07 | Low doses of Perampanel protect striatal and hippocampal neurons against in vitro ischemia

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Energy depletion caused by ischemic brain insults may result in persistent neuronal depolarization accompanied by hyper-stimulation of glutamate ionotropic receptors and excitotoxic phenomena, possibly leading to cell death. Thus, glutamate receptor antagonists, such as the novel AMPARs antagonist Perampanel (PER), might be useful to counteract the excessive over-activation of glutamate receptors providing neuroprotective effects. Using electrophysiological and molecular analyses, we investigated the effect of PER against in vitro ischemia obtained by oxygen and glucose deprivation (OGD) in rat slices of two brain structures particularly sensitive to ischemic insults, the nucleus striatum and the hippocampus. We found that in these regions, while PER reduced the excitatory synaptic transmission without altering the electric membrane excitability, it was neuroprotective against OGD at doses that did not affect physiological long-term potentiation (LTP). Furthermore, in both the analysed regions, PER blocked the pathological form of LTP, namely ischemic LTP. Finally, we proved that the neuroprotective effect of PER against OGD was due to its capability to normalize the subunit expression and function of AMPAR altered after an ischemic insult. Taken together these findings support the idea that PER is a drug potentially effective to counteract *in vivo* ischemic damage.

NI08 | Hydroxychloroquine modulation of RNA:DNA hybrids in lymphoblasts derived from patients with Aicardi-Goutières syndrome

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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 (*TREX1, RNASEH2A, RNASEH2B, RNASEH2C, SAMHD1, ADAR1* and *IFIH1*) lead to the accumulation of endogenous DNA or RNA:DNA hybrids, which are recognized as foreign DNA and RNA of viral origin by the organism. These free nucleic acids trigger the cGAS-STING pathway, which induces an Interferon-alpha (IFN-α) mediated immune response. Hydroxychloroquine (HCQ), hampers the cGAS-dsDNA binding and, therefore, the activation of STING. Moreover, HCQ facilitates autophagosome formation but accumulates within lysosomes blocking the autophagic flux. Aim of this study was to assess the effectiveness of HCQ treatments in modulating IFN-α response in lymphoblasts (LCLs) derived from AGS patients and its action on autophagy, which could be a mechanism involved in RNA:DNA hybrids discard. An abnormal RNA:DNA hybrids accumulation and colocalization of these hybrids with lysosomes of AGS LCLs was found compared to controls. AGS patients' LCLs also showed an increased cGAS protein level and an induction of ISGs-interferon stimulated genes (*IFIT1* and *IFI44*). After a 24 hours treatment with 25μM HCQ we saw a significant decrease of expression of ISGs and of cGAS protein levels. After treatment, a decreased level of RNA:DNA hybrids content in AGS LCLs suggested the removal of toxic nucleic acids by HCQ. However, after HCQ treatments we identified increasing levels of LC3 and p62, autophagy markers, which protein level were not different between controls and AGS LCLs. Colocalization of RNA:DNA hybrids with LC3 (autophagy marker) and with lysosomes was lost after HCQ treatment. Therefore, HCQ seems able to stop IFN-α activation and release, determining improvements in patients' condition. HCQ could also represent an effective method to decrease RNA:DNA hybrids, although further experiments are needed to better understand this mechanism.

NI09 | Role of auto-antibodies and Peripheral Blood Mononuclear Cells on cerebral excitability in encephalopathies with epilepsy: a new experimental approach

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Auto-antibodies (auto-Abs) directed against surface neuronal antigens are found in patients affected by autoimmune encephalitis associated with seizures. Their contribution in altering brain excitability and the circumstances associated with their entrance into the brain, remains unexplored and unknown. The extravasation of auto-Abs across a dysfunctional blood-brain barrier (BBB) and the subsequent recruitment of systemic immune cells, complement factors and inflammatory mediators release could have a role. The pathophysiological impact of healthy donors and patients–derived Peripheral Blood Mononuclear Cells (PBMCs) on BBB permeability and brain excitability are tested in a peculiar experimental setting: the in vitro isolated guinea pig brain. It represents a unique in vitro preparation in which the BBB, the vascular and the neuronal compartments are morphological and functional preserved up to 5 hours. We demonstrated that in a condition of BBB impairment (induced by arterial perfusion of Lypopolysaccaride), co-perfusion of human recombinant serum albumin and Concanavalin A-activated healthy PBMCs or patients-derived PBMCs stimulated endothelial expression of intracellular adhesion molecule-1 (ICAM-1) and evoked spontaneous seizure-like events (SLEs). Separate perfusion of human recombinant albumin or no-activated healthy PBMCs did not induce PBMCs endothelial adhesion or extravasation, adhesion molecules expression and any electrophysiological modifications. We defined the hypothesis that pre-existing BBB alteration facilitates immune system cells and other components of serum (e.g. albumin) entrance into brain parenchima modifying brain excitability by direct interference with neuronal-vascular integrity and pro-inflammatory processes. The effect of perfusion of autoantibodies extracted by plasma of patients underwent to plasma exchange will be tested in the future.

NI10 | Prokineticin system as a new target to counteract experimental vincristine induced peripheral neuropathy

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Chemotherapy Induced Peripheral Neuropathy (CIPN) is a side effect of several antineoplastic agents. Regardless their mechanism of action, chemotherapeutic agents induce an alteration of neural and glial function, mitotoxicity, cytokines and chemokines release which result in an enhanced nociceptive input. Among chemokines, Prokineticins (PK) have a fundamental role in other types of experimental neuropathies. The aim of our study was to elucidate the role of PK in CIPN induced by Vincristine (VCR) and whether the block of PK-receptors, using an antagonist (PC1), may represent a therapeutic approach. CIPN was induced in mice by the administration of VCR. Hypersensitivity was evaluated by measuring mechanical and thermal allodynia as well as thermal hyperalgesia. When hypersensitivity was established, PC1 was administered until the end of VCR schedule. Before starting PC1 treatment, and at the end of VCR/PC1 schedule, Prokineticin2 (PROK2), PK receptors (PK-R1 and PK-R2), cytokines, CD68, CD11b, TLR4 and ATF3 were evaluated as mRNA (RealTime-qPCR) in DRG and spinal cord. Neurite outgrowth was assessed in DRG neurons treated with different concentrations of VCR and PC1 and expression levels of PK system and ATF3 were evaluated by RT-qPCR. VCR induced a dose-dependent neuropathy in mice, which was correlated to an upregulation of PK system and neuroinflammation, both in peripheral and central nervous system. Activated macrophages/microglia could be key mediators in this condition. PC1 administration counteracted hypersensitivity. Its effect could be due to its ability to reduce PK system levels and contrast neuroinflammation probably by reducing macrophages activation and migration in DRG. Neurite outgrowth was reduced in cultured DRG neurons treated with VCR, these cells also showed an upregulation of PROK2, PK-R1 and ATF3 mRNA levels. Also *in vitro* PC1 counteracted the effect of VCR. These results show that PK antagonism may represent a new pharmacological strategy to handle CIPN.

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NI11 | Inflammasome in the pathogenesis of Parkinson's Disease

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Parkinson's Disease (PD) is an age-related neurodegenerative disorder, with progressive motor symptoms including uncontrollable tremor, muscular rigidity, slowness of movement, and postural instability as a result of loss of dopamine neurons in the substantia nigra pars compacta. PD brain is characterized by the presence of intracellular Lewy bodies, constituted mainly by the misfolded synaptic protein α-synuclein (α-syn), a cytosolic protein with unclear physiological function, whose abnormal folding and accumulation has been associated with neuronal death and neuroinflammation. It is suggested that α-syn is released early in the disease and by the activation of the inflammasome NLRP3 pathway it activates microglia to release pro-inflammatory molecules, such as TNF-α and IL-1β, which are detrimental to dopamine neurons. To better investigate whether different α-syn proteins were able to activate NLRP3 inflammasome, we primed with LPS (2 hours) and stimulated with monomeric or aggregate α-syn (24 hours) peripheral blood mononuclear cells (PBMC) of 10 PD individuals and of 10 healthy controls (HC), and evaluated the production of inflammatory IL-1β, IL-18 and of caspase-1 and caspase-8 by ELISA in supernatants of cell cultures.
Results showed that: 1) IL-1β production was significantly increased in immune cells from PD compared to HC either when primed with LPS and stimulated with monomeric and aggregated α-syn; 2) caspase-1 (p20) production was significantly increased in LPS primed and monomeric α-syn -stimulated as well as aggregated α-syn - stimulated PBMCs from PD compared to HC; 3) IL-18 and caspase-8 production was similar in supernatants from LPS primed and either monomeric or aggregated α-syn stimulated cells from HC compared to PD. These results suggest both α-synuclein proteins were able to promote the activation of NLRP3 inflammasome and suggest an involvement of the inflammasome pathway in PD-associated neuroinflammation.

NI12 | A glial side to the neurotrophin field: studying the effects of neurotrophins on glial cells in the CNS

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Glial cells are the support and sentinels of the central nervous system (CNS). If the neurotrophin field has been dominated by the neurocentric view that the primary target of these molecules must be neurons, the ever increasing evidence that glial cells are integral part of neuronal computation calls for a closer introspection as to whether glial cells themselves are capable of responding to neurotrophins. Using 2-photon microscopy on head-fixed mice expressing either GFP on microglia or GCAMP6 in astrocytes, we evaluated in vivo the response of glial cells to the presence of neurotrophins, in particular Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF). Interestingly, microglial cells increase their surveilling activity and motility under the influence of NGF, both in resting and injury conditions. Moreover, astrocytes respond with calcium transient when affected by BDNF. These results uncover a glial function for neurotrophins in vivo and call for a closer look to their role in glial physiology and their impact on the surrounding neuronal circuitry.

NI13 | Monomethyl fumarate signals through the hydroxycarboxylic acid receptor-2 via cell- and environment-biased activation of different pathways

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We demonstrated that monomethyl fumarate (MMF), the bioactive metabolite of the drug dimethyl fumarate (DMF), modulates microglia activation through a new pathway mediated by hydroxycarboxylic acid receptor-2 (HCAR2) that inhibits NF-κB, via the AMPK/Sirt1 axis. Increasing evidence associates HCAR2-signaling in dendritic cells (DC) with an anti-inflammatory phenotype. We have investigated through which pathway MMF affects the activation of DC, and show that MMF induces an anti-inflammatory phenotype in activated-splenic DC (sDC). Importantly, MMF does not exerts such effect in sDC isolated from HCAR2-KO mice. Since HCAR2 is also the butyrate receptor, an anti-inflammatory commensal metabolite, we have speculated that intestinal side effects associated with (DMF) treatment might be associated with competition of MMF vs butyrate for HCAR2 in intestinal epithelial cells (IEC). We propose that MMF would signal in IEC through the prostaglandin/cycloxygenase-2 (COX2) inflammatory pathway, whereas butyrate through the AMPK/Sirt-axis. Our experiments show that MMF increases the expression of Tnf and COX2 in IEC isolated from WT-mice, but not in HCAR2-KO mice. Otherwise, butyrate has no such pro-inflammatory effect. In in-vitro-activated IEC, both ligands exerted an anti-inflammatory effect, albeit HCAR2-dependent only for MMF. To confirm the pro-inflammatory effect of MMF also in vivo, we isolated IEC from WT and HCAR2-KO EAE-affected mice treated or not with DMF. We found that DMF administration induced an HCAR2-dependent increase in the expression of pro-inflammatory cytokines on IEC, which, is mediated by the activation of the ERK1/2 pathway, rather than the COX-2 pathway as might be expected. We have ongoing experiments performed using X-Ray Phase Contrast Tomography to evaluate in 3D possible morphological alterations induced by DMF at intestinal level. Altogether these data suggest a cell- and environment-biased activation of different pathways in HCAR2 signaling.

NI14 | A drug combination administered via an implantable, polymeric delivery system improves the functional recovery in rat spinal cord injury

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Spinal cord contusion injury (SCI) is an incurable condition, in which a cascade of cellular and molecular events triggered by inflammation and excitotoxicity impairs endogenous regeneration, namely remyelination and axonal outgrowth. We designed a treatment solution based on an implantable biomaterial (electrospun PLLA) loaded with ibuprofen (Ibu) and triiodothyronine (T3) to counteract inflammation, thus improving endogenous regeneration. In vitro study on RAW264.7 cell line, demonstrated the ability of our device to reduce both mRNAs and proteins levels of pro-inflammatory markers (iNOS and TNF-α) and stimulate the expression of oligodendrogenic markers (CNP-ase, MBP). In vivo efficacy was tested by implanting the drug-loaded-PLLA in the rat model of T8 contusive spinal cord injury. We observed the expected recovery of locomotion beginning on day 7. In PLLA-implanted rats, the recovery stabilized at 21 days post lesion (DPL), after which non-further improvement was observed. On the contrary, in PLLA-Ibu+T3 rats a further recovery and a significant treatment effect was observed and also confirmed by the gait analysis on 49 DPL. The glutamate release at 24 hours and 8 DPL is reduced in PLLA-Ibu+T3 compared to PLLA-implanted rats, such as the estimated lesion volume at 45 DPL. The myelin and 200-neurofilament-positive area-fraction is higher in PLLA-Ibu+T3 implanted rats, where the percentage of astrocytes is significantly reduced as demonstrated by flow cytometry analysis. Also pro-inflammatory cells (macrophages and lymphocytes) showed a significant reduction in animals implanted with PLLA-Ibu+T3 scaffolds compared to PLLA-implanted. The implant of a PLLA electrospun scaffold loaded with ibuprofen and T3 significantly improves the endogenous regeneration, leading to an improvement of the functional locomotion outcome in the spinal cord contusion injury.

NI15 | Vascular networks of rat choroid plexus and cochlear nucleus: do they communicate?

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The choroid plexus (CP) is a major player in neuroimmune communication. So far, however, few studies have focused on CP spatial organization, because of its fragile nature and deep location within the brain. Cellular and subcellular details of the CNS may be obtained using clarification protocols followed by fluorescent labeling [Vigouroux et al. 2017]. In our lab, we have developed an iDISCO-based whole clarified rat brainstem/temporal bone preparation, in which the entire lower auditory system may be imaged. In this preparation, the 4th ventricle CP appears associated with several structures of the auditory system [Perin et al. 2019], and in particular with the surface of the dorsal cochlear nucleus (DCN), where epiplexus macrophages may reach the DCN parenchyma. Within the rat brain, the CN [Gharagouzloo et al 2017] and CP [Keep and Jones 1990] are among the regions with the highest blood perfusion. Moreover, the CP displays peculiar vascular features [Miller and Wollam 1953, Zagorska-Swiezy et al. 2008, Mortazavi et al. 2013] with no clear functional role, and the immature DCN shows a glial pattern similar to that of circumventricular organs [Mao et al.2015]. Geometrical features of blood vessels (e.g. size, tortuosity, branching patterns) could affect CP functions: e.g., vascular bifurcations could increase immune cell extravasation probability [Jung et al. 2018]. Therefore, as a first step for performing realistic blood flow / molecular diffusion simulations, we reconstructed the vascular networks of CP and DCN *in situ*. Collagen IV was used as a basal lamina marker for both endothelium and choroid plexus epithelium, alpha-SMA for smooth muscle, Iba-1 for macrophages. Regional organization of macro-and microvasculature is analyzed.

NI16 | 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 synaptic pruning and clearance of pathogens and cell debris. In line with this, dysregulation in microglial function is linked with the onset of neuropathology. Increasing evidence is pointing towards the involvement of metabolism in the regulation of immune cells. Microglial metabolic pathways, especially glucose catabolism, are altered in neuroinflammatory processes and in neurodegeneration. The aim of our study is to elucidate how specific nutrients can modulate microglial function, potentially affecting brain development and homeostasis. In particular, we are interested in the role of lactate, one of the main energetic substrates in the brain, as well as in microglial functional adaptations to the availability of other nutrients. We aim to gain mechanistic information on this topic by taking advantage of both *in vitro* and *ex vivo* models, and to validate our findings in an *in vivo* model presenting a microglial-specific lactate transport impairment. We have first characterized the expression of a set of glucose and lactate transporters in BV-2 cell line and microglia primary cultures, confirming that they express a large, although not identical, set of these proteins. Additionally, we have observed that BV-2 microglia mainly depend on glutamine for their metabolism, but that they can at least partially rely on other metabolic substrates under glutamine starvation. Next, we analyze the impact of cellular metabolism on phagocytosis and on other immune-related processes. In summary, this study will potentially enhance our understanding of the crosstalk between metabolism and brain function. Given the established correlation between metabolic syndrome, neurodegeneration and microglial activation, the manipulation of the underlying mechanisms may allow to set the bases for targeted therapeutic interventions.

NI17 | Longitudinal molecular magnetic resonance imaging of endothelial activation after severe traumatic brain injury

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Traumatic brain injury (TBI) is a major cause of death and disability. Despite progress in neurosurgery and critical care, patients still lack neuroprotective treatment to counteract or attenuate injury progression. Inflammation after TBI is a key modulator of injury progression and neurodegeneration, but its spatiotemporal dissemination is only partially known. In vivo approaches to study post-traumatic inflammation longitudinally are pivotal for monitoring injury progression/recovery and the effectiveness of therapeutic approaches. Here, we provide a minimally invasive, highly sensitive in vivo molecular magnetic resonance imaging (MRI) characterization of endothelial activation associated to neuroinflammatory response after severe TBI in mice, using microparticles of iron oxide targeting P-selectin (MPIOs-α-P-selectin). Strong endothelial activation was detected from 24 hours in perilesional regions, including the cortex and hippocampus, and peaked in intensity and diffusion at two days, then partially decreased but persisted up to seven days and was back to baseline 15 days after injury. There was a close correspondence between MPIOs-α-P-selectin signal voids and P-selectin stained area, confirming maximal endothelial activation at two days. Molecular MRI markers of inflammation may thus represent a useful tool to evaluate in vivo endothelial activation in TBI and monitoring the responses to therapeutic agents targeting vascular activation and permeability.

NI18 | The intra and extra cranial veins in relationship with chronic migraine

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Background | Migraine with and without aura is a widespread disease characterized by recurrent pain involving only one side of the head matched by nausea, vomiting, photophobia and / or hyperacusia.[1] It is defined as chronic if present for at least 15 days / month for more than 3 months.
Methods | From 2013 to 2019, 30 persons were examined (13 males, 17 females, mean age 43 ys, SD ± 18.57) diagnosed with chronic cerebrospinal venous insufficiency (CCSVI) and migraine; 7 underwent jugular balloon angioplasty. 15 performed an intracranial MRI venography and an Echo Colour Doppler (ECD) [2] of the Internal Jugular Veins (IJV)[3,4], resulting positive for venous anomalies and stenosis; 11 performed an intracranial MRI venography with positive results for venous anomalies; 4 performed ECD of IJV, resulting positive for stenosis. Of the people observed, 23 are in medical-treatment, and 7 underwent jugular balloon angioplasty.
Results | In this group, abnormalities of the cranial venous circulation are associated with chronic migraine. In 3 persons (2 males, mean age 16 ys) with cyclical vomiting associated with migraine, it was found: in 2 positivity both to the MR venography and to the ECD, 1 was negative to MR venography, but positive to the ECD. Of this subgroup, 2 underwent balloon angioplasty of the IJV with symptom improvements. In 5 persons (4 females, mean age 45 ys) with migraine, it was found: positivity in both the MR venography and the ECD, 1 did not perform MR venography, but was positive ECD. All 5 underwent jugular balloon angioplasty as a therapy for CCSVI with symptom improvements in 4.
Conclusions | These preliminary observations suggest the hypothesis of a possible association between migraine and intra and extra cranial venous anomalies [4]. Further studies will be needed, to verify the efficacy of jugular angioplasty as an additional therapy for chronic migraine.

NI19 | Gene Expression Profiling Identifies Inflammatory Signatures in Peripheral Blood Monocytes of Primary Progressive Multiple Sclerosis Patients

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Multiple Sclerosis (MS) is an autoimmune disease that affects the Central Nervous System (CNS) leading to demyelination, axonal and neurological damage. In this study, we aimed to investigate the contribution of the Innate Immune System in the MS pathology. Peripheral blood monocytes from 3 groups of female individuals (1st cohort): healthy controls (HCs), relapsing-remitting (RR) and primary-progressive (PP) MS patients have been studied by using a Transcriptomic approach. We were able to identify a number of dysregulated pathways specifically induced in PP monocytes compared to RR patients and HCs. We observed the biggest variability between PP and HC monocytes gene expression profiles, and could detect a clear division between RR samples into two subgroups (renamed as RR1 and RR2). In particular, the RR1 patients' expression pattern was very similar to those of the PP, whereas the RR2 profiles were more similar to the HCs. Gene ontology analysis of the differentially expressed genes (DEGs) showed that pathways related to 'Inflammatory Response', 'Cellular Proliferation', 'Anti-Apoptosis' and 'Cholesterol Biosynthesis' were specifically enriched in PP monocytes compared to RR and HC samples. Analysis of these pathways revealed that PP monocytes display a unique inflammatory signature suggesting that this cellular population in the PP patient of this cohort, is metabolically active. To corroborate and validate our results, the selected signatures were validated in a 2nd cohort of 12 female MS and 7 HC subjects. Surprisingly, only a subset of genes within the selected pathways were upregulated, suggesting that these groups of patients might represent an additional MS phenotypical group. Therefore, our results strongly suggest to undertake a personalized medicine approach, in order to be able to better stratify MS patients and to identify the molecular mechanisms that are operating within each MS patient's subgroup.

NI20 | Effects of Extracellular Vesicles on Myelin Repair

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In response to injury, microglia (MG) can acquire multiple activated phenotypes, participating not only in mechanisms of damage but also in tissue remodelling. In multiple sclerosis, activated MG play a role in remyelination impairment and neuronal injury, but also exert beneficial effects in the restorative MS phase through secretion of neurotrophic factors and phagocytosis of myelin debris. Moreover, detrimental MG phenotypes are attenuated and directed toward pro-regenerative types by exposure to mesenchymal stem cells (MSCs). These effects may be mediated by extracellular vesicles (EVs) that have been proposed as mediators of intercellular communication between microglia and brain cells and as transporters of toxic agents in neurodegenerative diseases. In the attempt to develop effective strategies to prevent the deleterious effects of MG in MS and instruct neurosupportive phenotypes, we set out to examine whether and how the administration of EVs released from different phenotypes of MG affects myelin production and remyelination operated by oligodendrocyte progenitor cells (OPCs). Here, we injected EVs produced in vitro by pro-inflammatory MG (inflammatory EVs, i-EVs) or pro-regenerative MG (pro-regenerative EVs, IL4-EVs) or pro-inflammatory MG pre-conditioned with MSCs (MSCs-EVs) in lysolecithin-induced focal demyelinated lesions of the mouse corpus callosum. Immunofluorescence and electron microscopy analysis revealed that while EVs produced by inflammatory microglia inhibit OPC differentiation, EVs derived from MSCs-treated microglia favour both recruitment and differentiation of OPCs into mature post-mitotic oligodendrocytes and myelinating oligodendrocytes. These results show the relevant role of EVs in MG-oligodendrocyte cross-talk and that the phenotype acquired by MG greatly impacts the differentiation of OPCs.

NI21 | Correlations of inflammatory biomarkers with clinical features and onset patterns in an Italian sample of preschoolers with Autism Spectrum Disorders

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Background | Several studies tried to investigate the role of some inflammatory biomarkers in Autism Spectrum Disorders (ASD), and their correlations with clinical phenotypes. Despite the growing research in this topic, existing data are mostly contradictory.
Methods | Eighty-five ASD preschoolers were assessed with standardized tools for developmental level, adaptive functioning, gastrointestinal (GI), socio-communicative and psychopathological symptoms. Plasmatic levels of Leptin, Resistin, PAI-1, MCP-1, TNF-α and IL6 were correlated with clinical scores and compared among different subgroups of ASD individuals according to: (i) presence or absence of GI symptoms, (ii) the ASD onset patterns: regression +developmental delay (Reg+DD), regression without developmental delay (Reg-DD), early onset (EO).
Results | the range values of all biomarkers were lower than those reported in previous studies in children with and without ASD with systemic inflammatory conditions. TNF-α and PAI-1 levels negatively correlated with age (R 0.37 and R 0.25, respectively). Leptin levels positively correlated with Body Mass Index (R 0.34) and negatively correlated with Child Behaviour Checklist (CBCL)1.5-5 Internalizing problems (R 0.29). MCP-1 levels negatively correlated with CBCL1.5-5 Internalizing and Total problems (R 0.38; R 0.27) and with Repetitive Behaviour Scale-Revised total scores (R 0.21) and positively correlated with Vineland Adaptive Behaviour Scale-II Motor Skills (R 0.25). No significant differences in the levels of biomarkers were found in children with and without GI symptoms. Resistin and PAI-1 levels were found significantly higher in the Reg+DD group compared to the other ones ($p<0.01$ for all).
Conclusions | our results did not show the presence of a systemic inflammatory state in ASD subjects but partially confirmed previous findings about some significant correlations between plasmatic biomarkers and clinical patterns, giving new information about possible endo-phenotypes inside the heterogeneity of ASD.

NI22 | Pro- and anti-inflammatory phenotypes of acute microglia isolated from spinal cord of SOD1G93A mice during disease progression and effects of the partial deletion of mGluR5

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by a selective death of upper and lower motor neurons (MNs). ALS is also a multi cellular disease, due to the contribution of other cells, such as astrocytes and microglia, to MN damage. Although the aetiology of ALS is not completely understood and has been ascribed to numerous causes, glutamate(Glu)-mediated excitotoxicity is considered one major factor for MN death. Starting from evidences on the role of Group I metabotropic glutamate receptors (mGluR1 and mGluR5) in ALS, in our previous studies we generated double mutant mice carrying the $SOD1^{G93A}$ mutation and a partial or total mGluR5 deletion and we observed amelioration of disease progression and of its cellular and molecular features. The aim of this study was to investigate the effect of the in-vivo genetic partial deletion of mGluR5 in $SOD1^{G93A}$ mice ($SOD1^{G93A}mGluR5^{+/-}$) on ex-vivo acutely isolated microglia cells. Microglia was purified from motor cortex and spinal cord of the two mouse strains at different stages of the disease (30, 90, and 120 day-old mice, that represent pre-, early- and late -symptomatic phases) and in age-matched WT mice on a percoll gradient and analyzed by flow cytometry and Western blot. The balance between the pro-inflammatory (M1) and the anti-inflammatory (M2) microglia phenotype was studied. M1/M2 ratio did not change during disease progression in the motor cortex of $SOD1^{G93A}$ and $SOD1^{G93A}mGluR5^{+/-}$ mice; at variance, the M1/M2 ratio significantly increased in the spinal cord of $SOD1^{G93A}mGluR5^{+/-}$ mice at the late symptomatic phase of the disease. Our results suggest that the reduction of mGluR5 drives microglia toward a more pro-inflammatory phenotype in spinal cord at the advanced stages of disease progression.

NI23 | Evaluation of the impact of A20 deficiency in myeloid cells: a murine model study

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A20, codified by *TNFAIP3* gene, is an ubiquitin-ending enzyme involved in the inhibition of NF-kB and is a central gatekeeper in inflammation. Genetic variants in the *TNFAIP3* locus are associated with several autoimmune disorders, including multiple sclerosis (MS). A significant A20 down-regulation is frequently observed in blood cells from MS patients, particularly in monocytes. Based to the crucial role of myeloid cells in the pathogenesis of MS, we aim to investigate the effects of A20 deficiency in these cells *in vivo*. We generated the conditional knock-out (KO) murine model (A20lox::CX3CR1) crossing A20lox/lox mice with transgenic mice carrying the Cre recombinase under the control of a specific promoter for myeloid/microglial cells (CX3CR1). We performed cytofluorimetric and histologic analysis to characterize the phenotype of conditional homozygous KO mice (A20lox/lox::CX3CR1) compared to their wild type (WT) littermates (A20wt/wt::CX3CR1). KO mice show a mortality rate of 40% during post-natal development. Moreover, starting from 1 month of age they are smaller compared to the WT littermates, regardless of gender. At a cytofluorimetric analysis, spleens and lymphnodes from 3-months old KO mice display a reduction in the percentage of CD11c+CD86+ dendritic cells, CD11b+Ly-6C+Ly-6G+ monocytes and CD11b+F4/80+ macrophages compared to WT. Also common myeloid precursor in bone marrow are reduced. These findings are corroborated by the histological analysis, which highlights a massive hypertrophy of the spleen in KO mice. The phenotypical analysis of the A20lox::CX3CR1 murine model clearly suggests a crucial role for A20 in the generation and in the development of myeloid cells.

NI24 | Pentraxin-3 is present in a specific temporal pattern after traumatic brain injury, but its depletion is not sufficient to modify the outcome

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Pentraxin-3 (PTX3) is a pattern recognition molecule involved in the humoral immunological response, interacting with complement components and its regulators. PTX3 is up-regulated following pro-inflammatory stimuli and may be involved in central nervous system diseases, however its role in traumatic brain injury (TBI) is still unexplored. Wild-type (WT) or PTX3 knock-out (KO) male C57BL/6J mice underwent sham surgery or controlled cortical impact brain injury (velocity: 5 m/s; depth: 1mm). Plasma and brain PTX3 presence was assessed in WT mice, respectively by ELISA assay and immunofluorescence, at different time points after TBI. Immunostaining for PTX3 was quantified by segmentation of stained area using Fiji software. Sensorimotor deficits were assessed by neuroscore and beam walk test on a weekly basis for 4weeks after TBI. At 5week brains from both strains were harvested for histopathological analysis. Plasmatic PTX3 markedly increases at 24h after TBI (553%), decreases at 48h to pre-injury levels and then slowly increases again from 1week to 3week (max 262%). Brain PTX3 increases from 48h up to 5week post-injury. Genetic depletion of PTX3 does not induce any difference in sensorimotor deficits compared to WT mice. Lesion volume and neuronal count (cresyl violet), axonal damage (luxol fast blue), collagen presence (sirius red), astrocytosis (GFAP), microglia activation (CD11b) and fagocytosis (CD68) were not different in KO compared to WT mice at 5week post-injury. PTX3 increases in brain and plasma after TBI and its activation pattern suggests distinct functions in acute phases versus sub-acute or chronic phases. Its genetic depletion does not modify the outcome following TBI. The lack of a clear-cut phenotype in PTX3 KO mice may depend on the different roles of this protein, possibly involved in inflammation early after injury and in repair processes later on. Neuronal pentraxins could also be involved in TBI evolution and contribute to the overall outcome.

NI25 | SIRT6 inhibition as a therapeutic approach in multiple sclerosis

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Multiple sclerosis (MS) is a neurological autoimmune disorder sustained by self-reactive T lymphocytes and by aberrant microglia activation. Our previous studies indicate that lowering NAD^+ levels reduces demyelination and disability in experimental autoimmune encephalomyelitis (EAE), an animal model for MS. The NAD^+-utilizing enzyme SIRT6 plays a key role in inflammation, by regulating TNF-α, IFN-γ and IL-8 secretion. SIRT6 plays also an important role in dendritic cell differentiation and function. We identified small molecules selectively inhibiting SIRT6 activity. These compounds reduced T cells proliferation and the release of pro-inflammatory cytokines by T cells. Therefore, we aimed at unveiling the role of SIRT6 in MS through the use of our SIRT6 inhibitors. Our preliminary data indicate that the administration of a SIRT6 inhibitor (S6) at the time of the immunization to induce EAE, had a strong impact on the disease onset on all the treated animals. The total cell, as well as the dendritic cell (DC), number in lymph nodes from treated animals was significantly reduced, in line with SIRT6 having a major role in T cell and DC activation.
qPCR analysis on recovered spinal cords indicated that Sirt6 expression is upregulated in EAE animals (day 28 post-immunization). Notably, these observations are in line with a recent report, focused on SIRT1 role in autoimmune neuroinflammation, and also showing an upregulation of Sirt6 mRNA expression in cerebellum from EAE mice.
Preliminary in vitro studies to investigate the anti-inflammatory effects on microglia cells, indicated that, in N9 microglia cell: a) S6 is not toxic; b) S6 increases the levels of the H3K9 acetylated form, confirming SIRT6 inhibition; c) SIRT6 inhibition decreases the release of TNF-α and NO from LPS-stimulates cells; d) SIRT6 inhibition attenuates the LPS-induced CD38 up-regulation. Altogether, we propose that SIRT6 may represent an interesting target in MS.

NI26 | Lack of IL-1R8 Affects Interneurons Development and Generation

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There is a general consensus that immune system abnormalities and inflammation can modify the risk and/or the severity of a variety of brain diseases, from neurodevelopmental to neurological disorders. Recently, our group demonstrated that the lack of IL-1R8 in neurons and the hyperactivation of the IL-1Receptor pathway lead to morphological and functional impairments of excitatory synapses, as a result of the activation of mTOR pathway and the increase of MeCP2 protein levels. MeCP2 is an epigenetic regulator that is fundamental also for the development of GABAergic inhibitory circuits. However, whether IL-1R pathway hyperactivation affects this process is still an unexplored issue. This study is aimed at characterizing the generation of inhibitory GABAergic interneurons and inhibitory synapses in IL-1R8KO mice, as a model for IL-1Receptor pathway hyperactivation. Similarly to what we have found in excitatory neurons, Parvalbumin-expressing GABAergic interneurons of IL-1R8 KO mice display a significantly increased level of MeCP2. Moreover IL-1R8KO mice show a reduction of the density of inhibitory synapses and of the number of parvalbumin-positive interneurons in the hippocampus but a significant increase of the same neuronal population in the cortex. To investigate the molecular mechanisms underlying these alterations we performed RNAseq analyses on Medial Ganglionic Eminence (region which contains inhibitory neuron precursors) isolated at embryonic day 13.5 from IL-1R8 KO and WT mice. Alterations in the molecular pathway underlying the formation of the inhibitory network during brain development may be at the basis of the learning and memory defects observed in IL-1R8 KO mice.

NI27 | Intracerebral Injection of Extracellular Vesicles from Mesenchymal Stem Cells exerts reduced Aβ plaque burden in early stages of a preclinical model of Alzheimer's disease

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Bone marrow mesenchymal stem cells (MSC), due to their strong protective and anti-inflammatory abilities, are widely investigated in the context of several diseases for their possible therapeutic role, based on the release of a highly proactive secretome composed of soluble factors and Extracellular Vesicles (EVs). MSC-EVs, in particular, conveying many of the beneficial features of parental cells, including direct and indirect β-amyloid degrading-activities, immunoregulatory and neurotrophic abilities. Therefore EVs represent an extremely attractive tool for therapeutic purposes in neurodegenerative diseases, including Alzheimer's disease (AD). We examined the therapeutic potential of intracerebrally injected MSC-EVs into the neocortex of APPswe/PS1dE9 AD mice at 3 and 5 months of age, a time window in which the cognitive behavioral phenotype is not yet detectable or just starts to appear. We demonstrate that MSC-EVs are effective in reducing the Aβ plaque burden and the amount of dystrophic neurites, in both cortex and hippocampus. The presence of Neprilysin on MSC-EVs, opens the possibility of a direct β-amyloid degrading-action. Our results indicate a potential role for MSC-EVs already at early stages of AD, suggesting the possibility to intervene before overt clinical manifestations.

NI28 | iPSCs-derived neurons cultured on engineered substrates as an in vitro model for the study of Aicardi Goutières Syndrome

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Aicardi Goutières syndrome (AGS) is a rare early-onset monogenic inflammatory encephalopathy with elevated cerebrospinal fluid (CSF) interferon-α (IFN-α) level and specific neuroradiologic features. Locus heterogeneity in enzymes involved in nucleic acids metabolism to prevent activation of cell intrinsic responses to immunostimulatory DNA and RNA defects is well recognized in AGS. The main neuropathological feature of the disease is abnormal myelination, probably caused by increased expression of proteases like cathepsin D, able to degrade myelin and brain tissue matrix. To date, inaccessibility of central nervous system primary cells causes a pressing need for suitable, disease-relevant cell models for new potential therapies screening, particularly because gene specific knock-out mouse models do not recapitulate all aspects of the human pathologic phenotype. Moreover no specific read-out tests are available for these disorders, in order to analyze phenotype reversion. A recent suitable experimental tool to study neurological disorders is provided by induced pluripotent stem cells (iPSCs) derived neurons and neural stem cells (NSCs). We generated iPSCs from two healthy donors and from three AGS patients mutated in different genes: *TREX1* (AGS1), *RNaseH2B* (AGS2), and *IFIH1* (AGS7). After obtaining stable and fully characterized iPSCs lines, we performed NSCs induction and neuronal differentiation on different kinds of engineered bio-scaffolds to improve functional performances of derived neurons. Moreover, in order to monitor the diseased phenotype and eventual potential benefits resulting from drug treatments, we have developed an AGS1 specific read-out tests, which evaluate with cytogenetic tests the chromatin bridges formation in patients' binucleated cells.

NI29 | Standardized multi-centric flow cytometry demonstrates Fingolimod as the MS drug most impacting immune cell subsets

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Objective | To establish cytometric profiles possibly associated with disease stages and/or responses to therapies in multiple sclerosis (MS).
Methods | 227 MS patients and 82 sex and age-matched healthy controls (HC) were prospectively enrolled from four European MS centers (Spain, Italy, Germany and Norway). All subjects underwent collection of demographic/clinical data and peripheral blood samples. Assays were strictly standardized using specifically prepared antibody-cocktail lyotubes. Differences in terms of immune cell subsets were assessed between groups of untreated relapsing or progressive MS patients (RRMS or PMS) and HC and between groups of RRMS patients taking 6 commonly used drugs.
Results | Overall, the greatest deviation in immune profile of MS patients appeared to be induced by treatments rather than by the disease phase, with the strongest impact observed in patients treated with fingolimod. Fingolimod induced a decrease in total CD4+ T cells and CD19+ B cells (particularly B-mature and B-memory cells) and a clear increase in CD4+ and CD8+ T-regulatory and B-regulatory cells. In untreated patients, significantly higher frequencies of Th17 cells in the RRMS population compared to HC ($P = 0.01$), and lower frequencies of B-memory/B-reg as well as higher percentages of B-mature cells in PMS patients compared to HC ($P = 0.004$, $P = 0.009$ and $P = 0.01$, respectively) emerged.
Conclusions | Our highly standardized, multi-site findings support the role of flow cytometry as a powerful tool for understanding immunopathological mechanisms underlying MS and monitoring treatment response, and provide crucial information for the development and management of a personalized treatment strategy.

NI30 | The role of Endogenous retroviruses in the susceptibility to Autism Spectrum Disorders. A Pilot Study in Children and Mothers

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Recent studies hypothesize that some human endogenous retroviruses (HERVs) may predispose to the onset of Autism Spectrum Disorder (ASD), activating the host immune response. Above all, syncytin protein, encoded by a single member of HERV-W family, seems to regulate the formation of placental syncytiotrophoblasts and to inhibit the maternal immune activation during pregnancy. Consequently, a reduced expression of HERV-W could predispose to ASD, since low levels of syncytin would alter the development of the fetus. The aim of the present study's proposal is to test whether a dysregulated HERVs expression, along with specific anti-HERVs antibodies, has a possible implication in ASD. The sample collection will be conducted between 2019 and 2020 at the Child Neuropsychiatry Unit of the University Hospital of Sassari. The studied population will consist in 40 Sardinian ASD children aged between 6 and 17, and 40 age-matched Sardinian healthy controls (HCs). Blood will also be collected from mothers of the two study groups. Specific tests will be used to assess the clinical conditions and the cognitive level of ASD patients and HCs. The expression of retroviral gene sequences from different HERV families (H, K, E and W) will be evaluated in peripheral blood mononuclear cells from mothers, ASD patients and HCs by RT-PCR based on extracted mRNA. The study will also analyze the presence of serum autoantibodies against immunogenic regions of HERV proteins. Statistics: specific group comparisons will be studied with ANOVA and symptoms association with Pearson's correlation test. The expected result is a distinctive HERVs expression profile with higher levels of some HERVs families and the presence of specific anti-HERVs antibodies in ASD patients compared to HCs. Finally, it would be interesting to find a correlation between lower expression of syncytin in ASD children and their mother and diagnosis and symptoms of ASD.

NI31 | Inhaled Argon improves neurological outcome in experimental traumatic brain injury

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INTRODUCTION. While supportive treatment in the management of Traumatic Brain Injury (TBI) has progressed over the past 20 years, specific drug treatments are lacking. *In vitro* and *in vivo* models of ischemic heart and brain injury show that the gaseous agent Argon is endowed with neuroprotective potential. Whether Inhaled Argon (iAr) is protective in experimental TBI is presently unknown.

OBJECTIVES. To test the effects of inhaled Argon administered after experimental TBI in mice on neurological functions and structural outcome by longitudinal behavioural assessments and magnetic resonance imaging (MRI) including T2W and DWI sequences.

METHODS. Severe TBI was performed in anesthetized mice (C57BL/6J, 8 weeks old, male) by controlled cortical impact. Ten minutes after TBI, mice were randomized to 24h treatment by iAr 70%-O_2 30% (n=20) or air (n=20). Sensorimotor deficits were evaluated at 24h post TBI and at 1 week by neuroscore and simple neuroassessment of asymmetric impairment (SNAP) tests. MRI (7-T, Bruker)was performed at 3 days post TBI to evaluate contusion volume by T2W. The effect of iAr on acute brain edema, was analysed by DWI-MRI.

RESULTS. Argon inhalation significantly improved neurological function at 24 hours and 7 days after TBI (Neuroscore 24h post TBI iAr 6.1±0.5 vs. Air 3.7±0.7, p=0.0102). Vasogenic brain edema showed a clear reduction in iAr treated TBI mice (p<0.001). At 7 days post TBI iAr reduced microglial/macrophages activation in the contusional cortex evaluated by IBA1 staining. Moreover, shape parameters indicating an increase of ramified cells thus indicating a less toxic phenotype.

CONCLUSIONS. iAr induces an acute and persistent improvement of sensorimotor function when administered for 24h starting 10 minutes after TBI. This outcome is reinforced by preliminary MRI data showing decreased edema in iAr treated mice. Our data support future studies to understand the potential of iAr as an accessible treatment in TBI.

NI32|Metabolicdysfunctionasriskfactorforneuroinflammatory pathology disease

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Diet-induced obesity and associated metabolic effects can lead to neurological dysfunction and increase the risk of developing neurodegenerative diseases. The effects of a high-fat diet (HFD) on the central nervous system are not well-understood. The aim of this study is the evaluation of the influence of HFD on the metabolic profile and inflammatory pathway in the brain of a mouse model of Insulin Resistance (IR). C57BL/6J male and female mice were fed with standard chow or HFD (45%/60%) for 35 weeks. Animals were monitored weekly for body weight, food consumption and Glucose Tolerance Test (GTT). Positron Emission Tomography (PET) imaging studies were performed longitudinally in which glucose metabolism, microglia activation and dopamine receptor were assessed using respectively [^{18}F]FDG, [^{18}F]VC701 and [^{18}F]Fallypride as radiopharmaceuticals. HFD induces a significant increase in body weight with a gender effect. Metabolic tolerance test exhibited increased blood circulating glucose concentration in both male and female, indicating impaired insulin function. Regional [^{18}F]FDG uptake showed a significant increase in glucose metabolism within cortical regions in males while no difference were observed in females. A specific analysis performed on anterior cortex revealed a hyper-metabolism occurring in male mice at 12 and 35 weeks of diet, that could be associated with cognitive impairment. [^{18}F]VC701-PET showed a general trend toward an increase of tracer uptake all over the brain after diet consumption in both male and female HFD mice. The increased binding of the activated microglia associated TSPO radioligand suggest that obesity is able to induce a diffuse neuroinflammatory reaction in mice brain. Our model reproduce the peripheral metabolic modification typical of IR and type 2 diabetes. The PET imaging technique has permitted to identify the presence of metabolic derangement and neuroinflammatory response of mice brain induced by a HFD.

NI33 | Focus on NSC/progenitor cell fate during neuroinflammation in the adult hippocampal neurogenic niche

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During the adult life of mammals new granule cell neurons and astrocytes are continuously generated from multipotent neural stem cells (NSCs) in the dentate gyrus (DG) of hippocampus. The integration of new neurons in hippocampal neuronal circuitry contributes to new memory formation and learning, but multiple factors can positively or negatively modulate this process. For instance, neuroinflammation underlies a deregulation of adult hippocampal neurogenesis (AHN) and both chronic neuroinflammation and altered neurogenesis are common features in different neuropsychiatric and neurodegenerative conditions, as well as in physiological aging itself. While a growing number of studies have examined the effects of neuroinflammation on AHN, the current knowledge on this topic is far from being exhaustive. The aim of this study is to achieve a deeper characterization of the cellular/molecular mechanisms underlying the effects of neuroinflammation on adult DG NSC/progenitor cell fate. To this aim, we exploited a mouse model of neuroinflammation (by LPS-treatment) to characterize the changes occurring in the inflamed brain in terms of microglia reaction and altered balance of DG neurogenesis versus gliogenesis on brain tissues. Interestingly, we found that tamoxifen, an antitumor drug with antiestrogenic function, which is also used as activator of the inducible Cre-Lox technology in the context of AHN research, prevents the effect of LPS treatment on DG neurogenesis and attenuates microglia reaction. To better characterize the underlying mechanisms of tamoxifen action on AHN we are now assessing the expression profile of inflammatory markers, including pro- and anti-inflammatory cytokines, by qPCR. Moreover, we adopted an *in vitro* cell culture approach to dissect the direct (on NSCs) versus indirect (microglia-mediated) effects of tamoxifen treatment on AHN and we are now analyzing the data.

NI34 | CXCL16 as a possible modulator of inflammatory condition

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Microglia are patrolling cells of the brain innate immune system that constantly monitor brain microenvironment in order to sense changes in brain homeostasis. Upon brain injury they acquire different phenotypes: inflammatory microglia (*in vitro* LPS polarization) associated with the production of pro-inflammatory cytokines, damaging to neurons; anti-inflammatory microglia (*in vitro* IL-4 polarization), capable of producing anti-inflammatory cytokines, scavenge receptors and trophic factors, inhibiting inflammation and promoting tissue repair. Astrocytes are the most abundant cell type in the Central Nervous System (CNS), providing critical roles in the neuronal homeostasis. Upon acute brain insults or chronic brain diseases, these cells become reactive and undergo morphological, transcriptional and functional changes. A1 reactive astrocytes lose the ability to promote synapses formation, are defective in phagocytosis and release neurotoxic factors, being detrimental to neurons; A2 reactive astrocytes might have “helpful” functions, since they upregulate neurotrophic factors and thrombospondins, promoting synapse repair. Along with the classical direct cell-to-cell contact and the paracrine action of secreted molecules, brain cells can communicate by releasing and receiving extracellular vesicles (EVs). EVs, including exosomes and microvesicles (MVs), are small, nano-to-micrometer vesicles released from cells and contain lipids, proteins and RNAs, representing an efficient way to transfer functional cargoes from one cell to another. In our lab we have found that chemokine CXCL16 and its receptor CXCR6 are expressed in the CNS, promote neuroprotection against excitotoxicity and ischemia, and drive microglia polarization towards an anti-inflammatory phenotype. In this project we want to study the ability of CXCL16 to modulate phenotype of both microglia and astrocytes in order to provide neuroprotection and to analyze the possible role of MVs in promoting these effects.

NI35 | Predictive value of high titer of GAD65 antibodies in a case of limbic encephalitis

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We report the case of a 42-year-old woman who presented with vertigo and migraine and rapidly developed cognitive decline and seizures. Brain MRI showed mild hyperintensity, without contrast enhancement, in both mesial temporal lobes and left insula on T2 and FLAIR sequences. Cerebrospinal fluid (CSF) showed increased cell count (156 lymphocytes) and on immunoelectrofocusing an oligoclonal reaction type 2. Serum and CSF samples showed high titer of anti-GAD65 antibodies using Enzyme-linked immunosorbent assay (998881 UI/ml and 54687 UI/ml respectively). Limbic encephalitis was diagnosed, and treatment with methylprednisolone, 1 g daily for 5 days, was started. During one-year follow-up, without further immunomodulatory therapy, antibodies titer remained elevated, but the patient became seizure free and cognitive functions returned to normal. We discuss the prognostic and pathogenic value of high titer anti-GAD65 antibodies in our case of autoimmune limbic encephalitis. Variable titers have been identified in patients with different neurological disorders such as LE, stiff-man syndrome, severe dysautonomia, chronic epilepsy and cerebellar ataxia, and thus they may lack of specificity. Anti-GAD65 antibodies may impair GABAergic synaptic transmission by reducing GABA synthesis and interfering with exocytosis of GABA. Moreover, a down-regulation of GABA synthesis in basket-cell terminals, with a reduction of GABA release on postsynaptic Purkinje cells, has been demonstrated. The need for long-term treatment with immunomodulatory/immunosuppressants drugs more controversial, given the burden of possible severe adverse events. Based on our observation anti-GAD65 antibodies titers may not be an effective indicator to guide long-term immunotherapy, and should be considered with caution and strictly related to the clinical picture. Clinical response and 'relative' trend of the antibodies' titer over time rather than the 'absolute' value should be used to guide treatment decision and more studies are needed to understand pathogenic mechanisms.

NI36 | Mitochondrial dysfunctions trigger the onset of neuroinflammation in animal models of Parkinson's disease

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Recent findings demonstrated that mitochondria might have a role in neuroinflammation occurring in Parkinson's Disease (PD) due to their ability to release several pro-inflammatory factors. The present study was addressed to explore the role of mitochondrial function during ageing, in striatum and midbrain, in primary culture of neurons and astrocytes, obtained from mice expressing the human A53T variant of α-synuclein (A53T mice). Mitochondrial function, monitored with confocal microscopy shown an overload of Ca^{2+} ions and mitochondrial membrane depolarization in mesencephalic neurons and in striatal astrocytes. These effects were correlated with a reduction of Na^{+} and Ca^{2+} exchange (NCX3) (mitochondrial isoform) in neurons and with the increase of NCX1 expression (plasma membrane isoform) in astrocytes from A53T α-synuclein mice compared with WT mice. Western Blotting experiments, performed in the midbrain of 4 months old A53T α-synuclein transgenic mice reveals an increase of Cytochrome - c (Cyt-C). Moreover, in vivo experiments at 4 and 16 months old A53T α-synuclein mice demonstrated a rise up of Glial Fibrillar Acid Protein (GFAP) and Ionized Calcium Binding Adaptor molecule1 (IBA-1) in relationship with an increase in α-synuclein accumulation. These results let to hypothesize the activation of the neuroinflammatory process in A53T α-synuclein transgenic mice accompanied to mitochondrial dysfunction and synucleinopathy. Interestingly, preliminary proteomic experiments in isolated microglia obtained from 3 months old PDGF- α-synuclein WT animal mice confirmed that changes in about 350 proteins, most of them correlated to mitochondrial dynamics and metabolism, occurred. These last results, together with those above described further support the hypothesis that chronic mitochondrial damage might occur already in the asymptomatic stage of PD and that it might be responsible for the onset of neuroinflammation.

NI37 | Cholesterol 24-hydroxylase inhibition during epileptogenesis is neuroprotective, delays epilepsy onset and blocks seizures progression in a murine model of temporal lobe epilepsy

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Cholesterol 24-hydroxylase (CH24H) is a cytochrome P450 that converts cholesterol into 24S-hydroxycholesterol (24HC), a positive allosteric modulator of N-methyl-D-aspartate (NMDA) receptors. CH24H is constitutively expressed in neurons and induced in glia following epileptogenic brain injuries. Based on previous work showing that Soticlestat, a potent and selective brain-penetrant inhibitor of CH24H, delayed pentylentetrazol kindling acquisition, we tested whether it might have antiepileptogenic effects in a murine model of temporal lobe epilepsy (TLE). C57BL6N adult male mice were implanted with hippocampal and cortical electrodes to permit continuous EEG monitoring. One week after surgery, mice were intra-amygdala injected with kainate (0.3µg in 0.2µl) to evoke status epilepticus (SE). Soticlestat (30 mg/kg, subcutaneously) or its vehicle was administered once daily for 14 days beginning treatment 1h post-SE onset, then treatment was withdrawn. We measured spontaneous recurrent seizures (SRS) onset and their frequency (EEG recording: 1-14 days and 2.0-2.5 months post-SE). Brains were analyzed postmortem for histopathology. The treatment delayed epilepsy onset and the number of ensuing seizures was decreased by half compared to vehicle-treated mice. Notably, 2.5 months after drug wash-out the magnitude of therapeutic effects was increased by 2-fold. Soticlestat-treated mice showed neuroprotection of CA1 hippocampal neurons and a rescue of mossy cells that was associated with reduced seizure duration. In human epileptic foci, CH24H expression trends higher in neurons while the enzyme is ectopically induced in astrocytes compared to control specimens. In chronic epileptic mice mimicking the human condition, Soticlest reduced by half the number of SRS. CH24H contributes to SRS generation and recurrence and is involved in disease progression, thus representing a novel target for chronic seizures inhibition and for disease-modification therapy in epilepsy.

NI38 | The mTOR kinase inhibitor rapamycin enhances the release of the pro-inflammatory cytokine IL-6 modulating the activation of human microglial cells

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Emerging evidence suggest the potential use of rapamycin in treatment of several neurological disorders. The drug can rapidly cross the blood brain barrier and modulate the inflammatory processes in the brain. Microglia are the main innate immune cells of the brain, thus involved and responsible for the inflammatory processes at this level. However, there are conflicting data from rodent studies about the pharmacological effects of rapamycin on microglial inflammatory responses. Moreover, rodent microglia represent a model with different biochemical and pharmacological response compared to human microglia. Therefore, we tested the effects of rapamycin in an experimental model of human microglia (the human microglial clone 3 cell line, HMC3 cells). Rapamycin, tested in the nM range, significantly increased the stimulatory effect of a pro-inflammatory cocktail (a mixture of interferon-γ and interleukin-1β, II) on interleukin-6 (IL-6) in the HMC3 cells, while it reduced production of free oxygen radicals (ROS) both under basal conditions and in cells activated with II. Furthermore, rapamycin reduced the extent of mTOR (mammalian target of rapamycin) activation in the complex 1 (mTORC1) and the total amount of intracellular proteins. However, the drug did not alter cell viability and morphology nor inhibited cell proliferation. Thus, in contrast to rodent cells, rapamycin did not significantly affect human microglial cell viability under basal conditions. All together these data suggest that rapamycin displays complex immunomodulatory effects on human microglial cells, significantly reducing the mTORC1 activity as well as increasing the release of the pro-inflammatory IL-6 cytokine.

NI39 | Modulation of REST and galectin expression induced by fingolimod treatment during experimental autoimmune encephalomyelitis

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Fingolimod (FTY) treatment for relapsing-remitting multiple sclerosis prevents T-cell infiltration into CNS by inducing sphingosine 1-phosphate 1 (S1P1) receptor internalization and degradation that blocks their egression from lymphoid organs. Treatment of experimental autoimmune encephalomyelitis (EAE) with FTY reduces inflammation and neuropathological damage. As FTY crosses the blood-brain barrier and S1P receptors are expressed on many CNS cells, its direct effect on CNS cells has been suggested. We investigated the effect of FTY on galectins (Gals) and repressor element 1-silencing transcription factor (REST) expression. Gal1, 3 and 9, which are known to modulate microglia and astrocyte activation were shown to reduce (Gal1 and Gal9) or enhance (Gal3) inflammation-induced neurodegeneration in EAE. REST is a gene repressor that regulates neurogenesis; it is dysregulated in many neurological disorders and we have shown that it is upregulated during the acute phase of EAE. The impact of FTY treatment for EAE on mRNA expression of REST isoforms, full length (f)REST and REST4, of REST target genes, and of Gals was evaluated in striatum and in lower and upper spinal cord segments upon two treatment regimens (preventive and therapeutic). In EAE-affected mice, both FTY regimens led to a reduction in (f)REST overexpression in the spinal cord, but did not change REST transcripts expression in striatum. In spinal cord, REST4 expression was increased under preventive treatment, with a par reversal of Nav 1.2 and somatostatin downregulation. In the striatum, mRNA upregulation of relevant REST target genes observed in EAE-affected mice, was reversed by FTY treatment. Contrasting data were obtained for Gal transcripts that were downregulated in the spinal cord, but upregulated in the striatum upon both FTY treatments. These results suggest that FTY treatment could modulate Gal expression and affect REST pathway, possibly impacting on neuro-inflammation and -degeneration.

NI40 | Voluntary running wheel protects against brain damage and motor defects induced by cuprizone (CPZ)

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Multiple Sclerosis (MS) is a chronic disease that affects the central nervous system (CNS). It is thought to be an autoimmune disorder, characterized by demyelinating phenomena and by a complex interaction between inflammatory and neurodegenerative processes. Growing data indicate the beneficial effects of exercise on several clinical outcomes in patients MS and data in rodent model of MS suggest that it may slow down the disease progression, by inducing peripheral immunomodulation. However, the mechanisms involved are not fully elucidated. Aim of this study was to address the effects of voluntary running wheel in a toxic-demyelinating model of MS, in which demyelination and brain inflammation occur in response to cuprizone (CPZ) treatment, in the absence of a T cell-mediated autoimmune reaction. Mice were housed in standard or wheel-equipped cages starting from the day of CPZ or normal chow feeding for three or six weeks. Exercise prevented early weight loss caused by CPZ, and both neuromuscular function and motor coordination were significantly enhanced by exercise in CPZ-treated mice. Moreover, exercise induced an early protection against axonal damage and the loss of the myelin associated proteins, myelin basic protein (MBP) and 2',3'-Cyclic-nucleotide 3'- phosphodiesterase (CNPase), in the striatum and the corpus callosum, in coincidence of a strongly attenuated microglia activation in both brain areas. Further, during the phase of spontaneous remyelination exercise in CPZ mice reduced the recruitment of new OLs compared to sedentary CPZ mice, likely due to the precocious protection against myelin damage. Overall, these results suggest that exercise has a beneficial impact on the demyelinating-inflammatory processes occurring in the brains of CPZ mice and likely in MS patients, independently of the peripheral immunomodulation, and underlie the significance of diversified therapeutic strategies for a more efficacious treatment of MS.

NI42 | miRNA shuttled by mesenchymal stem cell-derived exosomes downregulate the activated phenotype of primary astrocytes from end stage SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting motor neurons (MN), is characterized by neuroinflammation supported by activated glial cells. We previously showed that intravenous administration of mesenchymal stem cells (MSC) to SOD1^{G93A} mice, an animal model for human ALS, ameliorated disease and reduced neuroinflammation, possibly through paracrine mechanisms. To understand such mechanisms, we studied the activity of exosomes derived from immunomodulatory IFNγ-primed MSC (Exo) on cultured astrocytes prepared from spinal cord of end-stage SOD1^{G93A} mice. A significant increase in glial fibrillary acidic protein (GFAP) and vimentin expression was observed in adult SOD1^{G93A} vs WT astrocytes, which was significantly reduced after exposure to Exo. Analysis of the inflammatory profile of SOD1^{G93A} astrocytes showed a significantly higher expression and production of the pro-inflammatory cytokines, IL-1b, TNF, and IL-6, by SOD1^{G93A} astrocytes. Exposure of these cells to Exo decreased the overexpression and release of the inflammatory cytokines, induced an upregulation of the anti-inflammatory cytokine IL-10, and reverted the increased expression of the neuroinflammatory marker NLRP3. This decreased inflammatory profile of SOD1^{G93A} astrocytes impacted MN survival. Thus, the viability of MN seeded on Exo-treated SOD1^{G93A} astrocytes was significantly increased when compared to co-cultures with untreated astrocytes. As per our speculation that the effect of the Exo might be attributable to their shuttled miRNA, we transfected SOD1^{G93A} astrocytes with nine miRNA that were upregulated in IFNγ-primed MSC and present in Exo. Seven of these miRNA significantly reduced GFAP, IL-1b and TNF expression. The results indicate that Exo-shuttled miRNA can reduce astrocyte reactivity with a positive impact on MN viability, paving the way to translational preclinical in-vivo treatments of SOD1^{G93A} mice.

NI43 | Different extracts from chestnut tree wastes downmodulate inflammation markers in a microglia cell model

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Initiation or exacerbation of neurodegeneration may result from dysregulation of the defence function by microglia. By controlling the balance of microglia activation neuronal survival will be preserved. The by-products of the chestnut grove represent the subject of a multidisciplinary study aimed at evaluating their potential against neuroinflammation. Extracts derived by leaves, spiny cupules and pericarps, collected from different chestnut trees and individually characterized for relevant metabolites have been evaluated in BV-2 microglia cellular model. Following inflammatory stimulation with LPS, cell viability and the expression of different pro-inflammatory factors were evaluated by MTT assay and qPCR respectively. The pre-treatment of the cells with chestnut extracts prevents LPS-induced cellular damages. Importantly, the extracts were able to reduce the mRNA levels linked to NF-κB signalling such as IL-1β, TNFa and iNOS. Since the LPS inflammatory response can be interfered at the level of its receptor TLR4, its surface expression was evaluated by flow cytometry. Samples pre-treated with the extracts before LPS stimulation reported levels of cell-surface bound TLR4 comparable to unstimulated controls. Moreover, preliminary results showed a modulation of LPS co-receptor, i.e. CD14 at the mRNA level. In conclusion, the results showed a promising anti-inflammatory effect and encourage the recycling and enhance chestnut waste as sources of active principles.

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NI44 | Mannan-binding lectin-associated serine protease-2 (MASP-2) depleted mice show a better outcome after traumatic brain injury

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Lectin pathway (LP) of complement activation is a key mechanism behind tissue inflammation after traumatic brain injury (TBI). Studies in TBI patients have reported that an increase in circulating mannan-binding lectin-associated serine protease-2 (MASP-2), the essential enzyme driving LP activation, and its presence in the brain, is associated with increased TBI severity. This work defines the neuropathological and functional responses of traumatized mice with a gene-targeted disruption of the MASP-2 gene (MASP-2 KO) to get a further insight in its role in TBI. Wild Type (WT) or MASP-2 KO male C57BL/6J mice underwent sham surgery or TBI by controlled cortical impact (velocity= 5 meters/sec, depth= 1 mm). The sensorimotor response was evaluated by neuroscore and beam-walk test on a weekly basis for 4 weeks. Brains were harvested 6 weeks after injury for histopathological analysis. A group of mice was sacrificed 30 minutes post-TBI to evaluate mannose-binding lectin (MBL) presence in the brain. MASP-2 deficient TBI mice showed reduced sensorimotor deficits (by 32.1% at 3 weeks and by 36% at 4 weeks, beam-walk test) compared to WT mice. At 6 weeks after TBI, MASP-2 KO mice retained higher neuronal density in the ipsilateral cortex with a 37.9% increase compared to WT mice. Lesion volume and inflammation markers such as glial fibrillary acidic protein (GFAP) and CD11b were not different. MBL deposition in brains of MASP-2 KO mice was similar to that in WT when assessed at 30 minutes post-TBI, a time point where significant MBL deposition is seen in the lesioned brain. This study demonstrates that the absence of MASP-2 or MASP-2 functional activity is neuroprotective after TBI, ameliorating the sensorimotor performance and limiting neuronal loss.

NI45 | Role of microglia in the regulation of sleep and circadian behavior

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Sleep is an involuntary process observed in all mammals. It is required to sustain good mental and physical health, and it plays a crucial role in learning and memory functions in the nervous system. Indeed, sleep promotes the re-establishment of synaptic homeostasis, which is challenged by the remarkable plasticity of the brain during the wake phase [1]. Moreover, sleep deprived mice show memory impairments [2]. Microglia are the resident immune cells of central nervous system, they actively monitor the tissue and perform different functions, such as the phagocytosis of cellular debris and the release of cytokines to modulate the synaptic transmission. In this context, microglial cells contribute to neural plasticity through the remodeling of brain circuits, including the formation, modification and elimination of synaptic structures [3, 4]. However, little is known about microglial functions during sleep. The purpose of this project is to study the role of microglia in the regulation of sleep and circadian behavior. To this aim, we analyzed the expressions of different genes related to microglial functions and the microglia morphology, during the wake and sleep phases, in specific brain areas involved in sleep regulation: hippocampus, hypothalamus and prefrontal cortex (PFC). To assess the role of microglia in the regulation of sleep phases and circadian behavior, we pharmacologically depleted microglial cells, then performed 24h electroencephalographic (EEG) recordings. Moreover, we monitored the motor activity for 72h in the home cage. Our data show a circadian modulation of mRNA expression and cell morphology in microglia, suggesting different physiological roles during the activity and the rest phases. Furthermore, our data show that microglia-depleted mice spend more time in non-REM (NREM) state, accompanied by an increase of EEG power density during the rest phase compared to control mice. Moreover, we show that lack of microglia causes an increase of motor behavior during the activity phase.

NI46 | Age-dependant changes of nAChRs and TNFa in learning- and memory-related brain regions in APPswe/PS1dE9 mice

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Alzheimer's disease (AD) and age-related brain disorders are characterized by deficits of learning and memory. Neuroinflammation is considered one of the cardinal features of AD, and amyloid deposition and AD progression are driven by an impaired cholinergic neurotransmission. Recent findings suggest that cholinergic nicotinic receptors (nAChR) play a key role in the regulation of immune function by modulating cytokine production by activated microglia and astrocytes. Our aim was to evaluate the differential and progressive involvement of nicotinic receptors together with TNFα in AD, and hypothesize new and early therapeutic strategies to promote cognitive maintenance. Double transgenic APPswe/PS1dE9 mice were used as model of AD, and Wild Type (WT) C57BL/6 mice were used as controls to study gene expression of anti-inflammatory and pro-inflammatory nAChR. Hyppocampus (H), and cortex (C) were dissected at 6-12 and 24 months and using Real-time PCR α7, α4, β2, α3, and β4 subunits were analyzed in relation to pro-inflammatory TNFα. Preliminary results showed that anti-inflammatory subunits α7, α4, β2 were reduced in C of 6- 12 ($p<0.05$)-24-months old AD mice. The impairment of anti-inflammatory receptor subunits in H seems to be later, with a significant 7- fold decrease of α7 at 24 months of age. Pro-inflammatory α3, β4 subunits were increased in both C and H area at all selected age, compared to age-matched WT mice. The expression of TNFα was up-regulated at all age and in both brain regions evaluated. Our findings support the early implication of cholinergic system dysfunction in neuroinflammation. In our AD mice model the anti-inflammatory receptors are precociously affected in cortex and only later in hyppocampus. Taken together, these results support the overtime increases of the inflammatory response other than the "cholinergic hypothesis of age-related cognitive dysfunction", providing a mean for studying the mechanisms and novel therapeutics for AD.

NI47 | Clinical relevance and laboratory management of MOG antibody testing in children presenting with acute neurological symptoms

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Acquired inflammatory demyelinating syndromes (ADS) of the central nervous system are a challenging diagnosis at onset of acute neurological disorders in children. With the exception of cerebrospinal fluid (CSF) oligoclonal bands and AQP4-Ab, diagnostic biomarkers are lacking. Several studies have detected MOG-Ab in children with AQP4-seronegative neuromyelitis optica spectrum disorders (NMOSD) and acute disseminated encephalomyelitis (ADEM); although partial overlaps with AQP4-Ab disease, a novel "MOG-Ab-disorder" phenotype has been suggested. In this observational case study, we tested the presence of anti-MOG antibodies and anti-AQP4 antibodies in paediatric patients with suspected ADS. Titration of MOG-Ab in an IIF assay was correlated to clinical status. We enrolled 30 with acute onset of a first episode of a suspected acquired demyelinating CNS syndrome and with other neurologic syndromes as control groups: 7 in the ADS group, 7 in the other immune-mediated disorders of the CNS and the peripheral nervous system group, 16 in the non-immune-mediated disorders group. Patients were consecutively admitted to the Neuropediatric Unit of University Hospital of Padova during a 12 months period. MOG-Ab and AQP4-Ab testing was performed with IIF microscopy on fixed CBA assay using human embryonic kidney 293 transfected cells (EUROIMMUN). Antibody testing was performed first at screen dilution 1:10 for serum and 1:1 for CSF. When positive, titration was performed at progressive dilution up to 1:160. Demographic, clinical and biological data were collected. MOG-Ab positivity was demonstrated in both serum and CSF of a 6-years-old female child presenting with bilateral optic neuritis. MOG-Ab serum titre at onset was >1:160 and CSF titre was 1:40. Laboratory follow-up on serum showed persistence of antibody titre at 1:160 during hospitalization; finally, three months after onset, while on second line immunosuppressive treatment, antibody titre was 1:40.

NI48 | Paraneoplastic polyneuropathy with multiple antibody detection, a case report

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A 71-year-old Caucasian female presented to Emergency Department due to the sudden worsening of a slow-onset gait impairment and instability, without back pain and sphincter dysfunction. Past medical history included high blood pressure, atrial fibrillation and recent weight loss. Neurologic examination revealed gait impairment with ataxia, reduced deep tendon reflexes, bilateral distal hypoesthesia in lower limbs with hypopallesthesia and asymmetric muscular hyposthenia of the legs. Neuroradiological study was negative for spinal or nerve roots alterations. Routine blood and CSF workup was unremarkable except for hyperproteinorrachia and modest increase in CSF leucocyte count. Neurophysiological study showed signs of polyneuropathy with asymmetric distribution and both sensory and motor impairment. Serum autoimmunity testing was requested to our Laboratory Department of tertiary care University-Hospital. Strong positivity was found for onconeural antibodies anti-Hu and anti-CV2 on linear immunoblotting (EUROIMMUN), moreover anti-GAD antibody in ELISA assay (EUROIMMUN) was >2000 KUI/L. Testing was repeated on line blot with expanded antigen-panel, confirming high anti-Hu, weak anti-CV2 and weaker anti-GAD65 reactivity and detecting a weak anti-Zic4 signal. Immunohistochemistry on fixed rat cerebellum tissue showed a neuronal nuclear pattern supporting blot results. A thoracic CT scan found multiple mediastinal and lobar lymphnodes with necrotic-colliquative appearance. Transbronchial biopsy of mediastinal lymphadenopathies was performed and diagnosis of small cell lung carcinoma was made. Subsequent PET/CT study diagnosed metastatic stage of disease. Patient's general conditions worsened and 3 months after first hospital admission she died in hospice care. While the diagnostic value of onconeural antibodies in PNS is well established, their prognostic correlation on survival is still debated, since they can be associated to particularly aggressive neoplastic diseases.

NI49 | Mannose-binding lectin elicits a direct toxic effect on ischemic endothelial cells from human brain vessels

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Mannose-binding lectin (MBL), an initiator of the lectin pathway, is detrimental in ischemic stroke. MBL deposition on the ischemic endothelium indicates the beginning of its actions, but downstream mechanisms are not clear yet. We investigated MBL interactions with the ischemic endothelium by exposing human brain microvascular endothelial cells (hBMECs) to protocols of ischemia. Cells were exposed to hypoxia or oxygen-glucose deprivation (OGD), and re-oxygenated with human serum (HS) or recombinant MBL (rhMBL). Hypoxic hBMECs re-oxygenated with HS showed increased complement system activation (C3c deposition, +59%) and MBL deposition (+93%) than normoxic cells. Superresolution microscopy showed MBL internalization in hypoxic cells and altered cytoskeletal organization, indicating a potential MBL action on the endothelial structure. To isolate MBL effect, hBMECs were re-oxygenated with rhMBL after hypoxia/OGD. In both conditions MBL reduced viability (hypoxia: -25%, OGD: -34%) compared to conditions without MBL, showing a direct toxic effect. Ischemic cells also showed greater MBL deposition (hypoxia: +153%, OGD: +126%) than normoxic cells. These results were confirmed with primary hBMECs exposed to OGD (increased MBL-induced cell death: +226%, and MBL deposition: +104%). The present findings demonstrate that MBL can exert a direct deleterious effect on ischemic brain endothelial cells *in vitro*, independently from complement activation.

NI50 | C9orf72 deletion anticipates motor onset, exacerbates denervation and increases immune response in SOD1G93A mouse model

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The hexanucleotide repeat expansion in C9orf72 gene (C9) is the most common genetic cause of ALS. In addition to the toxicity of the expanded repeat, the reduction in C9 transcript and protein levels in patient cells and brain tissue suggests that loss of function mechanisms may contribute to ALS. Recently, it has been shown that loss of C9 leads to neurodegeneration in human motor neurons (MNs) and hypersensitizes cells to stress. However, C9 knockout (C9KO) mice do not display neurodegenerative phenotype suggesting that both the loss and the gain of function mechanisms are required for MN pathology. Conversely, C9KO mice develop altered immune response with reduced autophagic and microglial function and increased inflammatory state, mechanisms involved in ALS progression. Thus, to determine whether a loss of function of C9 may have an impact on the disease course, in this study we developed constitutive C9KO mice on C57Bl/6 genetic background, which were crossbred with SOD1G93A mice, the best characterized ALS model. Behavioral tests were conducted from 11 to 22 weeks of age, when mice were sacrificed and histopathological and biomolecular analyses performed. The grip strength test showed an anticipation of the symptom onset and an exacerbated denervation in tibialis anterior muscle (TAM) in C9KO/SOD1G93A mice with respect to SOD1G93A. CD4+ and CD8+ T cell infiltrates were significantly increased, while Tregs levels did not change in both TAM and sciatic nerve of C9KO/SOD1G93A with respect SOD1G93A mice. Also in the lumbar spinal cord there was an increase of CD4+ and CD8+ T cell infiltrates while Tregs decreased. Furthermore, both microglial and astrocytes activation was exacerbated, despite proinflammatory cytokine levels as well as MN loss did not change. This suggests that the constitutive loss of C9 generally exacerbates the immune response induced by SOD1G93A mutation, making the peripheral compartment more susceptible to neurodegeneration.

NI51 | Infusion of human amniotic mesenchymal stromal cells improve functional recovery of aged traumatic brain injured mice promoting protective astrocytic polarization

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Traumatic brain injury (TBI) shows a second peak of incidence in the elderly, associated with worse outcome. Preclinical studies have investigated the effects of mesenchymal stromal cells (MSC) as a potential therapy for TBI treatment in young adult. But little is known about the potential effects on the aged population. In this study we assess the efficacy of human amniotic MSC (hAMSC) in TBI aged mice infused either intracerebroventricularly (ICV) or intravenously (IV). Aged C57Bl/6 male mice (15-18 months) were subjected to sham or TBI surgery. Twenty-four hours post TBI, aged mice were treated with PBS (control) or hAMSC infused ICV (150.000 hAMSC in 5µl of PBS) or IV (10^6 hAMSC in 200µl of PBS). hAMSC ICV, but not IV, treated TBI mice showed an early and persistent reduction of sensorimotor deficits (up to 5w post TBI) assessed by neuroscore and beam walk tests, and an improvement of recognition memory compared to TBI PBS mice, evaluated at 4w by novel object recognition (NOR) test. Histopathological analysis at 5w showed a decrease neuronal death (by Nissl staining) and an increase microvessel density (by CD31 marker) in the contusional cortex of TBI hAMSC ICV treated mice compared to controls. No effect was on overall glial TBI groups, evaluated by GFAP and CD11b markers. However, mRNA gene expression analysis at early stages (3 days post-TBI) showed a selective downregulation of genes associated to proinflammatory astrocytic activation (Serping 1, H2-T23, H2-D1, Ggta1) in the contusional cortex of TBI hAMSC ICV mice. In conclusion, local but not systemic hAMSC infusion is protective in aged TBI mice. hAMSC, when infused ICV modulate the inflammatory microenvironment reducing proinflammatory astrocytic activation thus contributing to the improvement of functional outcome.

NI52 | Sympathetic nervous system signals to beta-3 adrenergic receptor-expressing bone marrow cells promote lymphoid hematopoiesis in experimental autoimmune encephalomyelitis

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In bone marrow (BM), mesenchymal stem cells (MSC) contribute to the homeostasis of the hematopoietic niche, producing factors which promote a quiescent hematopoietic stem cell (HSC) state. The sympathetic nervous system negatively controls the expression of these factors through the neurotransmitter norepinephrine (NE), whose interaction with β3-adrenergic receptors (B3AR) expressed by MSC contributes to HSC mobilization, proliferation and myeloid differentiation in models of diabetes, stroke and stress, respectively. We have assessed how β3-adrenergic transmission affects hematopoiesis in experimental autoimmune encephalomyelitis (EAE), a T-cell-mediated model for multiple sclerosis. From 1 day after immunization (dpi) with the encephalitogen, we observed a significant increase of NE in the BM, followed by reduced expression of genes controlled by NE. Confocal analysis of BM displayed an increase of tyrosine hydroxylase (TH)-positive perivascular fibers and of TH+ lymphocytes in BM parenchyma. Cytofluorimetric analysis of HSC showed a significant increase of Lineage-/Sca1+/cKit+ (LSK) cells. Quantification of common lymphoid (CLP) and common myeloid (CMP) progenitors revealed a lymphoid bias of hematopoiesis from 3 dpi. Mobilization of LSK from the BM occurred from 7 dpi, concomitantly with an increase of c-Kit+ precursors in the thymus. Chemical blockade of B3AR impaired differentiation of HSC into CLP, while promoting CMP generation, and prevented both the mobilization of LSK and the increase of c-Kit+ precursors in the thymus at 7dpi. These results indicate that SNS signals early elicited upon EAE induction activate MSC through B3AR and shape hematopoiesis promoting the generation and mobilization of lymphoid precursors.

NI53 | Microglia depletion in a murine model of epilepsy: effects on seizures and neuropathology

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Activated microglia and astrocytes release inflammatory molecules and generate oxidative stress that contribute to seizures and neuronal cell loss in seizure-generating brain areas in epilepsy. However, despite this evidence, the role of microglia in seizure generation is still unclear. Our hypothesis is that targeting microglia in a murine model of epileptogenesis affect neuropathology and spontaneous seizures (SRS). We blocked Colony-Stimulating-Factor receptors that govern microglia survival with PLX3397 in food pellet. We confirmed that PLX3397 depletes microglia in naive C57BL6 adult mice by 95% within 3 weeks. We found no changes in hippocampal synaptic transmission and neuronal excitability in microglia-depleted mice, as assessed *ex vivo* in hippocampal slices and in *Xenopous oocytes* microtransplanted with hippocampal membranes from microglia-depleted mice. Upon diet withdrawal, microglia repopulates the brain within one week. We depleted microglia in two phases of the disease in mice exposed to status epilepticus (SE): 1. before the mice developed the first spontaneous seizures until the onset of the disease; 2. in chronic epileptic mice. Microglia depletion did not modify the severity and duration of SE and the onset time of epilepsy, or the SRS number and duration. However, mice showed a rescue in neuronal cell loss associated with prevention of cortical thinning in the entorhinal cortex, as assessed by post-mortem histology and MRI. Microglia depletion in chronic epileptic mice did not affect SRS or cognitive deficits. Since cortical thinning is a common hallmark of human epilepsies, our data establish a causal link between this histopathological phenomenon and the activation of microglia during the initial disease development. The data highlight microglia as a cellular target for neuroprotective intervention in human epilepsy.

NI54 | Soluble-TREM2 exerts a role on neurons independently of microglia interplay

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Triggering Receptor Expressed on Myeloid Cells-2 (TREM2) is a transmembrane protein expressed in the central nervous system selectively by microglia, and upregulated during neurodegeneration and even glioblastoma. Increased levels of its soluble form (sTREM2) are correlated to neuroinflammatory disease, and recent studies demonstrate that it exert a beneficial action on microglia mediated clearance in Alzheimer disease. The endogenous ligand remain unclear, then a possible target on other cell type cannot be exluded: in this view, our aim is to investigate the effect of sTREM2 specifically on neuron, and recognise the pathway through which it exerts the action. For this purpose we have treated primary hippocampal culture with different concentration of sTREM2, observing an increased phosphorylation of the Signal Transducer and Activator of Transcription 3 (STAT3), that leads to the reduction of synaptic markers expression, vesicular glutamate transporter (VGLUT1) and SH3 and multiple Ankyrin repeat domains (SHANK2), and to an altered spontaneous electric activity. We are going to strengthen our preliminary data quantifying by immunofluorescence the synaptic protein after culture treatment; in the same perspective we are exploring the neuronal site of action of TREM2. This project will contribute to understand how microenvironment drive neurons in health and disease.

NI55 | Altered expression of the Cerebral Cavernous Malformation gene CCM1 upon exposure to proinflammatory stimuli and aging: a study in animal and cellular models

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Cerebral Cavernous Malformation (CCM) is a major cerebrovascular disease characterized by abnormally dilated and leaky capillaries that may result in severe clinical symptoms, including neurological deficits, seizures, and intracerebral hemorrhage. Typically, CCM occurs in mid-age individuals carrying heterozygous mutations in any of three known CCM genes, including CCM1 (also known as Krev interaction trapped protein 1, KRIT1), a pleiotropic regulator of oxidative stress responses and cell-cell junction stability. Nevertheless, CCM gene mutations *per se* are not sufficient to trigger CCM development, suggesting the contribution of a second hit. Recently, the activation of the innate immune signaling via TLR4 has been proposed to synergize with the individual genetic background in the formation of CCM (Tang et al., 2017, *Nature*). However, the molecular mechanisms underlying this second hit effect are still obscure. Here, we made the hypothesis that proinflammatory stimuli may impact on the expression levels of CCM1, thereby inducing a complete loss of function in heterozygous subjects and eventually leading to CCM. Using qRT-PCR and Western Blot analyses, we found that lipopolysaccharide (LPS) injection resulted in CCM1 mRNA and protein downregulation in the nervous tissue of both WT and CCM1 heterozygous mice. Notably, reduced levels of CCM1 were also found in LPS-exposed mouse fibroblasts, suggesting that LPS-induced CCM1 reduction may be a general phemomenon. Interestingly, a time-course analysis revealed that, in WT mouse cortex, CCM1 mRNA levels progressively increased up to 6 months of age, and then significantly decreased during aging (i.e. at 18 months). Analyses are ongoing to assess whether such age-related downregulation may be appreciated also in human samples and whether epigenetic mechanisms (i.e. miRNAs targeting CCM1 transcript) may be involved in LPS-/age-related downregulation of CCM1. This study may shed light on novel contributors in CCM pathogenesis and progression and may provide new targets for the development of preventive and therapeutic options.

NO01 | ASCL1 phosphorylation regulates neuronal differentiation of glioma stem cells

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Glioblastoma (GBM) represents one of the deadliest cancers in the adult. Its growth is fuelled by a subpopulation of stem/progenitor cells, which are though to be the source of resistance and relapse after treatment. The presence of these cells stimulated the evaluation of their developmental capacity to differentiate into mature cell types as a therapeutic approach. One important factor driving neuronal differentiation in a subset of GBM cells is the proneural transcription factor ASCL1, a key player in embryonic neuronal development. However, different GBM lines respond differently to ASCL1 overexpression and endogenous ASCL1 does not seem to reach sufficient levels to drive full differentiation, so we asked what would restrain ASCL1 activity in GBM and whether we could manipulate ASCL1 post-translational modifications to trigger permanent cell cycle arrest. We show that ASCL1 is highly phosphorylated in GBM cells and that overexpression of a form of ASCL1 that cannot undergo phosphorylation on serine proline sites drives GBM cells out of cell cycle, more efficiently than its wild-type counterpart. We also found that this effect on cell cycle exit is coupled with increased neuronal differentiation. Our results indicate that ASCL1 phosphostatus plays an important role in regulating the differentiation of GBM cells and future studies should focus on the mechanisms that drive terminal differentiation and lock cells in a non-tumorigenic state.

NO02 | A game of clones: clonal dynamics of Glioblastoma progression suggests internal clonal competition

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Glioblastoma progression is not fully understood, and our knowledge is mainly derived from post-analysis and reconstruction of clonal evolution from fully progressed tumors. Thus, in vivo studies on clonal dynamics of glioma progression from healthy cells to high-grade glioblastoma can shed light on this disease and favor the ideation of new therapies. To address this, we used a model of gliomagenesis driven by overexpression of PDGF-B in embryonic progenitor cells by in vivo somatic gene transfer, mimicking a first hit of gliomagenesis. In mouse brain, transduced cells generate neoplasms that undergo tumor progression from a low-grade lesion to a high-grade stage resembling human glioblastoma. In order to univocally tag each PDGF-B expressing cell and track their clonal behavior, we created PDGF-B transducing vectors carrying a library of more than 10^4 genetic barcodes. Such 22-mer sequences can later be retrieved by Next Generation Sequencing of genomic DNA from tumor masses, allowing to infer the number and the relative proportion tumor subclones in different stages of glioma progression Our data suggests that the entire glioma progression is a very unlikely process, which imposes strong bottlenecks in the initial pool of thousands transformed cells. During progression, clones undergo a dramatic selection that is not constant but appears to decrease with clonal heterogeneity. We excluded massive cell death as the main responsible of the progressive loss of clonal heterogeneity, and hypothesized that internal competition is taking place between clones. Serial transplantation assays show that even fully progressed clones keep losing subclones with similar dynamics and suggest the stochastic nature of this process. Our data suggest that Glioblastoma evolution is a balance between the emergence of new clones, which leads to tumor heterogeneity, and a continuous internal clonal competition, that would lead to monoclonal tumors.

NO03 | Microcephaly gene inactivation induces Dna damage and radiosensitization in medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children. Current treatment for MB, consisting of surgery followed by irradiation of the whole neuraxis and high-dose multi-agent chemotherapy, is only partially effective and is associated with highly invalidating side effects. Therefore, the identification and validation of novel target molecules, capable of contrasting MB growth without disturbing brain development, is needed. The Citron kinase protein (CITK), encoded by primary microcephaly gene MCPH17, is required for normal proliferation and survival of neural progenitors. Constitutive loss of CITK leads to cytokinesis failure, chromosome instability and apoptosis in developing brain, but has limited effects on other tissues. On this basis, we hypothesized that CITK could be an effective target for MB treatment. We tested this hypothesis using the MB cell lines ONS-76 and DAOY. In these cells, CITK knockdown increases both cytokinesis failure and DNA damage, impairing proliferation and inducing cell senescence and apoptosis via TP53 or TP73. Similar effects were obtained in MB in vivo using the NeuroD-SmoA1 transgenic mouse model, in which CITK deletion increases apoptotic cells and senescence markers, such as P21CIP1, P27KIP1 and P16INK4A. Most importantly, CITK deletion decreases tumor growth and increases overall survival in these mice, with no apparent side effects. Despite these positive results, much remains to be done to consolidate CITK as a useful target for therapy. A crucial point is to investigate whether CITK inactivation may increase the effectiveness of established treatments. We addressed whether synergistic pro-apoptotic effects could be obtained by combining CITK depletion with other standard or experimental anti-MB agents. In particular, association of IR and CITK depletion synergistically reduced the growth potential of MB cells. We expect that the results of our studies will provide clear indications about the suitability of CITK as a target for combined therapy in MB and will increase our understanding of CITK functions.

NO04 | SALL4A is a new positive regulator of Hedgehog signalling involved in medulloblastoma tumorigenesis

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Hedgehog (Hh) pathway is essential for embryonal development and tissues homeostasis; its alteration causes several human cancers, including medulloblastoma (MB), the most common brain malignancy in childhood. The tumor suppressor REN^{KCTD11}, a Cul3/E3-ubiquitin ligase that resides on a genomic region most frequently lost in human MBs, has been previously identified by our team as negative regulator of Hh pathway. Given its relevance in MB tumorigenesis, the identification of REN^{KCTD11} interactors is really important to elucidate new molecular mechanisms whose deregulations can contribute to MB onset. Among REN^{KCTD11} interactors identified by mass spectrometry, we focused on the transcriptional factor SALL4A that plays a key role in maintaining pluripotency and self-renewal features of embryonic stem cells (ESCs) and whose expression is inhibited in the post-natal period in many adult tissues; SALL4A is reactivated in different tumors and its expression is often related to worse prognosis and lower survival rate. We observed that SALL4A is a substrate of REN^{KCTD11}, that induces its poly-ubiquitylation and its consequent proteasome-mediated degradation. Investigating its biological activity in Hh signalling, we demonstrated that SALL4A enhances Gli1 transcription activity working in complex with HDAC1, a well known Hh activator. Further, we observed that the proliferation ability of MB cells increases in presence of SALL4A, whereas the migration rate is reduced after its genetic depletion. Our findings identify SALL4A as a previously unknown regulator of Hh pathway able to promote Gli1 activity in complex with HDAC1 and to contribute to Hh-dependent tumorigenesis; its regulation is mediated by the tumor suppressor REN^{KCTD11}. Hence, SALL4A stands as a new molecular target involved in the onset and progression of Hh-dependent tumors and represents an interesting focus in cancer research.

NO05 | Cdh4 down-regulation impairs in vivo infiltration and malignancy in patients derived glioblastoma cells

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The high invasive phenotype of glioblastoma is one of the main cause of therapy inefficacy and tumor relapse. Cell adhesion molecules of the cadherin family are involved in cell migration and are known as master regulators of epithelial tumor invasiveness, but their role in glioblastoma is less understood. In particular, we recently demonstrated, in syngeneic murine model, the occurrence of a previously undescribed cadherin switch between Cdh2 and Cdh4 during gliomagenesis, which is necessary for the acquisition of the highly infiltrative and tumorigenic phenotype of these cells. In the present study, we tested the role of Cdh4 in human gliomas. Our results on patients-derived glioma cells demonstrate a positive correlation between Cdh4 expression levels and the loss of cell-cell contact inhibition of proliferation controls that allows cells to proliferate over confluence. Moreover, the silencing of Cdh4 by artificial microRNAs induced a decrease in the infiltrative ability of human glioma cells both in vitro and in vivo. More strikingly, Cdh4 silencing induced an impairment of the tumorigenic potential of these cells after orthotopic transplantation in immunodeficient mice. Overall, we conclude that also in human glioblastoma Cdh4 can actively contribute in regulating cell invasiveness and malignancy.

NO06 | Cellular prion protein controls stem cell-like properties of human glioblastoma cancer stem cells

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Glioblastoma (GBM) is the most aggressive brain tumour, also due to the presence of cancer stem cells (CSCs) which are responsible for its many malignant properties. GBM invasiveness and recurrence, and even drug resistance are sustained by CSCs which persist within tumor mass by self-renewing and, displaying cellular plasticity, give rise to a more "differentiated" non-CSC progeny. Cellular prion protein (PrP^C) is a highly conserved cell surface glycoprotein, abundant in the central nervous system whose misfolding is responsible for prion disease. The physiological role of PrP^C is not well understood: PrP^C has been proposed to be involved in self-renewal, pluripotency, proliferation and differentiation of neural stem cells. More recently, PrP^C was reported to regulate different biological functions in human tumors, including GBM. Therefore, we investigated the role of PrP^C in GBM pathogenic mechanisms focusing on CSCs. To this aim we analysed the role of PrP^C in different GBM CSC-enriched cultures demonstrating that PrP^C expression is directly correlated with CSC proliferation rate. In order to more precisely define PrP^C role in CSC biology, we knocked-down PrP^C expression in five GBM CSC-enriched cultures by specific lentiviral-delivered shRNAs. We provide evidence that CSC proliferation rate, spherogenesis, clonogenesis, migration and *in vivo* tumorigenicity are highly reduced in PrP^C down-regulated cells. Moreover, PrP^C down-regulation caused loss of expression of stemness and self-renewal markers, while the activation of differentiation pathways was also observed. At molecular level the WNT signaling pathway was inhibited in CSCs with reduced PrP^C expression. Our results suggest that PrP^C controls stemness properties of human GBM CSCs and that its down-regulation promotes the shift to a more differentiated phenotype.

NO07 | Functional interactions between Citron Kinase inactivation and microtubule targeting agents in medulloblastoma

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor. The current therapy consists of tumor mass surgical removal, followed by radiation and adjuvant chemotherapy. Citron Kinase (CITK) was recently found to be a new possible target for MB, since its knockdown induces DNA damage and cytokinesis failure in medulloblastoma cell lines and reduces tumor growth *in vivo*. Cell sensitivity to CITK depletion has been linked to tubulin-bIII isoform and microtubules' dynamics. On this basis, we investigated the relationship between CITK and Microtubule Targeting Agents (MTAs), which are classified as microtubule 'stabilizers' or 'destabilizers' and have emerged as a successful class of cancer drugs in solid and hematopoietic tumors. Combination of CITK knockdown with the destabilizing agent Vincristine shows an increase in the number of apoptotic cells. In contrast, the stabilizing agent Paclitaxel is able to recover the DNA damage induced after CITK knockdown and the percentage of binucleated cells. These results suggest that CITK depletion could be combined with destabilizer MTAs, but not with stabilizing agents, which could counteract its effects. We plan to further elucidate the molecular pathways leading from microtubule alterations to DNA damage in medulloblastoma cancer cells.

NO08 | Gut microbiota alterations affect glioma growth and innate immune cells involved in tumor immunosurveillance

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Glioblastoma (GBM) is a recurrent CNS tumor with few therapeutic treatments. One of the main reason of its recurrence is the presence of a tumor microenvironment that hampers the tumor suppressive functions of innate and adaptive immune cells. GBM secretes immunosuppressive factors responsible for the recruitment of cellular elements like microglia and perivascular macrophages, whose presence negatively correlates with patient's survival. The panel of GBM infiltrating immune cells comprises leukocytes, like tumor infiltrating lymphocytes, Tregs and natural killer (NK) cells. NK cells encompasses different subsets endowed with different effector functions and characterized by the different expression of CD27 (TNF receptor) and CD11b (integrin αM). NK cells, once activated, might have direct cytolytic activity against cancer cells. At present, host microorganis has been involved in microglial phenotype modulation and an unbalanced composition of microbiota is now recognized as a key element that impairs the host metabolism and the immune system, playing important role not only in systemic diseases, but also modulating several brain functions. Recently, gut microbiota has been involved in the immune modulation of different tumors, but nothing is known about the effect of gut-immune axis on tumor brain growth. Here we studied in a syngeneic (GL261) mouse model of glioma (GM), the effect of a broad spectrum antibiotics (ABX) treatment on tumor growth, microbiota composition, NK cell subsets distribution and microglial phenotype. We reported that antibiotics treatment changed the gut microbiota populations, reducing the relative ratio of Bacteroidetes and Firmicutes and increasing the Proteobacteria. The treatment also reduced the cytotoxic NK cell subset and altered the expression of inflammatory and homeostatic genes in microglia likely favoring glioma growth. These findings show that microbiota alterations modulate the immune system and tumor brain growth.

NO09 | AMBRA1 controls cerebellar morphogenesis through the regulation of N-Myc stability

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During embryogenesis, the expansion of cerebellar granule neuron precursors (CGNPs) pools relies on a meticulously regulated proliferation that controls and allows the shaping of the cerebellum. In this context, dynamic modulation of NMYC oncoprotein is indispensable for proper morphogenesis and to avoid brain tumor onset. AMBRA1 is a key regulator of autophagic process and its first characterization demonstrated that its absence during embryogenesis leads to severe nervous system deficits, including failure of the neural tube closure, extensive midbrain/hindbrain exencephaly, and spina bifida. Taking advantage of a newly generated Ambra1$^{flox/flox}$/Nestin-Cre conditional knockout (cKO) mouse model, we demonstrated that AMBRA1 absence impacts positively on cell proliferation by mean of NMYC increased levels in the expanding external granular layer of the developing cerebellum. Our *in vivo* and *ex vivo* data unraveled a new mechanism for AMBRA1 in regulating NMYC protein stability through the promotion of their proteasome-mediated degradation, thus influencing not only the balance between expansion and consumption of the progenitor pool but also the potential induction of brain malignant hyperplasia.

NO10 | A neurogenic model of adult brain cancer in Drosophila

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The poor prognosis associated with adult brain tumours accounts on resistance to therapy and consistent relapse. Primary glioblastoma, a severe form of brain cancer, displays early PTEN inactivation, and cancer stem cells show a dysfunctional PTEN/aPKC/Lgl axis. After showing this axis is conserved in Drosophila, I impaired its function in the fly's neural stem cells, causing neoplastic growth and formation of tumour masses that kept growing in the adult brain, leading to premature death. The glioblastoma cell of origin is still under debate; then, in order to understand what neural population is more susceptible to alterations in the PTEN/aPKC/Lgl axis, I induced its dysfunction in distinct progenitor subtypes of the Drosophila brain, so as to identify those responsible for cancer initiation. My results demonstrated that only the type II neuroblasts (NBs) are prone to undergoun restrained growth following dysfunction of this axis. Of note, those type II NBs generate mature neurons and glia through transit-amplifying progenitor cells, as it happens in the human brain. It is our interest to identify the contribution of the PTEN/aPKC/Lgl axis to the complex molecular pathogenesis of brain cancer: to this aim, we are going to carry out an RNAseq analysis of brain cancers from our fly model, in the hope to isolate some relevant molecoles which will be validated and studied in patient-derived glioblastoma cell lines. The manipulated cells will also be injected intracranially in SCID mice to observe major changes in cancer growth and aggressivity.

NO11 | Identification and functional characterization of long non-coding RNAs involved in the pathogenesis of glioblastoma

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Introduction: Glioblastoma (GBM) is the most frequent and aggressive primary tumor in the central nervous system. Adult GBMs and GICs (glioma initiating cells) can be classified according to their gene expression and epigenetic profiles into subtypes: glioma-CpG island methylator phenotype (G-CIMP), proneural (PN), neural, classical (CL), and mesenchymal (MES). In addition, the different subtypes are plastic and transitions from one subtype to another are often observed. These transitions might be regulated by long noncoding RNAs (lncRNAs) that are involved in a transcriptional regulation or epigenetic regulation.
Results: We performed RNA sequencing of 124 formalin-fixed paraffin-embedded GBM specimens, which were classified into GBM's subtypes. We selected one lncRNA from each subtype to study their role in the self-renewal capacity of GICs and their role in the subtypes' transitions. We generated knock-downs-GICs of lncRNA selected by short-hairpins RNAs produced by lentiviruses. We performed flow cytometry (FC) and RT-qPCR assay to study the transitions of subtypes. A significant decrease of the number of the primary and secondary neurospheres was observed both in shPAUPAR-CL-GICs and shENST00000547804-MES-GIC with respect to their controls. There were no significant differences between control and modified GICs regarding to gene expression. Thus, we hypothesized that these lncRNAs are involved in self-renewal capacity of GICs, but, they might not be involved in the transitions of GBM's subtypes.
Conclusions: Our results suggest that these lncRNAs might be regulating the self-renewal capacity of GICs which would be a possible target to inhibit the progression of GBMs, without promote transitions between subtypes, which in some cases might cause a resistance to the therapy.

NO12 | Engineered drug-loaded PLGA-PEG nanoparticles functionalized with an MMP-cleavable peptide for glioblastoma treatment

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Glioblastoma (GBM) is among the deadliest of all human cancers because recurrence is unavoidable and fatal, with only a few patients surviving beyond 5 years. Therefore, it is essential to develop novel treatments to selectively kill therapy- resistant cell populations. The radio- and chemo- resistance of GBM stem-like cells (GSCs) and their innate tumor-initiating aptitude, make these cells a crucial target for the design of effective therapeutic strategies. In this work, Poly(lactic-co-glycolic acid) (PLGA) - poly(ethylene glycol) (PEG) nanoparticles (PLGA-PEG NPs) were synthesized to target and selectively release the chemotherapeutic agent doxorubicin (DOXO) to GBM cells. PLGA-PEG NPs were further functionalized with a metalloproteinases (MMPs) Activable Low Molecular Weight Protamine (ALMWP) penetrating peptide, that can be triggered in the presence of high amount of MMPs as in GBM microenvironment. PLGA-PEG was synthesized by carbodiimide chemistry, the resulting copolymer was further functionalized with a maleimide group. Afterwards, it was conjugated with a cysteine-terminated ALMWP through a Micheal-type-addition. Finally, DOXO was encapsulated by a standard nanoprecipitation method. A PLGA-PEG NPs functionalized with a low molecular weight penetrating peptide (LMWP-NPs) was used as control for DOXO uptake and toxicity. By confocal microscopy, we demonstrated the ability of three different patient- derived GSC cell lines to cleave the ALMWP and allow the uptake of the NPs. Moreover, treating with NPs a human endothelial cell line (hCMEC/d3), that expresses very low amount of MMPs, we confirmed the MMPs involvement in NPs activation and uptake. By live cell fluorescent microscopy, we followed DOXO accumulation in the GSCs treated with ALMWP-NPs. Our results show that ALMWP-NPs can deliver the same DOXO amount as for LMWP-NPs. As expected, MTT assay demonstrated both activable and active NPs toxic activity on GSCs.

PN01 | Prenatal stress reshapes spinal myelination affecting BDNF signaling in the experimental autoimmune encephalomyelitis model of Multiple Sclerosis

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One of the most substantial and established environmental risk factors for neurological and psychiatric disorders is stress exposure. The long-term consequences of stressful experiences hinge on several variables, including timing. In this regard, the gestational period is known to present an intrinsic vulnerability to environmental insults and indeed stressful events during pregnancy can lead to severe consequences on the offspring's brain development and thereby having long-term repercussions throughout adulthood. In this study we investigated the long-lasting effect of prenatal stress exposure on the susceptibility to the experimental autoimmune encephalomyelitis (EAE), a murine model of Multiple Sclerosis (MS). Although stress is considered a triggering factor for MS, little is known about how adverse events during gestation can shape the susceptibility to MS in the progeny. To address this, EAE was induced by immunization with MOG_{35-55}/CFA and treatment with pertussin toxin (PTX) in adult female C57BL/6 mice born from control or stressed dams exposed to restraint stress during the last days of gestation (from gestational day 16 until delivery). Our results demonstrate that gestational stress induces a marked increase in the severity of EAE symptoms in adulthood. Further, we highlight an altered maturation of oligodendrocytes in the spinal cord of prenatally stressed mice, as depicted by higher levels of the G-protein-coupled receptor GPR17, a marker of immature oligodendrocyte precursor cells (OPCs). These behavioral and molecular alterations are paralleled by changes in the expression and signaling of the neurotrophin BDNF, an important mediator of neural plasticity that we hypothesize could contribute to the stress-induced impaired OPCs maturation and resultant exacerbated EAE symptomatology. Since several already marketed drugs are able to modulate BDNF levels, these results pave the way to the possibility of "repositioning" these drugs in Multiple Sclerosis.

PN02|Seleniuminearlylifeisabletopromoteneurodevelopment after Lead exposure

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Lead (Pb) is an environmental pollutant, to which humans are exposed primarily through the diet, that affects the development of the Central Nervous System (CNS) leading to long term effects with a relevant impact on brain plasticity and development. Most important, to date no safe blood Pb levels in children have been identified. *In vivo* and *in vitro* studies suggest that Pb targets molecular mechanisms implicated in neurogenesis, synaptic function, inflammation and oxidative stress. These mechanisms are also modulated by Se, a fundamental micronutrient for brain health. The aim of this study has been to characterize the role of Se in supporting brain development and plasticity after Pb exposure. Dams (*Rattus norvegicus*) were fed with a suboptimal (0.04 mg Se/kg feed) or optimal (0.15 mg Se/kg feed) Se diet, in the presence or absence of Pb (25 or 100 mg Pb acetate/L water), before mating and through pregnancy and lactation. At weaning, offspring were fed with the same Se diet without Pb. The expression of N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptor subunits, proteins involved in Se transport and neuroinflammation, was assessed in the hippocampi at Post Natal Day (PND) 23 and PND 70. All the analysis evaluated male and female rats separately to highlight the potential sexual dimorphism in Se and Pb effects. Our results demonstrate that, in the presence of a poor Se supply corresponding to the suboptimal Se diet, Pb increases GluN2B, while reducing AMPAR subunits distribution at the post-synapse only in males at PND 23; the effect disappears at PND 70. On the contrary, in presence of an optimal Se diet, Pb increases both GluN2A and AMPAR subunits distribution at the post-synapse in females, with no effects in males. In conclusion, Pb effect on the glutamatergic system is sex dimorphic and strongly depends on Se dietary supply.

PN03 | Early visual assessment and magnetic resonance in preterm and term infants: between structure and function

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Visual function plays a central role in human global maturation. Newborns are at a major risk of developing visual impairment that can cause retardation in their adaptive functions due to anatomical and functional immaturity of their visual systems. The aim of this prospective study was to identify white matter structures, beyond optic radiations, that could be related to visual performance. The correlation between structural maturation (assessed with magnetic resonance imaging, MRI) and early visual function (according to Ricci et al. battery of tests) has been studied in a cohort of term and preterm newborns assessed at term equivalent age between March and June 2019 at Neuroradiology unit of Gaslini Paediatric Hospital of Genoa. Exclusion criteria were the presence of cerebral congenital abnormalities, chromosomic mutations, infections or bad clinical conditions that did not allow visual assessment. Apart from standard MRI sequences, DTI (diffusion tensor imaging) was performed, analysing four parameters (Fractional Anisotropy – FA, Mean Diffusivity – MD, Radial Diffusivity – RD, Axial Diffusivity – AD) in several white matter areas. For multiple regression analysis, Jonckheere Terpstra test was used. The patients included in this study were born at a median post menstrual age of 34+2 weeks and had an MRI scan at a median age of 41+5 weeks. Multiple regression analysis showed a significant correlation between visual score and MRI-DTI in optic radiations (MD p=0,045, RD p=0,045, AD p=0,034), cingulate gyrus (MD p=0,004, RD p=0,002, AD p=0,016), and sagittal stratum (MD p=0,014, RD p=0,007). In conclusion, early visual assessment in newborns can be correlated with structural brain maturation not only in the optic radiations, but also in other white matter tracts, such as the cingulate gyrus and the sagittal stratum, which are known to be involved in visual function in post-neonatal period.

PN04 | Evolution of visual function in full term physiological newborns during the first 48 hours of life

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The assessment of visual function should be part of standard newborn neurological examination, however it usually includes only basic evaluations. The aim of our study was to examine the development of a more structured visual function in the physiological newborn at term during the first 48 hours of life, at 24 and 48 hours. Sixty physiological babies (mean gestational age 39,5±1,5 weeks, mean birth weight 3387±392 g) were assessed, divided in two populations: 30 newborns (17 males, 13 females) examined at 24±6 hours of life, and other 30 newborns (15 males, 15 females) examined at 48±6 hours of life. A visual assessment battery including 9 items was used: assessing ocular movements (spontaneous behaviour and in response to a target), the ability to fix and follow a black/white target; the reaction to a coloured target; the ability to discriminate a target with black and white stripes; the ability to keep attention on a target moved slowly away. Comparing the assessments performed at 24 and 48 hours of life, we observed a statistically significant increase in the share of neonates able to complete vertical tracking (increased by 23%, $p<0.05$), to discriminate stripes (increased by 37%, $p<0.05$) and to maintain attention at distance (increased by 44%, $p<0.05$). Our study shows an evolution of the visual performance in a short time of 24 hours, not dependent on the functional exercise. This evolution could be part of the physiological neuro-cognitive development, even if the role of environmental stimulations contributing to the early visual experience in this process remains to be further investigated.

PN05 | Corpus callosum growth in complex congenital heart disease

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Mortality rates in infants with congenital heart disease (CHD) have improved considerably due to advances in peri- and intraoperative care; however, morbidity remains a significant concern. Structural alterations in the corpus callosum (CC) may underlie adverse outcomes in CHD, even in the absence of brain injury (BI), and may be more pronounced in newborns with Single Ventricle physiology (SVp), who continue to have poor cardiac function in comparison to infants with transposition of the great arteries (TGA), whose cardiac function improves after early surgical correction. This study examines the association of preoperative CC volumes and growth with heart lesion type in newborns with CHD and whether BI influences the association between CC growth and heart lesion. 62 infants with CHD participated, 33 had TGA and 29 SVp. Participants underwent open-heart surgery and had serial MRI scans pre- and postoperatively at median post-menstrual ages (PMA) of 39.7 (IQR:39-41) and 42.1 (IQR:41-44) weeks, respectively. CC and total cerebral volumes (TCV) were obtained using a semiautomatic segmentation algorithm on T1-weighted images. Preoperative CC volumes and growth were assessed in relation to heart lesion type and preoperative BI, using a general linear model, adjusting for GA, PMA, birth weight, head circumference at birth, SNAP and TCV. Preoperative volumes did not differ by heart lesion type ($p=0.9$). However weekly growth of the CC was significantly reduced ($p=0.03$) in newborns with SVp (mean growth: -2.8mm^3, confidence intervals [CI]: -33.4-27.9) compared to newborns with TGA (mean growth 42.1 mm^3, CI:12.9-71.3). No significant effects of preoperative BI were evident ($p=0.4$). Thus, maturation of the CC was significantly impacted by heart lesion type, with SVp newborns having reduced growth relative to TGA newborns despite the presence of BI. Findings indicate that SVp newborns who do not regain cardiac function are at high-risk for structural alterations in the CC.

PN06 | The oxygen-glucose deprivation induced death in fetal neural stem cells-derived oligodendrocyte precursor cells is mainly driven by glucose metabolism perturbation

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Impaired myelination is a key feature in neonatal hypoxia/ischemia (HI), the most common perinatal/neonatal cause of death and permanent disabilities, that is triggered by the establishment of inflammatory and hypoxic environment during the most critical period of myelin development. This process is dependent on oligodendrocyte precursor cells (OPCs) and their capability to differentiate into mature oligodendrocytes. In this study we investigated the response of OPCs derived from fetal neural stem cells (NSCs) to inflammatory and HI insults, focusing on survival and differentiation. Since the same pathological mechanisms are in common with demyelinating diseases in adult life, we used the same protocols on OPCs derived from NSCs isolated from the sub-ventricular zone of adult brain. Both fetal and adult brain-derived OPCs were exposed to cytokines, to mimic inflammation, or to oxygen-glucose deprivation (OGD), to mimic HI condition. The differentiation of both fetal and adult OPCs is completely abolished after exposure to inflammatory cytokines, while only fetal-derived OPCs degenerate when exposed to OGD (Figure 1). We then investigated three possible mechanisms involved in OGD-mediated toxicity: i) T3-mediate cell cycle exit and maturation induction by deiodinase-3 upregulation; ii) glutamate excitotoxicity; iii) glucose metabolism. We found that a severe glucose metabolism perturbation occurs, while no contributions of deiodinases 3 activation and glutamate excitotoxicity were found. These results indicate that the biological response of OPC to inflammation and demyelination are different in fetal and adult cells, and that the glucose metabolism perturbation in fetal OPCs may heavily contribute in different neonatal CNS pathology. The understanding of the underlying molecular mechanism will strongly contribute to differentiate myelination enhancing and neuroprotective therapies for neonatal and adult CNS lesions.

PN07 | Differential immunomodulatory properties of oligodendrocyte progenitor cells and immature oligodendrocytes in a murine model of perinatal brain inflammation: focus on the role of TLR3 activation

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Perinatal inflammation is the leading cause of preterm birth and neurodevelopmental injuries including periventricular white matter injury (PWMI), the major form of preterm brain injury. Despite the prominent role exerted by microglia in orchestrating the response induced by infiltrating immune cells from the periphery it is now clear that oligodendrocytes (OLs) may play an important immunomodulatory role too. Here, we focus our study on toll like receptor 3 (TLR3), an endosomal -nucleic acid–sensing receptor that can be activated by sterile inflammation promoting neuroinflammation and whose expression and role in OLs remains unclear. Specifically, our goal is to study whether TLR3 activates different pathways in two critical populations of premyelinating OLs: the PDGFRa+ OL precursor cells (OPCs) and the O4+ immature OLs (immOLs). Firstly, our in vivo experiments in a mouse model of perinatal brain inflammation induced by IL-1b exposure from P1 to P5 shown the upregulation of Tlr3, Ccl2 and Cxcl10 in both PDGFRa+ and O4+ magnetically sorted cells. This upregulation was higher in O4+ immOLs as compared to PDGFRa+ OPCs, suggesting a different sensitivity to neuroinflammation of the two populations. These observations were confirmed in OLs primary cultures: cells treated with TLR3 agonist Poly (I:C) during differentiation showed a higher upregulation of Ccl2 and Cxcl10 compared to cells treated during proliferation which resulted in defects in reaching the more mature phenotype. Finally, we observed that conditioned medium derived from primary OLs treated with Poly (I:C) during proliferation induced the expression of anti-inflammatory Socs3 in microglia. In conclusion, we demonstrate important differences between the OPCs and immOLs populations in response to an inflammation induced-WMI. These differences are particularly important from a therapeutic point of view, since immunomodulatory treatment may affect the two populations differently over the course of the disease.

PN08 | Are oligodendrocyte progenitors all born equal? A lesson from a microcephaly model

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Vertebrate neurons are enormously diversified in morphology, neurochemical profile, function and susceptibility to injury. Diversity is instead much less established for neuroglia cells, despite observations of distinct functional properties and the identification of distinct embryonic sources for subsets of astroglia and oligodendroglia. In particular, whether, depending on their developmental origin, glial cell subpopulations may differ in molecular features or in their ability to contribute/respond to pathological conditions, is still not understood. We tackled this issue by studying a mouse model of human microlissencephaly, where the germinal ablation of Citron-kinase (Cit-K, a citoskeleton regulator involved in cell division and DNA repair) resulted in profound myelination defects and triggered distinct responses in telencephalic dorsal oligodendrocyte progenitor cells (dOPCs; i.e. populating the dorsal cortex and corpus callosum, and generated perinatally by $Emx1^{+}$ progenitors in the dorsal subventricular zone) and ventral OPCs (vOPCs; i.e. located in the striatum and hypothalamus and generated during the embryonic life in the preoptica area and ganglionic eminences). Both populations showed high levels of DNA damage. However, Cit-K KO dOPCs underwent depletion, whereas vOPCs persisted and displayed a senescent phenotype. Such differential sensitivity depended on a distinct intrinsic capability to set up Nuclear factor erythroid 2-related factor 2 (Nrf2)-mediated anti-oxidant defenses. Notably, also WT dOPCs showed a higher intrinsic vulnerability to DNA damage induced by cisplatin, that could be counteracted by an anti-oxidant supplementation. These data provide novel evidence of molecular and functional heterogeneity in OPC subsets and indicate that distinct postnatal OPC populations may be differentially vulnerable to pathological conditions associated with DNA damage and oxidative stress.

PN09 | The use of Fingolimod in a neonatal murine model of Krabbe's disease

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Krabbe's Disease (KD) is a rare, predominantly infantile neurodegenerative disorder with a rapid and invariably fatal progression. It is characterised by profound demyelination, oligodendrocyte death and neuroinflammation. These underlying histological abnormalities clinically translate into psychomotor and cognitive deterioration, with infants ultimately suffering from decerebrate posture. Currently, clinical practice lacks a curative treatment and is mostly directed towards symptomatic relief. Recent findings give rise to the sphingosine 1-phosphate receptor agonist fingolimod, a therapy for multiple sclerosis, as a potential therapeutic agent for KD. Findings from our lab show that fingolimod reduces demyelination and attenuates inflammation in the central nervous system of twitcher mice, a murine model of KD, when administered post-weaning. These findings prompted the current extension study administering fingolimod presymptomatically during perinatal stages, starting at postnatal day 5. Primary outcome measures include lifespan, weight and mobility. To identify potential underlying mechanisms we further investigate the myelination and inflammatory state using histological and biochemical analysis of central and peripheral nervous systems. The findings of this neonatal study hope to address the effect of fingolimod in a neonatal mouse model of KD and to shed light on the role of sphingosine 1-phosphate receptors during perinatal development.

PN10 | The role of selenium intake in brain development: focus on the glutamatergic system

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Selenium (Se) is a fundamental micronutrient for brain health promoting brain plasticity and development, although the underlying mechanisms are not clear. The European Food Safety Authority set an Adequate Intake of 70 µg/day for adults and 85 µg/day for lactating women, although in some EU regions the dietary intake is lower, with estimated values around 40 µg/day. The aim of this study was to investigate if an insufficient Se intake impacts on brain plasticity and development. We evaluated the effect of Se levels on the expression of the glutamatergic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subunit receptors and proteins involved in Se transport like Selenoprotein P (SEPP1), the key Se transporter, and Apolipoprotein E receptor II (ApoERII), responsible for the intraneuronal uptake of SEPP1. To this purpose, rat dams were fed with a suboptimal (0.04 mg Se/kg feed) or optimal (0.15 mg Se/kg feed) Se diet, before mating, through pregnancy and lactation. At weaning, offspring were fed with the same diet until adulthood. The expression of the selected proteins was evaluated in hippocampal total homogenate and post-synapse (PSD) from offspring at Post Natal Day (PND) 23 and PND 70. The analysis was performed on males and females separately to highlight the potential sexual dimorphism. Our results demonstrate that a suboptimal Se intake reduces glutamatergic receptors distribution in the PSD at PND 23 in both sexes. At PND 70, NMDAR recovers both in males and females, while AMPAR only in females. Preliminary results suggest a decreased expression of ApoERII in males and females at the PSD at PND 23 with a Se suboptimal diet. The lower expression of ApoERII is coupled with a decrease in SEPP1. These effects mirror Se analysis of early maternal milk and blood plasma, which showed that the suboptimal diet resulted in a poorer Se status of both dams and offspring compared to the optimal diet.

PN11 | Understanding the molecular bases of myelination defects in the microcephalic Citron-K KO mouse: a role for secreted Wnt inhibitors?

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Microcephaly is a condition in which, in consequence of brain development disruption, an infant shows a head size much smaller compared with other subjects of the same age/sex. Oligodendroglia defects have been reported in microcephalic patients and mouse models, although their contribution to the phenotype and the underlying molecular alterations are poorly understood. In humans and rodents, the loss of function of Citron-kinase (Cit-K, a citoskeleton regulator involved in cell division and DNA repair) results in severe microcephaly and myelination defects. Despite the presence of numerous axons, Cit-K KO mouse oligodendroglia comprises exclusively immature NG2+/PDGFRa+ oligodendrocyte precursor cells (OPCs) that do not progress toward more mature stages. To discriminate the contribution of cell-autonomous *vs.* environmental factors to such differentiation impairment, we cultured Cit-K KO and WT OPCs. In controlled differentiation conditions, most mutant cells expressed myelin proteins and acquired complex morphologies typical of differentiated cells, although WT cells displayed a higher propensity to mature. Moreover, when transplanted into the perinatal CIT-KO mouse brain, WT OPCs hardly ever differentiated into mielinating cells, at difference with OPCs grafted in the WT. These findings suggest that Cit-K KO OPCs are not intrinsically unable to undergo maturation, and that extrinsic factors in the mutant tissue hamper oligodendrocyte progression along the lineage. In search for these environmental signals, we re-analysed RNA sequencing data obtained from the Cit-K KO postnatal cerebellum (Bianchi et al., 2016 Cell Rep). We found increased levels of secreted Wnt inhibitors (e.g. Wif1, Sfrp1, Sfrp5) and decreased levels of soluble positive Wnt regulators (e.g. Rspo2). Experiments are ongoing to assess whether the manipulation of these signals or the pharmacological activation of the Wnt pathway can restore the maturation potential of Cit-K KO OPCs.

PN12 | In search for druggable mechanisms in MCPH17 primary microcephaly: a preliminary study on the effects of a postnatal antioxidant treatment

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Microcephaly is a rare neurological condition that results from brain development disruption. The microcephaly 17 (MCPH17) is caused by the loss-of-function of Citron-kinase (Cit-K), a cytoskeleton regulator involved in cell division and DNA repair. Consistent with MCPH17 phenotype, Cit-K KO mice display a strong reduction of brain size and widespread dysmyelination. At the behavioral level, Cit-K KO mice show ataxia and seizures that lead to precocious lethality (i.e. between postnatal day (P) 12 and P16). Cit-K KO neuronal and glial progenitors show multiple alterations, including DNA damage and multinucleation, and eventually undergo apoptosis or enter cell senescence. The activation of the DNA damage response is known to induce the production of reactive oxygen species (ROS), that, in turn, can affect cytoskeleton stability, thereby resulting in cytokinesis failure and multinucleation. Thus, in search for druggable mechanisms that could be exploited to rescue Cit-K KO mouse phenotypes, we focussed on oxidative stress. Indeed, Cit-K KO brain tissue showed reduced expression of Nuclear factor erythroid 2-related factor 2 (Nrf2), a crucial regulator of cell anti-oxidant defenses, and, consistently, increased levels of ROS. The postnatal (i.e. from P0 to P10) daily administration of N-acetylcysteine (*NAC*), an FDA-approved glutathione precursor, reduced oxidative stress and senescence-associated markers in Cit-K KO mouse brain. Percentages of multinucleated and apoptotic oligodendrocyte progenitor cells (OPCs) were also significantly reduced and resulted in a 2.5 fold increase in OPC density in Cit-K KO cerebral cortex. Despite that, myelination was not reinstalled in vivo at P10, whereas myelin was detected ex-vivo when NAC treated organotypic brain slices were maintained in vitro for other 7 days. Analyses are ongoing to assess NAC effectiveness in reverting more broadly Cit-K KO mouse neuroanatomical and behavioral phenotypes, and in extending their life span.

PN13 | Cracking Brain Development: Chick Embryo Model and Neuropharmacology Studies

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The background for this work is the present international lack of knowledge about the possible long-term effect of pharmaceuticals on neurodevelopment. We are conducting experimental research *in vivo* (chicken egg) and *vitro* (chick cortical and cerebellar neuron cultures). The project addresses adverse effects on the brain development of pharmaceuticals commonly used by pregnant women, including antidepressants (escitalopram, venlafaxine) and antiepileptic (valproic acid, lamotrigine) drugs. The main aim is to identify deviations and effects of the pharmaceuticals on neurodevelopment. The whole-brain and cerebellar histological analysis following after drugs at the different stages of the development and for different exposure periods *in ovo* was applied. This model will bring us to a more systemic approach in this scientific problem. *In vitro* studies with different conditions (single-cell and neurospheroid seeding) was utilized here as well. The neurite outgrowth and branching, together with clustering and migration – assessed with time-lapse IncuCyte system. Cell viability, glutathione assay, ROS-assay, Ca-influx, mRNA and protein changes in neurons were examined during neuronal maturation and drug treatments. Even though the 3D approach to enable mimic physiological microenvironment seems to be appealing for pharmacotoxicological investigation, it's still lacking the systemic conditions i.e. whole body surroundings. Chicken embryo provides a trustable and adequate alternative for *in vivo* studies of pharmaceuticals, and with additional *in vitro* supplementation, we access new directions in developmental neuropharmacological studies.

PN14 | SINEUPs: a novel molecular therapeutic strategy for the treatment of developmental and epileptic encephalopathies caused by pre-synaptic genes haploinsufficiency – preliminary in vitro results

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STXBP1-encephalopathy is a broad neurological disorder caused by mutations in the pre-synaptic gene STXBP1, crucially involved in neurotransmitter release at the synapse. The core symptoms of this disorder include intellectual disability, epilepsy, movement disorders and autism spectrum disorders. Nowadays, the therapeutic approach is mainly symptomatic. We propose a new targeted therapeutic approach in the wake of precision medicine. SINEUPs are a class of natural and synthetic non-coding RNA composed of a Binding Domain (BD) specifically binding an mRNA's ATG and an Effector Domain (ED) which increases the translation by 1,5-5 fold. These compounds can be used to specifically increase the protein levels in case of haploinsufficiency due to loss-of-function mutations. Here we present the preliminary results of an in vitro study of these compounds to rescue STXBP1 protein levels in case of haploinsufficiency. Transfection controls experiments (n=3) with GFP and SINEUP-GFP (positive controls) in a human neuroblastoma cell line showed a 3-fold increase in GFP levels at Western blot at 48h. The pilot transfection with 3 STXBP1-SINEUPs at 48h showed a 1.2 to 1.8 fold increase in STXBP1 levels (normalized per GAPDH and related to the NCs), when compared to the NCs and the Not-Treated samples. These preliminary results prove the SINEUPs' potential to specifically increase the protein levels without impacting on the genome. We are going to perform several tests at different time-points and then test in mutated patients' neurons differentiated from induced pluripotent stem cells, to evaluate the functional rescue potential of SINEUPs on the synaptic vesicles release and recycling. This is an extremely flexible approach to target many developmental and epileptic encephalopathies caused by haploinsufficiency, and therefore to address these diseases in a more tailored and radical way.

PN15 | Unravelling the role of electrical activity on cerebral cortex development and disease

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Cerebral cortex development relies on spatiotemporally coordinated events that involve the execution of intrinsic molecular programs and activity-dependent processes, both contributing to the extraordinary cell diversity and circuit complexity characteristic of the mature cortex. While activity-dependent processes were known to be involved in neuronal maturation and circuit assembly, recent data on bioelectric membrane properties of cortical progenitors/stem cells – non excitable cells- provide new insights on how electrical activity can modulate the transcriptional programs of developing cortical neurons. Our project aims at dissecting the contribution of activity-dependent processes on both cortical progenitors' commitment and specific neuronal subtype differentiation trough *in vivo* genetic manipulation of distinct channel activity. Specifically, by increasing (m*NaChBac,* voltage-gated sodium channel) or silencing (*Kir2.1,* inward-rectifier potassium channel) cell intrinsic excitability, we will investigate the effect of distinct electrical shifts on neuronal laminar positioning, molecular differentiation, morphological features and circuit properties of cortical subtypes. In addition, by finely modulating the expression of specific channels (hyperpolarization-activated, cyclic nucleotide-gated, non-specific cation channel 1, HCN1) known to control synaptic excitability and associated with epileptic infantile early encephalopathy 24, we will dissect their effects on overall cortical development both at the molecular and electrophysiological level, to eventually unravel the molecular bases of this disorder. By combining single cell RNA sequencing technology and *in utero* experimental activity perturbations, we will also identify cell-extrinsic effects on neuronal and non-neuronal cell types, providing a comprehensive map of activity-dependent processes in cerebral cortex development and disease.

PN16 | When perinatal programming goes wrong: a case of intra-ventricular hemorrhage

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The Developmental Origins of Health and Disease (DOHaD) states that status of health and disease of an individual is affected by any adverse event that may occur before and during pregnancy. Also known as perinatal programming, it means that mother's conditions (diseases, diet, drugs assumption) during pregnancy may shape the health outcomes of her offspring. Here we present a case of perinatal programming that had severe adverse effect on newborn's brain: ventricular hemorrhage at birth. The mother had a very severe chorioamnionitis with smelling amniotic fluid at birth and the newborn was born prematurely due to membrane ruptures. An ultrasound performed during pregnancy showed an asymmetry between the right and the left heart ventricles. The newborn was born at 27 weeks of gestational age and was admitted to the Neonatal Intensive Care Unit of the Policlinico di Monserrato (Cagliari) due to respiratory distress. The neonate was intubated and experienced sepsis a couple of days after birth (treated with two antibiotics). A sonogram performed immediately after birth showed an intra-ventricular hemorrhage on the right dilatation of ventricular body and a parietal-occipital periventricular venous infarction on the left. During the hospitalization the newborn presented tri-ventricular post-hemorrhagic hydrocephalus, cystic leukomalacia and intra-cranial hypertension. The neonate was then transferred to another hospital to receive proper surgical treatment to drain the blood and to lower the intracranial hypertension. In our case the adverse event is a severe pathology that affected the placenta that was crucial in determining such an adverse neurological outcome of the newborn. In the future, it would be desirable to monitor the women at risk of developing placental pathologies even during the preconceptional period in order to prevent these episodes, shape the perinatal programming for the better and guarantee the best possible health status for these little patients.

ND01 | A CXCR4 receptor agonist strongly stimulates axonal regeneration after sciatic nerve damage

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The peripheral nervous system has retained through evolution the capacity to regenerate, but little is known on the inter-cellular signals involved in its functional recovery from trauma, autoimmune attacks, or neurotoxins. We recently found that the CXCL12α-CXCR4 axis plays an important role in the functional recovery of the neuromuscular junction (NMJ) after nerve terminal degeneration, and that its exogenous stimulation potently promotes NMJ regeneration after localized nerve damage induced by the pore-forming toxin alpha-Latrotoxin. This evidence prompted us to test the possibility that the expression/activation of the CXCL12α-CXCR4 axis takes place also in more severe peripheral nerve injuries. We chose the sciatic nerve cut/crush models as they closely recapitulate the most frequent nerve lesioning occurring in humans. We found that both CXCL12α and CXCR4 are re-expressed in the proximal part of the lesioned nerve at an early stage after injury. The former is specifically produced by Schwann cells, the latter is in the re-growing axons. Notably, they are involved in axon regeneration as either AMD3100, a CXCR4 antagonist, or an antibody neutralizing CXCL12α significantly delay the process. Most importantly, we found that the exogenous stimulation of this axis by NUCC-390, a specific small molecule agonist of CXCR4, strongly promotes the recovery of neuromuscular activity and the anatomical regeneration of axons of the crushed nerve. Given the ongoing intense research for novel therapies for injured peripheral neurons, the present results have important implications in the effort to find novel protocols to improve recovery of function after different forms of motor axon terminal damage, and propose NUCC-390 as a strong candidate to be tested in human therapy.

ND02 | Combined RNAi and gene therapy targeting MFN2 for the treatment of Charcot-Marie-Tooth 2A (CMT2A)

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Charcot-Marie-Tooth type 2A (CMT2A) is an inherited sensory-motor axonopathy caused by missense mutations in the *MFN2* (*Mitofusin2*) gene, transmitted with an autosomic dominant pattern. *MFN2* mutations seem to induce disease through a "dominant-negative" mechanism, meaning that the expression of wild-type *MFN2* allele is negatively regulated by the mutant protein. MFN2 mutations have been characterized and numerous mechanisms proposed to unravel CMT2A pathophysiology; however, no cure for this devastating disease is available up to now. Gene therapy for dominantly inherited diseases with RNA interfering (RNAi) requires mutant allele-specific suppression when mutated gene normally have an important role. Here, we propose a strategy for selective suppression of mutant alleles; both mutant and wild-type alleles are inhibited by most effective shRNA, and wild-type protein is restored using mRNA designed to be resistant to the shRNA. In particular, we demonstrated the effective silence of the endogenous MFN2 and the its replacement with an exogenous copy of the wild-type MFN2 gene in CMT2A human induced pluripotent stem cells (iPSCs)-differentiated motor neurons (Rizzo et al 2016) and in Mitocharc1, a mouse model of CMT2A (Cartoni et al., 2010). To evaluate the amelioration of the disease phenotype after this strategy, we analyzed key motoneuronal features relevant to CMT2A, observing an enhancement in mitochondrial distribution and function, and in apoptotic and autophagic parameters. Our data confirm the feasibility of combined RNAi and gene therapy approach as potential therapeutic strategy for treating CMT2A and other genetic neurological disorders.

ND03 | A novel HCN2 mutation associated with progressive epileptic encephalopathy

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A novel mutation was found in *HCN2* gene encoding for the hyperpolarization-activated cyclic nucleotide-gated channel 2. The proband, now aged 7 years old, presents a congenital encephalopathy characterized by drug resistant epilepsy, severe developmental delay, ataxia, dystonia and cerebral visual impairment. The onset of epilepsy was marked by a convulsive status epilepticus at 5 months of age; since then, the patient presented recurrence of fever-triggered generalized seizures, in many cases featuring status epilepticus. The mutation affected an aminoacid located in the transmembrane S6 helix (p.Gly460Asp) and is carried in heterozygosis. To describe the functional consequences of the mutant channel, whole-cell patch-clamp experiments were performed in HEK293 cells expressing HCN2 wild type (wt) or mutant channel. Heterozygotic condition was mimic through a coexpression of the same amount of plasmid encoding for the wt or the mutant form of the channel. A complete abolishment of the current density was observed considering the mutant compared to the wt channel (-9.9±1.2 pA/pF n=20 vs -31.2±8.4 pA/pF n=44 respectively; $p<0.05$). A significant reduction of the current density was also found when heterozygotic condition was tested compared to the wt one (-20.1±5.1 pA/pF n=35; $p<0.05$). Both wt and heterozygotic channels share overlapping activation curves ($V_{1/2}$ and k -92.9±0.3 mV and 5.6±0.3 n=29 vs -91.6±0.2 mV and 5.6±0.2 n=16 respectively). No significant differences were present in the kinetics of both activation and deactivation of the wt and of the heterozygotic channel. In conclusion, the mutation seems to act as a loss-of-function that could potentially affect the control of neuronal excitability and therefore be linked to the proband pathological condition.

ND04 | Investigating the role of disease risk genes as modulators of microglial function

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Microglia are the innate immune cells of the central nervous system (CNS). Microglia not only play crucial roles in brain development, repair and plasticity, but they are also critically involved in neurodegenerative processes, with recent studies suggesting that these cells can be responsible for pathological synapse loss. Genome wide associations studies (GWAS) reveal that hundreds of genetic variants associated with neurodegeneration are indeed highly expressed in microglia. However, how these variants regulate microglia-mediated synapse loss in neurodegeneration, thus affecting synaptic function, remains to be elucidated. Therefore, we selected a set of highly expressed microglial genes, whose SNPs (single nucleotide polymorphisms) have been associated with dementia, to investigate their role as modulators of microglial functions. Here we use a loss-of-function approach to assess how each specific candidate regulates basic microglial properties, and whether such alterations may impact on microglia-mediated synapse remodeling. To this aim, CRISPR/Cas9 knockout (KO) is performed on primary microglia isolated from mice expressing Cas9 upon CRE-recombination, under the microglial CX3CR1 specific promoter. First, cell-autonomous effects of the KO are investigated by means of phagocytic and intracellular degradation assays; in addition, cytokines expression and release are measured. Second, co-culture of KO microglia with wildtype mouse primary neurons is performed to investigate the consequent effects of microglial alterations on synaptic integrity and function. In conclusion, this project aims at dissecting the microglial specific contribution to synapse loss in the pathogenesis of neurodegeneration.

ND06 | Role of Sox2 in the neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion

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After Quinolinic Acid lesion in mouse, parenchymal astrocytes in the striatum undergo a spontaneous neurogenic activation and generate neuroblasts locally. Yet, the mechanisms that drive this response are unclear. Through genetic lineage tracing we show that, after neurogenesis onset, striatal astrocytes continuously and asynchronously transit from quiescence to a neurogenic active state giving rise to sparse independent niches. Moreover, we provide evidence that the switch of striatal astrocytes from a quiescent to a neurogenic state depends on the transcription factor Sox2 within an early post-injury time window, after which Sox2 is dispensable. These data suggest that Sox2 is necessary to prime astrocytes for the neurogenic competence and that after the acquisition of this competence, Sox2-independent mechanisms govern the execution of the neurogenic program. Mechanisms implicated in Sox2-dependent priming and in the further execution of the neurogenic competence are currently under investigation. Of note, the abrogation of Sox2 in astrocytes profoundly modifies the whole striatal neuroinflammatory profile, suggesting that also Sox-2 dependent non-cell autonomous factors may take part in the regulation of neurogenesis from striatal astroglia. Overall these results support a model where the awakening of striatal astrocyte neurogenic competence and the transition to a neurogenic active state are dissociable components of a complex multi-step process.

ND07 | Deciphering the complex interplay between LRRK2 and p21-activated kinase 6 (PAK6)

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Leucine-rich repeat kinase 2 (LRRK2) is a serine-threonine kinase widely expressed in the brain as well as in peripheral tissues. Mutations in *LRRK2* gene cause autosomal dominant forms of Parkinson's disease (PD). The most common G2019S mutation, which occurs in 5% of familial cases of PD, increases kinase activity, but also other pathogenic mutations confer a gain of kinase function. We previously showed that there is a close relationship between LRRK2 and p21-activated kinase 6 (PAK6), a kinase highly expressed in nerve cells. PAK6 interacts with the ROC/GTPase domain of LRRK2 to stimulate neurite complexity in mammalian brain. Intriguingly, a constitutively active form of PAK6 rescues the G2019S LRRK2-associated neurite shortening phenotype. Based on these findings, we investigated whether PAK6 activity is altered in mutant LRRK2 mouse brains. Interestingly, our data show that PAK6 phosphorylation is significantlyaltered in cortex, midbrain and striatum of different mutant LRRK2 mice in which LRRK2 is hyperactive, estimated by increased S1292 autophosphorylation. Furthermore, we recently showed that PAK6 promotes LRRK2 dephosphorylation at Ser935, via phosphorylation of 14-3-3 gamma, a well-characterized LRRK2 interactor. Independent studies also show that LRRK2 dephosphorylation leads to the relocalization of the kinase into defined cellular clusters of uncertain identity. Based on this, we investigated whether PAK6-mediated LRRK2-dephosphorylation is responsible for LRRK2 subcellular localization. Our data show that LRRK2 relocalizes into perinuclear clusters in the presence of PAK6 and that this effect is mediated by PAK6 kinase activity. Taken together these data suggest that there is a tight crosstalk between these kinases since they are able to influence the phosphorylation state and the activity of each other. Understanding the molecular mechanism underlying these processes may help to find novel therapeutic approaches for the treatment of PD.

ND08 | Melatonin promotes regeneration of injured motor axons

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Melatonin (Mel) is a hormone produced by the pineal gland in a photoperiod controlled mode, and released in the blood flow. It is a major regulator of the sleep/wake cycle via its binding to G-protein coupled receptors (MT_1/MT_2). Once in the body fluids Mel displays a broad range of actions: circadian rhythm regulator, free radical scavenger, anti-oxidant and immunoregulating molecule. A neuroprotective activity of Mel has been described in a variety of neuronal models, mainly attributed to its direct antioxidant action, but less attention has been dedicated to its possible contribution to nerve regrowth and neuroregeneration. To dissect the role of Mel in peripheral nerve regeneration we first exploited an innovative experimental model set up, based on the neurotoxic action of the α-latrotoxin. This presynaptic neurotoxin causes the rapid and selective degeneration of motor axon terminals without inflammation, with complete recovery within a week, thus providing an ideal model to investigate the molecular determinants of nerve repair. Indeed, using this model system we recently identified hydrogen peroxide and chemokines as important molecules involved in the rescue of function of the injured neuromuscular junction. We have also tested the activity of Mel in well-established forms of prolonged damage (compression and transection of the sciatic nerve). In both models Mel promotes motor axon re-growth and peripheral neuroregeneration in a receptor-mediated fashion. This pro-regenerative action is at least in part due to a sustained activation of the ERK1/2 pathway, and it is mediated by the interaction with Mel receptors, as incubation with luzindole, a non-selective receptor antagonist, slows down neurotransmission rescue upon peripheral damage. Our study reveals a receptor-mediated, pro-regenerative action of Mel which holds important clinical implications, as it posits Mel as a safe candidate molecule for the treatment of peripheral neurodegenerative conditions.

ND09 | IGHMBP2 related pathological pathways in Spinal Muscular Atrophy with Respiratory Distress type 1 (SMARD1) in vitro models

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Spinal Muscular Atrophy with Respiratory Distress type 1 (SMARD1) is an infant neurodegenerative disease manifesting within the first month of life with progressive distal muscular weakness and irreversible diaphragmatic palsy. Since no therapy is available, the deepening of its pathogenic mechanisms is crucial to assess new therapeutic targets and strategies. SMARD1 is caused by mutations in the *IGHMBP2* gene that determine deficiency of the encoded protein Immunoglobulin μ-binding protein 2 (IGHMBP2), eventually leading to motor neurons (MNs) degeneration and disease onset. IGHMBP2 appears to have many different functions, but the actual mechanism through which its mutations cause the disease remains elusive. Hence, we explored the effects derived from IGHMBP2 reduction in *in vitro* models, that are induced-Pluripotent Stem Cells (iPSCs), iPSC-derived MNs and *IGHMBP2*-silenced *SH-SY5Y.* We observed an increase of apoptosis, and enhancement of DNA damage response pathway, presumably related to R-loops aberrant persistence, indicating a possible connection between IGHMBP2 deficiency and genome instability that finally lead to cell demise. In addition, gene therapy with *IGHMBP2* delivery determined a decrease of apoptotic rate and DNA damage response-related protein levels, as well as less R-loops accumulation, further suggesting a link between IGHMBP2 and genome instability. Our findings lead to speculate the involvement of IGHMBP2 in aberrant R-loops resolution, a novelty that may contribute to address research towards new therapeutic targets for SMARD1.

ND10 | Deep RNA and DNA sequencing to support the clinical diagnosis in neurodegenerative diseases

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The differential diagnosis between Alzheimer's disease (AD) and Dementia with Lewy Body (DBL) remains a big challenge to prognose correctly patients with dementia. Here we analyzed through Next Generation Sequencing (NGS) and RNA-sequencing (RNA-seq) two patients, mother and son, diagnosed for AD in life but resulted to be AD and DBL respectively after neuropathological examination, to unravel the molecular factors that may support clinical diagnoses. NGS testing (SureSelect^{QXT} Target Enrichment, Agilent Technology) was performed on peripheral blood DNA, using a customized panel of over 6000 genes associated to mendelian disorders and to inherited neurodegenerative diseases. Variants were screened according to hereditary hypothesis. Total RNA from hippocampus, parietal lobe, substantia nigra and basal ganglia was extracted and RNA-seq analysis was done (SENSE Total RNA-Seq, Lexogen). Differentially Expressed Genes (DEGs) were identified via R package DESeq2. We found seven variants shared by mother and son. Among them a disruptive inframe insertion c.12948_12950dupAAG in RYR2 gene. We also found six variants present only in the son. In particular, the missense variant c.2990T>C in HDAC4 gene and the splice variant c.328+6A>G in GALC. DEG analysis from RNA-Seq revealed a dysregulation in the mother's hippocampus and in the son's substantia nigra, with a 10-fold higher number of DEGs. With this study we identified either genetic factors that may explain the AD and the DBL phenotypes and deregulated transcripts in AD- and DBL-specific brain areas, highlighting the importance of DNA and RNA deep sequencing to support differential diagnoses of complex neurodegenerative pathologies.

ND11 | Assessing fibre specific myelin content using microstructure informed tractography

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Diffusion magnetic resonance imaging tractometry is a widely used tool to investigate differences in white-matter tracks integrity between healthy subjects and neurological patients. Its potential is due to the capability of averaging microstructural tissue properties (obtained from voxel-wise maps of any imaging modality) along streamlines recovered with tractography. Nonetheless, the average of a microstructural measure is a weak information about an entire bundle of streamlines if this crosses with other bundles in a significant portion of voxels. The recently proposed Convex Optimization Modeling for Microstructure Informed Tractography (COMMIT) framework is able to recover bundle's specific tissue properties by estimating the actual contribution to the signal of each individual streamline using convex optimization. The only hypothesis behind is that the microstructural property under investigation is constant along a given streamline (which is the representative trajectory of a coherent set of axons) and additive in each voxel traversed by the streamlines. We extended COMMIT by considering the myelin water fraction (MWF), that can be estimated from T2 images. The new model assigns MWF contribution to every streamline proportionally to its length inside each voxel and the total amount of streamlines traversing a voxel must sum up to the MWF value measured in it. We tested our approach on a synthetic phantom with crossing bundles having different MWF contributions as well as in-vivo data of a healthy subject. Results on synthetic data show that COMMIT estimates the correct MWF of each individual streamline, while the values computed with tractometry are different from the ground truth. On in-vivo data the obtained tractometry connectomes were flat (clear consequence of the averaging operation) whereas COMMIT ones can distinguish bundles with more MWF than others. For the first time we decoupled the MWF contributions of each individual streamline in a tractogram.

ND12 | Urocortin 2 promotes functional recovery of degenerated nerve terminals

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The neuromuscular junction (NMJ) is a specialized synapse controlling locomotion, composed of a motor axon terminal (MAT), perisynaptic Schwann cells (PSC) and a muscle fibre. An intense interplay among them takes place during injury, allowing regeneration to occur. The NMJ has retained the ability to regenerate, at variance from central synapses; hence the search for molecules that promote regeneration is ongoing. We recently performed a transcriptome analysis of murine NMJs after degeneration induced by a spider neurotoxin, α-latrotoxin, that elicits an acute, localized and reversible MAT degeneration. This model is not accompained by inflammation, facilitating the identification of molecules involved in the process. Among the many differentially expressed transcripts, the mRNA encoding urocortin 2 (Ucn2) is strongly up-regulated during degeneration and decreased upon regeneration, suggesting a possible role of the molecule. Ucn2 is a neuropeptide of the corticotropin-releasing factor peptide family, involved in the physiological response to stress. It exerts a peripheral function by binding selectively to a G protein-coupled receptor, the corticotropin releasing hormone receptor 2 (CRHR2), apparently associated to neural structures within skeletal muscles. We found expression of CRHR2 on cultured Schwann cells, where Ucn2 induces MAPK activation, a signaling pathway essential to guide nerve regeneration. We also observed that Ucn2 promotes axon growth of cultured spinal cord motor neurons. Thus we tested the ability of exogenous Ucn2 to promote neurotransmission rescue of α-ltx degenerated MATs, finding that it accelerates NMJs functional recovery. We are currently trying to identify the target of Ucn2 in vivo and to extend our study to additional models of peripheral injuries such as cut or crush of the sciatic nerve. These findings may have important implications in the effort to promote functional recovery after different forms of motor axon damage.

ND13 | CPPs-conjugated antisense nucleotides: a new therapeutic strategy for Spinal Muscular Atrophy symptomatic patients

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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*, the paralogous gene, to produce a functional SMN protein. In our laboratory, a specific sequence of the antisense oligonucleotide Morpholino (MO) against the ISS-N1 region of SMN2 has been successfully tested in pre-symptomatic SMA mice. However, our MO-10-34 treatment, like other treatments tested for SMA, shows efficient results only if administered in a pre-symptomatic phase. To develop a functional treatment for symptomatic patients, cell-penetrating peptides (CPPs) can be conjugated to ASOs, allowing the crossing of the blood-brain barrier (BBB). This approach has been preliminarily explored with MO conjugated with four different peptides that were administered in pre-symptomatic mice, demonstrating the major efficacy of r6 and RXR, on which we focused further experiments. To verify the ability of MO-conjugated to CPPs to cross the BBB and ameliorate SMA mice phenotype and neuropathological features compared to unconjugated-MO, two group of symptomatic mice 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 the second group was sacrificed at p30, spinal cord and intercostal muscles were harvested and analysed by immunofluorescence for the presence of motor neurons and innervated neuro muscular junctions (NMJs). 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.

ND14 | The antipsychotic amisulpride utilises plasma membrane monoamine transporter at the blood-brain barrier: implications to the heightened sensitivity to antipsychotics observed in Alzheimer's disease

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Alzheimer's disease (AD) patients with psychosis show an increased sensitivity to antipsychotics, including amisulpride. There is evidence that disruption of the blood–brain barrier (BBB) integrity with AD mediates the heightened sensitivity. We studied the impact of the solute carrier transporters: plasma membrane monoamine transporter (PMAT), multi-antimicrobial extrusion proteins 1 and 2 (MATE1 and MATE2) on amisulpride transport in human cerebral microvessel endothelial cells/D3 (hCMEC/D3) *in vitro*. hCMEC/D3 cells were incubated with [^{3}H] amisulpride (3.8-7.7 nM) and [^{14}C]sucrose (0.7-1.5 µM) with or without inhibitors for PMAT, MATE1 and MATE2. PMAT inhibition led to a significant increase in [^{3}H] amisulpride cell accumulation ($F(1, 58) = 16.33$, $p=0.0002$) after 20, 30, 60 and 120 minutes ($p=0.038$, $p=0.0237$, $p=0.0010$, $p=0.0001$, respectively). MATE1 and MATE2 inhibition did not cause an effect. *In vivo*, we investigated the BBB transport of amisulpride in 5xFamilial Alzheimer's mouse model (5xFAD), and in age matched wild type mice (WT, C57/BL6). The presence of amyloid plaques was confirmed in 5 and 12 months old 5xFAD mice by transmission electron microscopy. No difference in the endothelium was observed between WT and 5xFAD mice. 5xFAD and WT mice (12-15 months old) were anaesthetised and perfused with artificial plasma, containing [^{3}H]amisulpride (6.5 nM) and [^{14}C]sucrose (9.4 µM). Compared to WT, the 5xFAD mice showed increased [^{3}H]amisulpride uptake in the striatum by 79% ($t=1.975$, $df=11$, $p=0.0370$), whereas the passive permeability measure [^{14}C]sucrose was not significantly changed. Western blots confirmed PMAT expression in hCMEC/D3 cells, and in WT and 5xFAD brain capillaries. PMAT is implicated in the BBB transport of amisulpride. The increased brain permeability to amisulpride in 5xFAD mice suggests altered BBB transporter function, possibly due to changes in PMAT expression with AD. This may explain the heightened sensitivity to antipsychotics in AD.

ND15 | P2X7 activation enhances skeletal muscle metabolism and regeneration in SOD1G93A mouse model of Amyotrophic Lateral Sclerosis

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Muscle weakness plays an important role in neuromuscular disorders comprising Amyotrophic Lateral Sclerosis (ALS). However, it is not established whether muscle denervation originates from the motor neurons, the muscles or more likely both. Previous studies have shown that the expression of the SOD1G93A mutation in skeletal muscles causes denervation of the neuromuscular junctions, inability to regenerate and consequent atrophy, all clear symptoms of ALS. In this work, we used SOD1G93A mice, a model that best mimics some pathological features of both familial and sporadic ALS, and we investigated some biological effects induced by the activation of the P2X7 receptor in the skeletal muscles. The P2X7, belonging to the ionotropic family of purinergic receptors for extracellular ATP, is abundantly expressed in the healthy skeletal muscles, where it controls cell duplication, differentiation, regeneration or death. In particular, we evaluated whether an in vivo treatment in SOD1G93A mice with the P2X7 specific agonist 2'(3')-O-(4-Benzoylbenzoyl) adenosine5'-triphosphate (BzATP) just before the onset of a pathological neuromuscular phenotype could exert beneficial effects in the skeletal muscles. Our findings indicate that stimulation of P2X7 improves the innervation and metabolism of myofibers, moreover elicits the proliferation/differentiation of satellite cells, thus preventing the denervation atrophy of skeletal muscles in SOD1G93A mice. Overall, this study suggests that a P2X7-targeted and site-specific modulation might be a strategy to interfere with the complex multifactorial and multisystem nature of ALS.

ND16 | Posttranslational modifications of tubulin in dementia

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Alzheimer's Disease (AD) and Vascular Dementia (VaD) are the two most common causes of dementia in older people. Together they account for 80-90% of all dementia cases. Previous work in my laboratory has shown that the hallmark of AD, accumulation of amyloid beta (Aβ) protein is also present in VaD, The second hallmark of AD are tau tangles and hyperphosphorylated tau, a microtubule associated protein (MAP). When tau is phosphorylated, it loses its ability to bind and stabilise the microtubules (MTs), the main protein filaments of the cytoskeleton of all eukaryotic cells. Although there is no hyperphosphorylation of tau in VaD, there is a decrease of total tau in the temporal lobe. However, the fate of MTs in either AD or VaD is still unknown. Apart from that, my group has also shown a decrease in neuronal volume in the dorsolateral prefrontal cortex (DLPFC) of people with dementia when compared to controls. In addition, there was a loss of tubulin but not tau in patients with VaD. Thus, we hypothesize that loss of tubulin could be an early molecular marker of VaD. Not only can MAPs stabilise MTs, but MTs can stabilise themselves due to the tubulin posttranslational modifications (PTM). There are more than ten tubulin PTMs, either specific or non-specific. Out of all of them, accumulation of polyglutamylation has been observed in regions of the nervous system that undergo degeneration in the *pcd* mouse model with a significant loss of Purkinje cells. Furthermore, it has been shown that if there are more than three glutamyl residues on the tubulin, tau's ability to bind to MTs decreases. I want to determine whether there is accumulation of polyglutamylation in the temporal and frontal lobe of different types of dementia, and if so, if there is a specific pattern in each of them. I also want to study the enzymes taking part in the modification to know whether there is overexpression of glutamylating enzymes or downregulation of deglutamylating enzymes.

ND17 | Circular RNAs from Alzheimer´s disease-related genes in entorhinal cortex

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Introduction: circular RNAs (circRNA) are a novel class of noncoding RNAs characterized by a covalently closed loop structure between 3'and 5' end, which makes them more stable than linear RNAs. circRNAs are abundant in different human tissues but they are particularly enriched in neuronal tissues. Several lines of evidence suggest that circRNAs have important regulatory functions such as microRNA sponge, transcriptional regulators, interaction with proteins and so on. Since epigenetic mechanisms are involved in Alzheimer's disease (AD), we hypothesized that circRNAs generated from AD-related genes are expressed in brain regions vulnerable to AD, such as entorhinal cortex. Indeed, a few circRNAs have been described for *APP* and *ADAM10* through next generation sequencing in different cells lines and tissues.

Methods: We isolated RNA from entorhinal cortex of human brain and from HMC3 cells with miRNAeasy mini kit (Qiagen). Next, we performed a retrotranscription (RT)-PCR for circRNAs of *TREM2*, *ADAM10* and *APP,* as follows. For cDNA conversion we used 500ng of RNA and SuperScript™ III First-Strand Synthesis SuperMix Transcriptase kit (Invitrogen). For PCR we used Go Taq® G2 DNA Polymerase (Promega) and primers were designed with primer3 software. Agarose gel electrophoresis (1.8%) was used to select the candidate bands which were further purified with Wizard® SV Gel and PCR Clean-Up System (Promega) and sequenced by Sanger method. UCSC Genome Browser software was used to align the circRNA sequence.

Results: We validated one circRNA each from *APP* (hsa-circ-0007556) and *ADAM10* (hsa-circ-00056) genes. Moreover, we identified 2 novel circRNAs from *TREM2* gene, an exonic circRNA and an exonic-intronic circRNA.

Conclusion: The reliability of our method was confirmed by validating previously described circRNAs in the entorhinal cortex and in HMC3 cells. Interestingly, we described for the first time circRNAs originated from *TREM2*, a risk gene for AD.

ND18 | A Smart Region Growing algorithm for isolating single neurons in confocal datasets

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Accurately digitizing the brain at the micro-scale is crucial for investigating brain structure-function relationships and documenting morphological alterations due to neurodevelopmental or neurodegenerative diseases. Doubtless, confocal and two-photon microscopy are the best candidates to image defined cellular populations in three-dimensional (3D) biological specimens. Their imaging depth, as well as the quality of the acquired datasets can be further improved thanks to recent tissue-clearing solutions, which render brain tissue transparent to photons by reducing the source of scattering, allowing confocal acquisitions with enhanced Signal to Noise Ratios and Contrast to Noise Ratios while maintaining low laser power. While these emerging technologies and protocols, combined with fluorescence-based labelling techniques, enable the imaging of the brain's intricacies at the microscale, single-cell segmentation algorithms able to deal with these datasets are still lacking, despite targeted initiatives such as the DIADEM (DIgital reconstructions of Axonal and DEndrite Morphology) challenge in 2009-2010 and the BigNeuron project in 2015. Indeed, many algorithms for neuron segmentation primarily focus on sparsely labelled data, while few tools were purposely developed for processing images (or volumes) representing densely packed neurons, typical of mammalian brains. Here we present a new Smart Region Growing (SmRG) algorithm for the segmentation of single neurons in their native arrangement within the brain. Its Region Growing procedure is based on a homogeneity predicate determined by describing the pixel intensity statistics of confocal acquisitions with a mixture model, enabling an accurate reconstruction of complex 3D cellular structures from high-resolution images of neural tissue. The algorithm's outcome is a 3D matrix of logical values identifying the voxels belonging to the segmented structure, thus providing additional useful volumetric information on neurons. To highlight the algorithm's full potential, we compared its performance in terms of accuracy, reproducibility, precision and robustness of 3D neuron reconstructions based on microscopic data from different brain locations and imaging protocols against both manual and state-of-the-art reconstruction tools.

ND19 | Herpes simplex virus-1 (HSV-1) infection induces a potent but ineffective IFN-λ production in immune cells of AD and PD patients

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Herpes Simplex Virus type 1 (HSV-1) is suspected to play a role in Alzheimer's (AD) and in Parkinson's (PD) diseases: several evidences lead to hypothesize that an immunological responses incapable to counteract viral reactivation might contribute to AD pathogenesis. IFN-lambda (IFN-λ), one of the cytokine endowed with a robust antiviral activity, might play a crucial role in containing HSV-1 reactivation. Productive HSV-1 infection requires the sequential activation of different families of genes, immediate early (IE), early (E) and late (L) genes. HSV-1-induced IFN-λ, IL-10 and IL-1β cytokines responses as well as the expression of viral IE, E and L genes were analyzed *in vitro* in peripheral blood mononuclear cells (PBMCs) obtained from in AD patients, PD patients as well as in healthy controls (HC), infected by one multiplicity of infection (1 MOI) HSV-1. Results showed that upon HSV-1 infection: 1) transcription of IE (*ICP0*, *ICP27*) genes was reduced whereas that of E (*UL41, UL29*) and L (*UL48, LAT*) genes was increased in AD and PD compared to HC, and 2) IFN-ʎ mRNA and serum concentration were increased in AD and PD compared to HC. Analyses of pro- and anti-inflammatory cytokines production by the same cells showed that IL-1β was increased and IL-10 was reduced in unstimulated AD and PD; HSV-1 stimulation resulted in a significant increase of IL-10 production in HC alone. Data herein show that a pro-inflammatory condition is present in AD and PD, in whom attempts to obstacle viral replication via an initial, possibly more potent IFN-ʎ mediated control of IE viral genes is unsuccessful.

ND20 | VARS2-linked mitochondrial encephalopathy: two case reports enlarging the clinical phenotype

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Mitochondrial respiratory chain consists of five complexes encoded by nuclear and mitochondrial genomes. Mitochondrial aminoacyl-tRNA synthetases are key enzymes in the synthesis of such complexes. Bi-allelic variants of *VARS2*, a nuclear gene encoding for valyl-tRNA (Val-tRNA) synthetase, are associated to several forms of mitochondrial encephalopathies or cardiomyoencephalopathies. Among these, the rare homozygous c.1100C>T (p.Thr367Ile) mutation variably presents with progressive developmental delay, axial hypotonia, limbs spasticity, drug-resistant epilepsy leading, in some cases, to premature death. Yet only six case harbouring this homozygous mutation have been described worldwide. Hereby, we report two additional cases of two non-related young girls from Sardinia, born from non-consanguineous and healthy parents, carrying the aforesaid variant. At onset both the patients presented with psychomotor delay, muscle hypotonia and brisk tendon reflexes. Standard genetic tests were normal, as well as metabolic investigations. Brain MRI showed unspecific progressive abnormalities, such as corpus callosum hypoplasia in one patient and cerebellar atrophy in both. Diagnosis was reached by adopting massive parallel next generation sequencing. The clinical phenotypes of the two patients differ. Notably the first patient appears to harbour a milder phenotype compared to previous known cases. The second patient eventually developed refractory epilepsy and currently presents with severe global impairment. Given the paucity of clinical data about this very rare mitochondrial encephalopathy, our report might contribute to broaden the phenotypic spectrum of the disorder. Moreover, noteworthy, three out of five pedigrees so far described belong to the Northern Sardinia ethnicity.

ND21 | Whole transcriptome analysis comparison of Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis patients

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Alzheimer's disease (AD), Parkinson's disease (PD) and Amyotrophic Lateral Sclerosis (ALS) are neurodegenerative disorders characterized by the progressive degeneration of the structure and function of the central or peripheral nervous system. A central role of RNA metabolism has emerged in these disease, concerning mRNAs processing and non-coding RNAs biogenesis. In this study we aimed to identify possible crossroads or deviations in the dysregulated pathways of AD, PD and ALS through RNA-seq technology. We performed a whole transcriptome analysis to investigate the regulation of both coding and non-coding RNAs in patients affected by sporadic ALS, AD and PD and matched controls (CTRL) in Peripheral Blood Mononuclear Cells (PBMCs). Gene enrichment analysis was performed on coding genes through Gene Ontology (GO) and pathway analysis through Kegg (Kyoto Encyclopedia of Genes and Genomes http://www.genome.ad.jp/kegg). A total of 293 differentially expressed (DE) lncRNAs was found in ALS patients, whereas a total of 87 mRNAs was DE. GO analysis showed that affected genes showed an association with transcription regulation, immunity and apoptosis pathways, while KEGG pathways enriched by dysregulated genes include cancer-related pathways. In AD patients a total of 23 DE genes has emerged, 19 protein coding genes and 4 non-coding genes, 3 of them are long-intergenic RNAs (lincRNA). GO analysis principally resulted in phosphorylation activity and exocytosis. KEGG analysis concerned the coagulation cascade and, again, cancer related pathways. In PD patients only 5 genes were found DE, 4 of which were non-coding RNAs (2 lincRNAs). Thus, we did not conduce any GO or KEGG analysis. Our data brought the light on the different importance of lncRNAs and mRNAs regulation in the principal neurodegenerative disorders, offering starting points for new investigations about pathogenic mechanism involved in them.

ND22 | Doxycycline treatment in transgenic fatal familial insomnia mice

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Fatal familial insomnia (FFI) is a genetic prion disease linked to the D178N/M129 mutation in the *PRNP* gene encoding the cellular prion protein (PrP^C). Symptoms, including insomnia, motor abnormalities and memory loss, appear in the 5th decade of life, leading to death within two years. No treatment is available. *PRNP* mutations promote PrP misfolding, eventually leading to formation of an infectious isoform (PrP^{Sc}) which propagates by imprinting its abnormal conformation onto PrP^C molecules. Doxycycline (doxy) favors PrP^{Sc} degradation and extends survival of prion-infected hamsters. However, two clinical trials did not show significant beneficial effects of doxy in symptomatic prion disease patients. A preventive treatment with doxy is ongoing in asymptomatic carriers of the FFI mutation. The study will last 10 years with results expected by 2023. We tested doxy in transgenic Tg(FFI) mice, which develop a fatal neurological illness with clinical and neuropathological abnormalities highly reminiscent of FFI. Mice were treated daily with 10 ml/kg by intraperitoneal injection and scored periodically for motor and memory function by an operator blind to the experimental groups. In a first experiment, treatment was started from a symptomatic stage. Interim analysis found no significant differences in progression of motor dysfunction between vehicle- and doxy-treated Tg(FFI) mice, and the experiment was terminated after three months. In a second experiment, mice were treated starting from a pre-symptomatic stage. Analysis after six months, when the treatment was terminated, found no significant difference in onset and progression of motor dysfunction between vehicle- and doxy-treated Tg(FFI) mice, but a protective effect of doxy on memory deficits. Some mice of each experimental group were culled for biochemical and histological analyses. The remaining mice will be monitored to see if the treatment had any effect on development of late stage symptomatology and survival.

ND23 | Photoreceptors rescue in Retinitis Pigmentosa. Emerging treatments exploiting knowledge of pathogenetic mechanisms by using MicroRNA therapeutics

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Retinitis Pigmentosa (RP) comprises a group of retinal dystrophies characterized by progressive degeneration of photoreceptors and abnormalities in retinal pigment epithelium. RP is an orphan disease, for which an effective pharmacological approach is still missing. Several evidences identified microRNAs (miRNAs) e.g. miR-155, as deregulated in development and progression of RP. In this context, targeting miR-155 could represent an innovative and attractive therapeutic strategy. The aim of this study is to evaluate the pharmacological activity of an innovative eye drops formulation containing antimiR-155 (molecules targeted miR-155) loaded on nanosized colloidal carrier, nanosponge (NS). In order to evaluate the delivery and functional activity of antimiR-155/NS formulation in an animal model of autosomal recessive RP (rd10), we administered antimiR-155/NS from postnatal day (P) 18 up to P30 in treated eye and antimiR-scramble/NS in control eye. The *in vivo* delivery was evaluated by using NS coniugate with fluorophore and fluorescence microscopy evaluation of retinal tissues. Moreover, real-time PCR was used to evaluate miR-155 levels after treatment. Fluorescent microscopy analysis showed the ability of antimiR-155/NS-fluorophore to efficiently reach the retinal layers. Furthermore, chronic treatment was able to decrease miR-155 expression in treated eye compared to control one and preliminary results by western blot analysis shows that the eye treated with antimiR-155/NS formulation has rhodopsin and cone-opsin levels increased compared to the control eye, indicating a reduction in degenerative processes. Further that, experiments aimed at deepened its activity on retinal neurons (electroretinogram recording), inflammatory and apoptotic pathways (real-time PCR and western blotting) are ongoing. Our preliminary data suggest that antimiR-155/NS could represent a potential therapeutic agent for RP.

ND24 | Function and expression of spinal cord metabotropic glutamate receptors 1 and 5 are enhanced in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis during disease progression

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by muscle wasting, weakness and motor neuron (MN) death. Although the aetiology of the disease is still unclear, glutamate (Glu)-mediated excitotoxicity is a major cause. Our previous work highlighted that abnormal Glu exocytosis, triggered by membrane depolarization, represents a leading mechanism for excessive synaptic Glu, both at pre-symptomatic and symptomatic stages of the disease. We demonstrated that Group I metabotropic Glu receptor (mGluR1, mGluR5) activation also produced abnormal Glu exocytosis in SOD1^{G93A} mouse spinal cord at a late disease stage (120 days), indicating a role for excessive Glu in ALS. The aim of the present study was to investigate whether mGluR1 and mGluR5 also affect Glu release in pre-symptomatic (30 and 60 days) and early-symptomatic (90 days) SOD1^{G93A} mice. Our results showed that the mGluR1/5 agonist (S)-3,5-Dihydroxyphenylglycine (3,5-DHPG) evoked the release of [^{3}H] D-Aspartate ([^{3}H]D-Asp) in a concentration-dependent way; an effect comparable in 30 and 60 day-old WT and SOD1^{G93A} mice. Noteworthy, 3,5-DHPG evoked [^{3}H]D-Asp release in 90 day-old SOD1^{G93A} mice, which was augmented respect to WT mice. Both mGluR1 and mGluR5 were involved. The 3,5-DHPG-evoked [^{3}H] D-Asp release was of vesicular origin and induced by Ca^{2+} released from intra terminal stores. The expression of mGluR1 and mGluR5 was increased in Glu nerve terminals of 90 day-old SOD1^{G93A} mice, but not in the whole nerve terminal population of the spinal cord. Interestingly, mGluR1 and mGluR5 expression was augmented in the total spinal cord tissue already at 60 days, supporting a modification also of postsynaptic and/or non-neuronal mGluR1 and mGluR5. In conclusion, the function and the expression of mGluR1 and mGluR5 are enhanced in early-symptomatic SOD1^{G93A} mouse spinal cord, possibly participating to the excessive Glu transmission. Our data support their implication in ALS.

ND25 | Glutamate mGlu5 receptor as a target to modulate the reactive phenotype of astrocytes in the SOD1G93A mouse model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a non-cell-autonomous neurodegenerative disease characterised by the rapid and progressive motor neuron (MN) death, sustained by damage of non-neuronal cells. Unveiling the precise role of each cell population turns out to be crucial to design a targeted therapy. Group I metabotropic glutamate (Glu) receptors (mGluR1, mGluR5) play an important role in ALS. In this contest, we have demonstrated that the reduced expression of mGluR5 in the $SOD1^{G93A}$ mouse model of ALS significantly prolongs survival and ameliorates the clinical progression of the disease. We investigated here the reactive phenotype of spinal cord astrocytes cultured from late symptomatic $SOD1^{G93A}$ mice and age-matched mice heterozygous for mGluR5 ($SOD1^{G93A}mGluR5^{+/-}$). The mGluR5 down-regulation significantly decreased ($p<0.05$) the cytosolic Ca2+ concentration ([Ca2+]) that was augmented in $SOD1^{G93A}$ spinal cord astrocytes. The over-expression of astrogliosis markers (GFAP, vimentin, S100β) in $SOD1^{G93A}$ astrocytes was significantly ($p<0.05$) diminished in $SOD1G93AmGluR5^{+/-}$astrocytes. The same, a reduction of misfolded-SOD1 accumulation was observed ($p<0.05$). The impaired oxygen consumption and ATP synthesis, as indexes of a bioenergetic deficit, were partially recovered ($p<0.05$) in $SOD1G93AmGluR5^{+/-}$astrocytes. The excessive release of neuroinflammatory cytokines (IL1b, TNFa, IL6) was reduced ($p<0.01$) in $SOD1^{G93A}$ astrocytes heterozygous for mGluR5. Finally, to verify the impact of mGluR5 down-regulation on MN viability, we co-cultured spinal cord MNs and astrocytes and observed that MN death was strongly reduced ($p<0.05$) when they were seeded on $SOD1G93AmGluR5^{+/-}$astrocytes, compared to $SOD1^{G93A}$ astrocytes. The present data demonstrate that lower constitutive levels of mGluR5 overall attenuate the reactive phenotype of astrocytes that, in turn, can reduce the MN damage. These results sustain the positive in-vivo effects of mGluR5 genetic ablation in ALS mice.

ND26 | Tourette Syndrome in Wilson's disease: causal or casual association? A case report

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Wilson's disease (WD) is a rare autosomal recessive disorder of copper metabolism. The accumulation of copper in liver, brain and cornea in this pathology is commonly associated to a wide range of psychiatric and neurologic manifestations. Children usually develop hepatic manifestations before 10 years, whilst neuropsychiatric symptoms are usually expressed only after this age. Neurologic involvement before 10 is rare. Moreover, at ophthalmologic evaluation, 98% patients with neurologic or psychiatric WD-related symptoms have the Kayser-Flesher ring. Herein, we report the case of a young male boy presenting with chronic vocal and motor tics, later also diagnosed as having WD. Brain MRI and ophthalmologic studies showed no neurological involvement and his tics did not change after specific therapy for WD. The patient was then diagnosed with Tourette Syndrome (TS) in comorbidity with WD. Repeated MRI and opthalmologic studies didn't show any CNS (Central Nervous System) involvement. After different therapeutical approaches for tics, a reduction in such symptoms was noticed adding clonidine to Habit Reversal Behavioral training. To the best of our knowledge TS in comorbidity with WD has not been previously described in literature, raising the question on whether the two conditions are etiologically related or only casually associated.

ND27 | The role of HDAC6 in ALS pathogenesis and its interaction with TDP43

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Background. Histone deacetylase 6 (HDAC6) is usually located in the cytoplasm, where it deacetylases alpha-tubulin, regulating the stability of microtubules (Hubbert et al., Nature 2002). Recent studies suggest that HDAC6 is involved in the regulation of autophagy pathway, because microtubules actively participates to it (Lin et al., Oncotarget 2017; Chen and Cohen, J Biol Chem 2019). Moreover, TDP43 was found to bind the mRNA of HDAC6, inhibiting its RNA translation (Xia et al., J Alzheimers Dis 2015). Thus, aim of this work was to evaluate the binding between TDP43 and HDAC6 in Amyotrophic Lateral Sclerosis (ALS) and the effect of the inhibition of HDAC6 in cellular model (SH-SY5Y cell line).
Methods. We firstly evaluated the binding of TDP43 to HDAC6 by RNA immunoprecipitation technique in peripheral blood mononuclear cells 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.
Results and discussion. We found that TDP43 binds HDAC6 mRNA in sALS patients, while this binding was not found in healthy subjects. Our data suggest that the increase of TDP43 in cytoplasmic compartment, which is usually found in ALS, leads to a decreased RNA translation of HDAC6 protein. 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, which is a hallmark of sALS patients' cells.
Conclusions. Our work provides new insight in the pathogenesis of ALS, by investigating a new pivotal role of TDP43 in autophagy impairment.

ND28 | Increased iron amount in old mice causes an inflammatory condition that activates Hepc/Fpn1 pathway and Ferritin heteropolymers changes

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Systemic body iron amount and availability are regulated by Hepcidin (Hepc), a peptide produced mainly by the liver as a response to increased iron amount or inflammation, that acts on the cellular iron exporter Fpn1, causing its degradation and decreasing the amount of available iron. On the other hand, very few is known about iron regulation in CNS. Nevertheless, it has been evidenced that iron amount increases during ageing and that several neurodegenerative disorders (ND) are associated with inappropriate iron accumulation. NCOA4 is a cargo protein which promotes autophagic ferritin degradation through its binding to ferritin H chains (Ft-H), modulating intracellular iron regulation that may be involved in ND. To verify a possible involvement and role of Hepc and NCOA4, in the elderly, we evaluated their brain levels in WT mice at different ages, starting from 4 until 72 weeks of age. In old mice brain iron content (BIC) and Ft-L, as marker of tissue iron amount increased, as well as Hepc and NCOA4, while Ft-H and Fpn1 showed a significant decrease. We hypothesized that the increased iron amount found in old mice was due to a higher iron flux in CNS because of a BBB misfunction. As a matter of fact, ZO1, a marker of BBB integrity, decreased significantly during animals ageing. Increased iron amount in a tissue could trigger an inflammatory process and in fact the expression of SAA1, an acute phase protein, raised significantly in brains from old mice. We conclude that brain iron increase in the elderly is due to a BBB misfunction that allows more iron to pass from systemic circulation to brain. Here, it induces an inflammatory signal that triggers Hepcidin expression and, as a consequence of this, Fpn1 degradation and iron retention into the cells. Cells responds to higher iron amount increasing NCOA4 that causes a selective Ft-H degradation promoting in this way the formation of Ft-L rich ferritin heteropolymers, more effective for iron storage.

ND30 | Synthetic neuromelanins: structural characterization and potential biomedical applications

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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. 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. However, important information about the structure, properties, and reactivity of the NMs can be obtained through the investigation of synthetic NM models. We have previously shown that it is possible to synthesize suitable models of the NM structural core by reacting DA, a fibrillar protein and iron under controlled conditions, leading to soluble and therefore tractable compounds where the melanic, protein and iron contents can be controlled and quantified. 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 brain with Parkinson Disease (PD). A main objective of this work was to obtain water-soluble pigments starting from NE in order to mimic NMs present in LC, or using a neuronal protein 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. Good models of NM could be employed in cellular or animal models that better reproduce the progression of PD *in vivo* with respect to models currently in use, which are based on the administration of paraquat or MPTP.

ND31 | In-vivo pharmacological blockade of metabotropic glutamate receptor 5 as a potential therapeutic approach to ALS

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron (MN) death, the aetiology of which is not clear, although glutamate(Glu)-mediated excitotoxicity represents one major factor. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in ALS, since they are largely over-expressed during disease progression and involved in altered cellular processes. In this scenario, we recently demonstrated that mGluR1 and mGluR5 at Glu synapses produces abnormal Glu release and that reducing mGluR1 or mGluR5 expression in SOD1^{G93A} mice significantly prolongs survival and ameliorates disease progression as well as several biochemical, cellular and functional parameters. The aim of this work was to strengthen the hypothesis that mGluR5 could represent a therapeutic target in ALS. Thus, we investigated the effects of the in-vivo pharmacological treatment of SOD1^{G93A} mice with 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP), an orally bioavailable mGluR5 negative allosteric modulator with favorable pharmacokinetics. We treated 90 days-old symptomatic SOD1^{G93A} mice with CTEP (2mg/kg/48h or 4mg/kg/24h) or vehicle until death. CTEP, dose dependently, ameliorated the clinical features in SOD1^{G93A} mice. The lower dosage only barely produced positive pharmacological effects while the higher dosage significantly postponed the disease onset, increased survival and improved motor abilities in treated mice. CTEP treatment also preserved motor neurons from death, decreased astrocyte and microglia activation and reduced the abnormal Glu release in the spinal cord of treated mice. These effects were more marked in female than in male SOD1^{G93A} mice. In conclusion, our previous and present results suggest that mGluR5 represents a promising target for the treatment of ALS and the CTEP *in-vivo* effects in SOD1^{G93A} mice support this translational perspective.

ND32 | Neutralization of interleukin-17 in experimental mouse model of Alzheimer's disease mitigates behavioural deficits and neuroinflammation

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Alzheimer's Disease (AD) is defined by amyloid beta (Aβ) and tau pathology, neurodegeneration, neuroinflammation, and cognitive dysfunction. In particular, neuroinflammation is both a contributing factor to and a consequence of neurodegeneration that contributes to memory dysfunction and decline of cognition. Several studies report the involvement of T helper 17 cells in the pathogenesis of AD-related neuroinflammation, however the role of the main cytokine, IL-17, remains elusive. Herein, we decided to examine whether the IL-17 neutralizing antibody (IL-17Ab) injected via intracerebroventricular (ICV) or intranasal (IN) routes could ameliorate amyloid-induced neuroinflammation and memory impairment in mice. In details, mice received IL-17Ab either via ICV at 1 hour prior to Aβ1–42 injection or IN 5 and 12 days after Aβ1–42 injection. In both experimental groups, 7- and 14-days after Aβ1-42 administration, we evaluated olfactory, spatial and working memory and performed biochemical analyses on whole brain and specific brain areas. The main finding in this study is that neutralization of IL-17 cytokine (both ICV and IN) is able to reverse Aβ1–42-induced memory and olfactory deficits in mice and to suppress the reactive gliosis and neuro-inflammation, by the inhibition of the typical pro-inflammatory cyto-chemokines, in total brain, hippocampus and prefrontal cortex. In conclusion, these data suggest that the neutralization of IL-17 results in substantial reduction of neuroinflammation and behavioral symptoms induced by Aβ1–42 injection, confirming the key role of IL-17 is a detrimental factor for AD. In addition, results point out a possible future therapeutic approach in patients with AD considering in a future perspective, IN as a non-invasive route that could offer patient compliance.

ND33 | Study of neuroprotective agents in a model of retinal neurodegeneration: a comparison between cord blood serum eye drops and saffron treatment

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Retinal degeneration induced in albino rats by high intensity light exposure (light damage, LD) provides a good model to study the pathways involved in neurodegenerative processes, beginning with oxidative stress and progressing to retinal pigment epithelium (RPE) damage, gliosis, photoreceptor death, microglia activation etc. Two different neuroprotective strategies were tested in LD rats; 1) eye drops from Cord blood serum (CBS) donated at delivery after consent (extract rich in chemokines and trophic factors, 8µL/3times/day); 2) Saffron supplement (1mg/kg/day). By comparing the neuroprotective efficacy we aimed to identify whether common protective pathways were shared between these treatments . Neurodegeneration was induced by exposure to 1000 lux for 18hrs. Rats were continuously treated starting a week before LD; one group with saffron agar jellies and the other with CBS eye drops. The treated rats were compared with those untreated. We monitored retinal function by recording flash electroretinography (f-ERG) before and 15 days after LD. We evaluated: a) retinal and RPE morphology; b) gliosis; c) microglia activation; d) self-protective mechanisms. Both CBS eye drops and saffron treatment mitigate the decline in the f-ERG response after LD. Furthermore, the retinal morphology was maintained in both treatments together with a reduction in microglia activation. Interestingly, compared to saffron, the CBS treatment did not show any modulation of reactive gliosis or activation of self-protective mechanism (fibroblast growth factor, FGF2). In conclusion, our results show that both CBS and saffron treatment can mitigate retinal neurodegeneration likely by modulating different pathways. Further studies are needed to clarify this hypothesis. Accordingly it suggests that a combination of these treatments might be a useful strategy to enhance neuroprotective effects.

ND34 | The mGluR5 knock out in SOD1G93A mice leads to a striking amelioration of Amyotrophic Lateral Sclerosis disease progression

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Amyotrophic lateral sclerosis (ALS) is a complex and progressive neurodegenerative disease leading to the death of upper and lower motor neurons (MNs) and to glial cells damage. To date, no effective treatments are available because the aetiology of the disease is still poorly defined. In this context, glutamate (Glu)-mediated excitotoxicity is assumed to represent one major cause of MN damage. Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may be implicated in Glu-mediated excitotoxicity in ALS, since they are involved in prominent cellular processes and largely over-expressed during disease progression. We recently demonstrated that activation of mGluR1 and mGluR5, present at Glu nerve terminals, produced abnormal Glu release in the SOD1^{G93A} mouse model of ALS. Moreover, halving mGluR1 or mGluR5 expression in SOD-1^{G93A} mice significantly prolonged survival and ameliorated the disease progression, as well as several biochemical and cellular characteristics of the disease. While the downregulation of mGluR1 positively affected motor skills in both males and females SOD1^{G93A} mice, the downregulation of mGluR5 ameliorated motor performances in males only. To further validate the role of mGluR5 in ALS we investigated here the effects of knocking out mGluR5 in SOD1^{G93A} mice, producing homozygous SOD1^{G93A}mGluR5$^{-/-}$ mice. SOD1^{G93A}mGluR5$^{-/-}$ mice showed delayed disease onset ($p<0.05$) and prolonged survival ($p<0.001$) vs. SOD1^{G93A} mice, accompanied by spinal cord MN preservation ($p<0.01$) and decreased astrocytic and microglial activation ($p<0.001$). Moreover, SOD1^{G93A}mGluR5$^{-/-}$ mice showed a stronger amelioration of motor skills respect to hemizygous mice and, most importantly, these improvements were present both in male and female SOD1^{G93A}mGluR5$^{-/-}$ mice ($p<0.05$). Thus, silencing mGluR5 has a positive impact in SOD1^{G93A} mice, supporting the idea that this receptor may represent a potentially effective pharmacological target in ALS.

ND35 | Assessment of amyloid pathology and anti-amyloid treatment in the 5xFAD mouse model of Alzheimer's disease

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Synaptic loss is an early and key event in Alzheimer's disease (AD) driven by soluble oligomeric forms of the amyloid b (Ab) peptide. However, the molecular mechanisms underlying Ab synaptotoxicity are not clear, nor has any therapeutic intervention been identified. Previous studies reported that a drug in clinical use, Fasudil, a ROCK inhibitor, is able to protect synapses and cognition in rodents from the effects of Ab. In this study, we investigated the therapeutic potential of Fasudil to reduce the Ab load in vivo in the well-characterized 5xFAD transgenic mouse model of AD, as well as the utility of manganese-enhanced MRI (MEMRI) for *in vivo* visualization of Ab plaques. For this purpose, 5xFAD mice (6-7 months old, n=16) and age-matched wild type littermates (wt, n=16) received four daily subcutaneous injections of $MnCl_2$ (cumulative dose 0.6 mmol/kg). One day after the final injection, all animals were scanned *in vivo* on a Bruker BioSpec 9.4T MRI scanner before and after 4 weeks of Fasudil (10mg/kg) or saline (vehicle) daily intraperitoneal injections. For all mice, 3D multi-gradient echo (MGE) images were acquired for plaque visualization as well as MP2RAGE structural images. Following the second MRI scan all mice were transcardially perfused with 4% paraformaldehyde, their brains were removed and one hemisphere was used for radioligand binding studies ([^{3}H]-PIB) and the other for histochemical analysis (anti-Aβ antibody 4G8). Plaque-like hypointense spots were visible in greater numbers in MGE images of 5xFAD mice compared to wild type littermates. [^{3}H]-PIB radiolabeling and 4G8 immunohistochemistry of sections demonstrated an intense punctate pattern in cortical (frontal, temporal, parietal and entorhinal) and hippocampal regions (CA1 and subiculum). Both showed immunoreactivity reduction in Fasudil treated mice only. Taken together these results suggests that Fasudil treatment substantially and significantly reduced amyloid plaque burden in 5xFAD mice.

ND36 | Innovative approach to discover new markers of Alzheimer's Disease for state/stage diagnosis by Phage Display technology

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Alzheimer's disease (AD) is the most common cause of progressive debilitating neurodegenerative dementia worldwide. The discovery of new diagnostic markers able to identify the several forms of Aβ-42 is pivotal. Conformation-dependent antibodies (Ab), have been reported able to recognize epitopes specific for several types of amyloid fibrils, regardless of their amino acid sequences. Since, the conformational epitopes are exposed only in peculiar aggregation states of proteins, the detection of Ab correlated with the aggregation states of amyloid fibrils *in vivo* could be useful in diagnosis. At this purpose we investigate the "conformational similarities" among several Aβ-42 forms and other amyloid-like proteins by bioinformatics tools. Conformation similarity was discovered with Caf1 protein of *Yersinia pestis* and the innovative phage display selection by alternate biopanning cycle "double binding" procedure was carried out in four steps. In the first round, the phage library was screened against YPf19 (monoclonal Ab that recognized Caf1), then eluted phages (restricted Caf1 library), were used in the second selection round against a pool of sera from patients' AD (IgG-AD). A third round was carried out against the YPf19 again, and finally with IgG-AD. The reactivity and specificity of phage clones were evaluated in E.L.I.S.A. assay with YPf19 and IgG-AD. 12III1 phage clone, displaying RWPPHFEWHFDD peptide, was used as mimotope of Aβ-42 and tested against IgG sera from healthy and AD patients in E.L.I.S.A. and Phage-Immuno-PCR (PI-PCR). Significant IgGs levels, detected by 12III1, allowed to discriminate AD-patients from non-AD subjects. Moreover, IgG-AD levels were significantly correlated with the progression state of the disease. The obtained results may be of significant impact on the development of diagnostic assay for AD stage/state. This approach could be extend to discover new markers for other neurodegenerative diseases.

ND37 | Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline

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Increasing evidence suggests an association between metabolic disorders, notably insulin-resistance and type 2 diabetes, with cognitive decline and Alzheimer's Disease. In fact, diet-induced changes in peripheral insulin sensitivity contribute to alterations in brain insulin signalling and cognitive functions. Deranged glucose metabolism in the brain accompanied by elevated fatty acids levels and chronic low grade inflammation could be the pathogenetic mechanisms associating type 2 diabetes with Alzheimer's Disease. We developed a preclinical animal model of diet induced-glucose impairment. We fed mice with 45% or 60% high fat diet for several weeks, and measured the effects on body weight, glucose-, pyruvate- and insulin-tolerance. Behavioural tests were used to asses the presence of cognitive impairments. The effect of insulin resistance on neurotrasmission, myelination and endoplasmic reticulum stress were studied by western blot of hippocampus and prefrontal cortex. Finally, palmitic acid treatments were used *in vitro* on primary cell cultures of neurons, astrocytes and microglia to mimic the metabolic condition determined in the brain by high fat diet. Our results suggest that even a short period of exposure to high fat diet can alter relevant brain functions, including neurotransmitter sensing and myelination. The molecular mechanisms are currently under analysis in our cellular model. Our work will lead to the identification of novel pathways affected by exposure to high fat diet, and may have clinical implications in halting cognitive decline in subjects at risk.

Topic: Neurodegeneration and Metabolism

ND38 | A new cellular system to uncover the prion-like properties of Tau P301L

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Tauopathies are a family of neurodegenerative diseases defined by the accumulation of fibrillar deposits of the microtubule-associated protein Tau. One hypothesis for the progression of Tauopathies is a prion like mechanism, where Tau aggregates in one cell spread to another to trigger aggregation of previously soluble Tau. Tau oligomeric aggregates are the most toxic Tau aggregates for cells and it has been hypothesized that are the cause of spreading. Despite these hypotheses the molecular mechanism underlying this process are still largely unknown. In this work using a cell system with an inducible expression of Tau P301L, a mutated form of Tau associated with Frontotemporal dementia, we studied the different propensity of aggregated and not aggregated forms of recombinant human Tau P301L (rhTau P301L) to spread and induce toxicity. We also studies seeding activity and toxicity of Tau P301L in hippocampal primary neurons culture. We created a reproducible and easy to use cell system to study the prion-like properties of TauP301L. We showed that exogenous rhTauP301L, either in monomeric or oligomeric form, can be uptaken by recipient cells and, once inside the cells, oligomeric TauP301L, but not monomeric, can trigger aggregation of endogenous TauP301L and induce toxicity. In addiction, oligomers appear resistant to degradation and the enhanced half-life of the aggregates correlates with a general decrease of proteasome activity. These studies provide new insights into the molecular mechanism of the prion-like spreading of Tau pathology.

ND39 | Hippo and necroptosis pathways as possible players in the neuronopathic Gaucher

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The Gaucher Disease (GD), is the most common lysosomal disorder, and arises from alterations in the *GBA1b* gene, which encodes the β-glucocerebrosidase acid enzyme. The same type of mutations can lead to the systemic or the neuronopathic form, which is orphan of therapies. This observation opens to the possible involvement of other genes and pathways. Recently emerged the evidence that the up-regulation of the Hippo pathway, is associated with neuron inflammation and death, both in mammals and in other species. Necroptosis is a form of cell death activated in inflammatory conditions. Hippo activity has been studied in a *Drosophila* loss of function (LOF) model, whereas human model of iPSC differentiated towards the mature monocyte/macrophage fate have been used to investigate the aberrant inflammation of the hematopoietic compartment. The *Drosophila* model shows a strong reduction of Ex, CycE, dIAP and MYC, direct targets of Yki, both in terms of transcript and protein, suggesting a deregulation of the Hippo pathway and a defect of the cell growth regulation mechanism. In the same context we found Dally and Dally-like protein, two soluble glypicans negatively regulated by Fat-cadherin and involved in glia and synapse development, deeply downregulated in the *dGBA1b* LOF background, supporting a role in the neurological damage. While GD iPSCs were able to differentiate into CD43+/CD45+ progenitors and mature CD14+/CD163+ monocyte/macrophages, they showed a decreased in proliferative potential compared to healthy donor iPSC, either in semisolid and liquid culture, therefore exhibiting a growth impairment. This effect was also evident in healthy donor iPSC treated with the β-glucocerebrosidase inhibitor CBE. The activation of necroptosis pathway with a significant upregulation of RIPK3 and MLKL was evident in both pluripotent and differentiated state in the GD model. These data support the role of Hippo and necroptosis components as potential therapeutic targets for GD.

ND40 | Translation efficiency is upregulated in hAPP mice before and immediately after the onset of cognitive impairments: insights for anticipating Alzheimer Disease diagnosis and treatment

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Introduction. Overexpression of full-length Amyloid Precursor Protein (APP) is a common feature of familiar or sporadic Alzheimer Disease (AD) patients and mouse models of AD. Which dysfunction of translational efficiency mediates APP overexpression is, however, unknown.
Methods. A polysome gradient analysis was carried out in hippocampal extracts taken from Tg2576 mice at 1, 3 and 6 months of age using quantitative real time PCR Hippocampal levels of eukaryotic initial translation factors (p-eIF2a/eIF2a, p-eIF4E/eIF4E, and eIF4G), APP, Ab. BACE-1 and caspase-3 levels were assessed by western blotting in 3-month old Tg2576 mice receiving intracerebral or peripheral injections of salubrinal, a blocker of eiF2a de-phosphorylation which inhibits overall translation. Synaptic plasticity, dendritic spines, memory, and memory-induced hippocampal *c-fos* immunoreactivity were investigated in the same 3-month old salubrinal injected Tg2576 mice.
Results. While upregulation of eIF2α phosphorylation in fully symptomatic patients and mouse models of AD is well documented, we found that elF2a phosphorylation is downregulated when Tg2576 mice are pre-symptomatic and early symptomatic. Decreasing translation efficiency by salubrinal injections in early symptomatic mice rescued AD-related molecular, neural, and behavioral alterations
Discussion. Our findings agree with the suggestion that p-eIF2a regulation is a promising therapeutic target for AD. The observation that p-eIF2a levels in AD mice are initially downregulated and then upregulated suggests that p-eIF2a should be regulated in directions which differ according to stage of the pathology.

ND41 | VAPB or not VAPB? Looking for a correlation between VAPB and neuronal excitability

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Amyotrophic Lateral Sclerosis (ALS) is a complex neurodegenerative disease that affects upper and lower motoneurons (MNs). To date, about 10% of ALS patients present a positive family history of the disease and more than 20 genes have been casually linked to familial ALS (fALS). One of these genes is the VAPB (vesicle-associated membrane protein-associated protein B) gene that encodes an endoplasmic reticulum protein implicated in multiple cellular functions such as intracellular trafficking and MN differentiation. The VAPB P56S mutation is linked to a dominant inherited form of fALS (ALS8) and recent studies pointed to the haploinsufficiency of VAPB as a possible mechanism for ALS8 pathogenesis. Interestingly decreased level of VAPB were found both in the spinal cord of ALS sporadic patients and in MNs of the superoxide dismutase 1(SOD1)G93A mice. To investigate about the putative pathogenic role of a reduction in the level of expression of VAPB, we analyze its expression in Sod1G93R transgenic zebrafish tissue homogenates. This model represents a new and consolidated animal model that recapitulates the major hallmarks of ALS in patients. Indeed, also in this model, we observed a reduction of VAPB protein level in the spinal cord of adult mutant fish compared to control animals. Defects in neuronal excitability, mainly hyperexcitability, are considered as potential trigger for MNs degeneration in several ALS model systems. Recent studies have shown that VAPB modulates the intracellular trafficking of the subunits of the Na^+ channel that is responsible for the neuronal pacemaker current (INaP). Since we have shown that Sod1G93R zebrafish embryos were characterized by a marked hyperexcitability of spinal neurons due to alteration of the INaP current, (possibly mediated by the Nav1.1 channel) we decided to look for a correlation between VAPB levels and Nav1.1 expression in both zebrafish Sod1G93R embryos and NSC34, a motoneuron-like cell line, silenced for VAPB.

ND42 | LncRNAs and ALS: the role of MYC-induced Non-Coding RNA MINCR

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Different studies in the past years highlighted the importance of RNA metabolism in Amyotrophic Lateral Sclerosis (ALS). An RNA-seq analysis of Peripheral Blood Mononuclear Cells (PBMCs) of sporadic ALS (sALS) patients revealed a great number of deregulated lncRNAs, including MINCR. MINCR is lncRNA which expression is induced by the transcription factor c-MYC. C-MYC acts in complex with MAX and binds also MYCBP, which stimulates the activation of E-box-dependent transcription by c-MYC. Also MYCBP was down-regulated in sALS. RNA levels of MINCR, MYCBP, c-MYC and MAX were investigated by qPCR in PBMCs and spinal cord of sALS patients and controls. Co-immunoprecipitation and immunofluorescence have been performed to assess the c-MYC-MAX heterodimer formation in PBMCs. We created an *in vitro* model for MINCR downregulation through siRNA-mediated silencing in SH-SY5Y. The sub-cellular localization of MINCR in PBMCs and silenced SH-SY5Y was studied with ddPCR. RNA-Seq results were confirmed by qPCR in PBMCs and spinal cord with the same trend: MINCR and MYCBP are downregulated in sALS patients, c-MYC is upregulated and MAX is not modified. Co-immunoprecipitation of c-MYC and MAX in PBMCs showed an impaired heterodimer formation in sALS patients. Immunofluorescence of c-MYC and MAX in PBMCs showed the co-localization only in controls. In silenced SH-SY5Y we investigated the RNA levels of c-MYC, MAX and MYCBP: there were no significant results. ddPCR in untreated and silenced SH-SY5Y and in PBMCs from patients and controls showed a predominantly nuclear localization of MINCR. In conclusion, c-MYC-MAX heterodimer formation is impaired in sALS, probably causing an altered transcriptional activity of the complex that could cause the MINCR downregulation. The *in vitro* model shows that MINCR doesn't act directly on c-MYC, MAX and MYCBP RNAs, but rather could be a downstream effector in the cascade. The nuclear localization of MINCR suggests the compartment of its function and targets.

ND43 | Tackling complexity in neurodegenerative disease

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Conformational diseases, such as serpinopathies and Huntington's diseases are characterized by the aggregation of aberrant conformations of proteins. Understanding common molecular mechanisms underlying protein aggregation, and the identification of possible biological processes contributing to the establishment of an aggregation-prone pathological state are intriguing unsolved issues with potential therapeutic implications. In the last years, our group using an interdisciplinary approach that combines quantitative biology and wet lab, investigated these aspects. In the present talk, I will discuss our recent results showing the critical role of cholesterol homeostasis in neuroserpin aggregation and the presence of heterogeneous oligomerization between wild type huntingtin proteins and the mutated ones with long polyQ tracts (Q > 36). Overall, our data opens up new perspectives on possible therapeutic targets.

ND44 | Study of the oncogenic lncRNA ZEB1-AS1 in sporadic ALS: identification of a new deregulated 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. Among these, 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. BMI1 has been reported to be downregulated in other neurodegenerative diseases (Alzheimer's Disease) suggesting a role for this molecule in neurodegeneration. Interestingly, it has been shown that FUS plays a role in the biogenesis of mir200c. 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. To investigate the role of ZEB-AS1/ZEB1 in sALS, we created an in vitro model silencing ZEB1-AS1 in SH-SY5Y. This downregulation does not influence ZEB1's levels, mimicking what observed in sALS. On the contrary, we observed an increase of mir200c and a decrease of BMI1, in an opposite pattern to what is reported 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. Concordantly, we found that BMI1, p53 and GSK3b present the same deregulations in PBMCs of sALS patients. We demonstrated that ZEB1-AS1 can bind the ALS-implicated RNA binding protein FUS, and we demonstrated this both in SH-SY5Y cells and in PBMCs. Interestingly, we saw that there is a reduction in the amount of ZEB1-AS1 bound to FUS in sALS patients. Our results strongly suggest the implication of the ZEB1-AS1 pathway in sALS.

ND45 | New nature-inspired hybrids modulating BDNF: a novel multi-target pharmacological approach to counteract neurodegeneration

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Brain-derived neurotrophic factor (BDNF) is the most abundant and widely distributed neurotrophin in the central nervous system (CNS). Initially isolated as a secretory protein capable of promoting the survival of peripheral neurons, BDNF is now recognized as a plethoric factor able to regulate a wide repertoire of neural functions. Nature has always been a rich source of products showing therapeutic properties. In our previous papers, we combined molecular fragments deriving from garlic and curcumin into new chemical entities, producing nature-inspired hybrids, previously tested for their ability to counteract Ab-aggregation and oxidative stress. Since data from literature reported that natural compounds increase BDNF levels targeting TrKB/CREB pathway, the aim of our project is to evaluate if our nature-inspired molecules, beside their well-established antioxidant activity, are capable to exert neuroprotective effects acting on BDNF in a view of multi-pharmacological compounds. We investigated the capability of our compounds to modulate BDNF protein levels by western blot in a cellular model of human neuroblastoma (SH-SY5Y). To better disclosure the molecular mechanism through which the compounds might affect BDNF levels, the expression of TrKB, CREB and BDNF mRNA has been evaluated by RT-PCR. Moreover, we tested whether our compounds may rescue the reduction of BDNF levels induced by cortisol. Our results suggest that the new nature-inspired molecules are capable to significantly increase both BDNF protein amount and mRNA expression, either in basal conditions and following cortisol-induced stress conditions, thus suggesting that our compounds may exert neuroprotective effect.

ND46 | The specific JNK inhibitor peptide (D-JNKI1) prevents motor deficits and dendritic spine dysfunction in Angelman Syndrome mouse model

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Angelman Syndrome (AS) is a rare neurodevelopmental disorder characterized by severe mental retardation, ataxia, susceptibility to seizures and autistic features. AS is linked to defects of the UBE3A gene located on chromosome 15. The UBE3A gene encodes for E6AP, an ubiquitin ligase and transcriptional coactivator protein. Recent studies show that E6AP plays an important role in synaptic function and plasticity, although the molecular mechanisms underlying these processes is largely unknown. By using the Ube3a +/- mouse model that well replicates the human pathological phenotype of AS, we characterized the synaptopathy and the metabolic profile of AS mice, by body weight measurement. Then, we examined the cognitive and locomotor impairments using behavioral tests: NORT, Open Field and Rotarod Tests, from 7 to 23 weeks of age. The Ube3a m-/p+ mice presented severe worsening of the behavioral impairments from 7 to 23 weeks. We then correlated the cognitive-motor dysfunctions to synaptic dysfunction, characterizing the biochemical changes of the post synaptic elements. PSD95, Drebrin and Shank3 levels were moderately altered in Ube3a m-/p+ at 7 weeks compared to controls of matching age, while these alterations become more marked in 23 weeks old AS mice. Thus, we examined the possible activation of the JNK signaling at the spine level, knowing its key role in synaptopathy. The biochemical analysis confirmed a powerful activation of JNK in Ube3a+/-. Lastly, we evaluate the effect of an in vivo chronic treatment with the cell-permeable specific inhibitor of JNK, D-JNKI1, in Ube3a+/- mice. The treatment recovered cognitive and locomotor impairments and, at the cellular level, the synaptic alterations/dysfunction. Thus, inhibition of the JNK signaling could represent a new therapeutic strategy for this disease. It is important to emphasize that there is still no effective therapy against AS and D-JNKI1 could be a realistic pharmacological opportunity for this disorder.

ND47 | Indeexing arousal with pupillometry and EEG: implication for normal vs. pathological aging

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The Locus Coeruleus (LC) is a brainstem nucleus with a fundamental role in arousal, attention and memory. The LC is impaired in neurodegenerative disorders, including Alzheimer's disease, and its degeneration may arise since the prodromal stage of 'mild cognitive impairment'. Thus, evaluating the functionality of the LC system could provide a novel early marker of cognitive decline. This poses the need for non-invasive tools for evaluating LC function in humans. One promising tool is pupillometry; work in animals suggests a tight coupling between the dynamics of pupil diameter and the moment-to-moment fluctuations in the activity of noradrenergic neurons in the LC. Here we aim to clarify this coupling by combining pupillometry with electroencephalography (EEG) to evaluate cortical activity and excitability. We record pupil variations and EEG during rest and during two tasks that are known to induce transient increases of arousal levels: Multiple Object Tracking (MOT) and Auditory Oddball. MOT is a visual divided attention task, whereby observers covertly track of a set of N targets (N=2, 3, 4, or 5) moving randomly among 10 distracters. In the Auditory Oddball task, observers are presented with a stream of repetitive sounds (1940 Hz tones) embedded with infrequent distracters (500 Hz) and infrequent targets (2000 Hz), which they should discriminate. We find increasing pupil dilation with increasing load during the tracking phase of the MOT task, and stronger pupil dilation for the Oddball tones vs. the repetitive tones. We aim to correlate these data with the simultaneous EEG recordings, to examine the association between pupil size changes and known EEG arousal indices and to develop a combined EEG-pupillometric index to track arousal states. The ultimate goal is to achieve a reliable estimate of the integrity of the LC system, obtained in healthy adult individuals, and designed to be extended to the elderly population with normal and pathological aging.

ND48 | The involvement of serotoninergic system in Parkinson's Disease: a morphological characterization of Tph2 mouse model

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Parkinson's disease (PD) is a neurodegenerative disorder, characterized by the loss of the dopaminergic neurons in the Substantia Nigra pars compacta (SNpc), and consequently, decreasing levels of striatal dopamine (DA) and the appearance of motor symptoms, representative of the disease, for example tremors, rigidity, bradykinesia, and postural instability. In recent years, many studies have focused on the characterization of the serotonergic system, which appears to be involved in PD. In fact, Serotonin (5-hydroxy-tryptamine, 5-HT), a neurotransmitter extensively distributed in the brain, plays an important role in the regulation of many physiological processes (development, synaptic plasticity, neuroendocrine function, sleep) as well as in neurodevelopmental and neuropsychiatric disorders of the brain. Additionally, 5-HT is involved in the altered mechanisms causing motor and non-motor symptoms of PD, but, in particular, it seems to be responsible for the L-Dopa-induced dyskinesias (LIDs) in 6-hydroxydopamine (6-OHDA)-lesioned rats. To better understand the possible involvementof the serotonergic system in the affected pathways of LIDs in PD, we performed a morphological study, to evaluate in particular the inflammatory state as well as the GABAergic and dopaminergic content, using the transgenic mouse model Tph2, concomitantly displaying lack of 5-HT synthesis and an intact serotonergic innervation, with or without 6-OHDA lesion. Our electrophysiological data (unpublished data not shown) suggest that the serotonergic system is necessary

for intact striatal function, confirming its role in corticostriatal plasticity. Then, to deepen this data, we have evaluated: GAD67 alteration and neuroinflammation markers,dopaminergic neurons (TH^+) and their progenitors cells ($Pitx3^+$), in SN and VTA brain regions, by immunofluorescence and immunohistochemistry techniques, in Tph2 mice, 6-OHDA lesioned, with normal content of 5-HT (Wt), partial (Het) or total absence of 5-HT (KO).

ND49 | A New Mechanism of Action for Saffron Repron®: The Importance of an Orderly Visual Cycle

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Background: Dysregulation of the visual cycle can lead to lipofuscin accumulation, a hallmark of several retinal diseases and a source of photo-oxidative stress. Antioxidants are able to reduce levels of lipofuscin and its main component, N-retinylidene-N-retinylethanolamine (A2E), as shown by several papers. Saffron can slow the onset of many diseases of the retina. It is known to have antioxidant properties and can modulate over 160 genes in the degenerating retina. As some of these genes affect crucial metabolic pathways, it prompts the question whether and how saffron can alter lipofuscin build-up.
Aim: We set out to investigate the role of saffron in protecting retinal pigmented epithelium (RPE) cells from lipofuscin accumulation and photo-oxidative stress.
Methods: Human adult RPE (ARPE19) cells were given increasing concentrations of *all-trans* retinol (precursor of A2E) and exposed to bright light to induce photo-toxicity. After obtaining the LD50 (lethal dose, 50%) for retinol, we pre-treated the cells with Saffron Repron® before inducing the same damage at the LD50. MTS dye was used as a cell viability assay, and immunocytochemistry was performed to assess levels of cytochrome C. Autofluorescence from lipofuscin was also analysed.
Results and conclusions: Our results suggest that saffron can reduce lipofuscin accumulation and apoptosis in RPE cells. Interestingly, saffron improves RPE cell viability even without any damage but can greatly protect from cell death induced by *all-trans* retinol and light. Furthermore, *all-trans* retinol and saffron both seem to act in a dose-dependent manner. We also confirm the importance of saffron's composition, using saffron with different chemical profiles. In the future, we will investigate the damage of A2E compared to *all-trans* retinol and test if saffron can also protect against this.

ND50 | Myelin alterations in Elovl5 knock-out mice, murine model of Spinocerebellar Ataxia 38 (SCA38)

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ELOVL5 (Elongase of Very-Long Fatty Acid 5) gene encodes for an enzyme that elongates long chain fatty acids, with a marked preference for polyunsaturated molecules. It has an important role in the biosynthesis of omega-3 and omega-6 fatty acids, precursors for longer polyunsaturated fatty acids (PUFAs), including arachidonic acid and docosahexaenoic acid. Mutations of ELOVL5 cause the spino-cerebellar ataxia type 38 (SCA38), a rare autosomal neurological disease which affects patients with both central and peripheral deficits (Borroni et al., 2016; Di Gregorio et al., 2014). ELOVL5 is highly expressed in cerebellar Purkinje cells and in many other central nervous system structures. We studied the role of Elovl5 in myelin by assessing the consequences of its loss in mice with a targeted deletion of the gene, which well represent SCA38 pathology (Hoxha et al., 2017). At the structural level, the central white matter showed a reduced area in histological sections. Moreover, Transmission Electron Microscopy (TEM) ultra-structural analysis of myelin sheaths showed that periodicity is enlarged in Elovl5 KO nerves compared to wild-type littermates. A lipidomic analysis of peripheral white matter of Elovl5 knock-out mice showed marked alterations of phospholipids containing polyunsaturated fatty acids, in which the forms with 20 carbon acids or more were strongly reduced. In contrast, phospholipids with shorter fatty acids, both saturated and unsaturated, were increased. The main function of myelin, to increase the speed of action potentials conduction along nerve fibers, was impaired in a peripheral nerve and showed a trend towards lower velocities in a central fiber, the axon of cerebellar Purkinje cells. These data taken together suggest that Elovl5 is important to allow a correct myelin structure and to enable fast action potential conduction along nerve fibers.

ND51 | Establishing the turquoise killifish Nothobranchius furzeri as a model for neurodegeneration

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All neurodegenerative disorders share ageing as major risk factor. Most of them are characterized by atypical protein aggregation as well as neuronal cell death. The most prevalent and studied neurodegenerative disorders are Alzheimer and Parkinson disease. *Notobranchius furzeri* (turquoise killifish) is a small teleost fish that originates from southern-east Africa, having as major feature an extremely short lifespan in captivity of only 40 weeks. Professor Cellerino's group proposed *Notobranchius furzeri* as animal model to study ageing due to its phenotype that shows most of the aging aspects of mammals, humans included. The brain undergoes major changes during ageing, and these changes set the conditions for the evolution of age-related neurodegenerative diseases. In particular, there is an increase in iron content, gliosis, and lipofuscin accumulation. The same molecular events also occur during *Norhobtanchius furzeri* brain ageing. Seen the valuable advantages that *Notobranchius furzeri* offers in studying age related disorders, our goal is to assess if it can be used as a model of neurodegenerative diseases, in particular to investigate Parkinson's disease. Using RNA-seq data analysis and immunofluorescence, we assessed that alpha-synuclein is conserved and expressed in *Nothobranchius furzeri*. Moreover, using an anti phospho-synuclein antibody we were able to observe the presence of aggregates in the brains of wild-type animals and we could observe a consistent age-dependent increase in alpha-synuclein aggregation in TH positive cells. We are also currently working in reconstructing the map of TH positive nuclei in *Nothobranchius furzeri* brain using whole brain clarification techniques.

ND52 | Assessment of the neuroprotective potential of the new HSP70 inducer in the aging brain of rats in the model of Parkinson's disease

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Parkinson's disease (PD) is the incurable neurodegenerative disorder of the elderly people. PD is characterized by the accumulation of toxic α-synuclein species in dopaminergic substantia nigra pars compacta neurons, followed by extensive neuroinflammation and neurodegeneration within nigrostriatal and other monoaminergic brain systems. It has been demonstrated that the expression of chaperones, e.g. Hsp70, is reduced within nigral neurons in PD, reflecting a reduction in the conformational control of neuronal proteins and cellular defense mechanisms. This led us to hypothesize that the expression of Hsp70 is required for the survival of DA neurons, especially in aging brain. The aim of this study is to assess the neuroprotective potential of the Hsp70 inducer U133 in the aging brain of rats in the model of preclinical PD stage. To mimic neuropathological pattern of PD intranasal injections of proteasome inhibitor lactacystin were performed to aged male Wistar rats. In order to increase the level of inducible Hsp70 in the SNpc neurons, the intraperitoneal injections of chaperone inducer, small molecule U133 were carried out. It has been shown for the first time that U133 treatment results in elevation of Hsp70 level in nigrostriatal system of aged rats, lead to the attenuation of neurodegenerative process in the locus coeruleus, nigrostriatal and mesolimbic systems comparing with the non-treated animals in PD model. It has been established that the neuroprotective effect of U133, caused by an increase in Hsp70 level, is due to the ability of the chaperone to reduce neuroinflammation and the number of α-synuclein aggregates in the brain structures relevant for PD. The results obtained indicate that elevation of Hsp70 level induced by U133 possesses neuroprotective and anti-inflammatory activities in the model of PD in aged rats. The research was supported by RSF (16-15-00278) and partly within the state assignment of FASO of Russia (theme № AAAA-A18-118012290427-7).

ND53 | Multisensory Temporal Binding Window in multiple sclerosis

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Multisensory integration reflects the ability of synthetizing information from different sensory modalities. An optimal *temporal "window"* exists, within which the binding of multisensory inputs is likely to occur: the *temporal binding window (TBW).* A defective TBW impacts on sensory and cognitive functions, in autistic, dyslexic, and schizophrenic patients. Little knowledge is available as to whether demyelinating disorders may disrupt multisensory integration, with an impact on clinical symptoms. We address this issue assessing the TBW extension in patients with multiple sclerosis (MS), featured by impaired unisensory processing, with multisensory analyses having been so far less investigated. Fifty-five neurologically healthy participants (control group) and 21 MS patients completed three simultaneity judgment tasks (SJ2), two unimodal (acoustic, visual) SJ2, one bimodal, visuo-acoustic. Couples of unimodal or bimodal stimuli were presented simultaneously or separated by variable *stimulus onset asynchrony, SOA*. In each trial, participants judged whether the two stimuli were simultaneous or not. Percentage of perceived simultaneity at the SJ2 tasks was analyzed. No difference between groups emerged in the unimodal SJ2, whereas SM patients were less accurate than controls in the bimodal SJ2 task ($F=18.90$, $p<.001$). MS patients show a disproportionate enlargement of the multisensory TBW, as compared to controls, with a reduced ability to bind together visuo-acoustical stimuli. The altered TBW suggests the existence of a specific multisensory integration deficits in MS. Besides the well-known modality-specific sensory impairments, MS also features a disruption of multisensory integration, likely related to abnormalities in cross-modal neural interactions. This might contribute to the clinical symptoms of MS patients.

ND54 | Investigation of molecular pathological hallmarks and therapeutic strategies in C9orf72 human lines

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The 40% of familial Amyotrophic Lateral Sclerosis (ALS) cases and 5%-10% of sporadic ALS can be attributed to the hexanucleotide repeat expansion in the C9orf72 locus. This mutation impairs different key molecular mechanisms giving rise to the complex etiology of the disease, even if many of them still remain to be clarified. Patient-derived induced pluripotent stem cells (iPSCs) can be a useful tool to model the disease and its relevant phenotypes. In our Lab, among the pathogenic features analyzed in C9orf72 iPSCs and derived motor neurons, we found the alteration of DNA damage response, the increase in DNA damage and the accumulation of DNA damage-inducing structures called R-loops. We also confirmed the presence of C9orf72 expanded transcripts RNA foci, structures known to sequester RNA-binding protein. Moreover, we found Pur-α, a molecular actor believed to play a role in R-loops homeostasis, together with RanGAP, a protein involved in nuclear trafficking, to accumulate and mislocalize. From a different pathogenic prospective, by a high-throughtput gene card assay, we identified and validated a subset of miRNA deregulated in C9orf72 lines compared to healthy controls. Investigation of the downstream target genes and their roles in the pathology is currently ongoing. Beside the research on ALS pathological mechanisms, we designed antisense oligonucleotides with a particular chemistry, morpholino (MO) oligomers, to target C9orf72 as a therapeutic strategy. Preliminary results on MO efficacy on pathological hallmarks observed are promising. In addition, we are exploiting the CRISPR\Cas9-mediated gene editing technique to produce isogenic mutation-corrected iPSC lines as tool for basic research and as a platform to test the feasibility of a gene editing-based therapeutic approach. Overall we demonstrated that patient specific iPSC-derived lines are a valuable tool to deepen the knowledge of C9ORF72 pathogenic mechanisms and to validate new therapeutic strategies.

ND55 | Chitosan-based hydrogel as a promising tool to support the paracrine activity of mesenchymal stem cells in spinal cord injury

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Advanced therapies which combine cells with biomaterial-based carriers are recognized as an emerging and powerful method to treat challenging pathologies, such as spinal cord injury (SCI). By enhancing transplanted cell survival and grafting, biomimetic hydrogels can be properly engineered to encapsulate cells and locate them at the injured site in a minimally invasive way. To this aim, a chitosan (CS)-based hydrogel was developed to host mesenchymal stem cells (MSCs), since their paracrine action can therapeutically enhance the spinal cord regeneration, limiting the formation of a glial scar and reducing cell death at the injury site. An injectable and highly permeable CS-based hydrogel was produced having a rapid gelation upon temperature increase from 0 to 37 °C. CS was selected as former material both for its high biocompatibility that guarantees the proper environment for MSC survival and for its ability to provide anti-inflammatory and anti-oxidant cues. The cells were mixed with the hydrogel solution prior to gelation. MSC viability was not affected by the CS hydrogel and encapsulated MSCs were able to release MSC-vesicles as well as to maintain their anti-oxidant features. Moreover, preliminary in vivo observations on SCI mice revealed good handling of the CS solution loading MSCs during implantation and good MSC survival 7 days after graft.

ND56 | Nano metal chelators: A new therapeutic approach in neurological disorder associated with metal imbalance

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Human health is severely hampered by many neurological disorders such as brain tumors, degenerative Alzheimer's disease (AD), Parkinson's disease (PD) and those involving inflammatory component. The brain is the central organ of the body and it is protected by series of multiple barriers such as the blood-cerebrospinal fluid (CSF) barrier and blood brain barrier (BBB). CSF reflects the central nervous system biochemical state under different physiological and pathological conditions. This makes CSF a potential candidate for identifying novel early biomarkers for neurological diseases. Disturbed iron homeostasis and mitochondrial dysfunction play important roles in the development of an increasing number of neurodegenerative diseases. Although the etiology of Alzheimer's disease is largely unknown, oxidative damage mediated by metals is a likely significant contributor since metals such as iron, aluminium, zinc, and copper are dysregulated and/or increased in AD brain tissue and create a pro-oxidative environment. This role of metal ion-induced free radical formation in AD makes chelation therapy an attractive approach for of dampening the oxidative stress burden in neurons. In recent years, growing evidence indicates that iron accumulation in the brain plays an important role in AD onset and progression and therefore, iron-chelation agents have been suggested as new potential drug able to counteract the neuronal deterioration in AD. Here, we propose a novel system of chelation therapy through the use of nanocarriers. Nanocarriers conjugated to chelators show unique ability to cross the blood–brain barrier (BBB), chelate metals, and to escape across the BBB with their corresponding complexed metal ions. This method may provide a safer and more effective means of reducing the metal load in neural tissue, thus attenuating the harmful effects of oxidative damage and its sequelae.

ND57 | Dysbiosis induces an alteration of microglial function and synaptic signaling

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The communication between the microbiota of the gastrointestinal tract (GIT) and the central nervous system (CNS), known as the gut-brain axis (gut-brain axis, GBA), is represented by bidirectional signals, mediated by neuro-endocrine and neuro-mechanisms immune. Different studies have shown that alterations in the intestinal microbial composition or its development, known as dysbiosis, determine changes in brain function and actual effects on the genetic pathology of various diseases affecting the central nervous system, the methods used in which the microbiota influences functionality cerebral and synaptic and contribution to the development of neurological pathologies are still little known. Recently it emerged that the intestinal microbiota component is involved in modulating microglial cell function, an immunocompetent population of the central nervous system. In this regard, we studied the effects of dysbiosis on both microglial function and on the synaptic activity of CA1 pyramidal neurons in acute hippocampal slices, of CX3CR1 + / GFP mice treated for two weeks with two wide-screen antibiotics in order to consider the dysbiosis function. The results obtained from the morphometric and functional analysis of microglia showed an effective alteration of the density and functionality of microglia in terms of both voltage-dependent K + currents (IKir and Ikv) and of the movement of cellular processes induced by ATP. The functionality of CA1-CA3 pyramidal neurons also appears to be influenced by the condition of dysbiosis. These in fact represent a reduced connectivity, described in terms of spontaneous activity, and alterations of the post-synaptic component, evaluated in terms of responses mediated by AMPA and NMDA receptors. No changes were observed in the presynaptic component.

ND58 | Human induced pluripotent stem cells as model to study two forms of familiar Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurological disease characterized by the progressive loss of upper (connecting the brain to spinal cord) and lower (connecting the spinal cord to muscle) motor neurons, leading to muscle weakness and paralysis and subsequent respiratory failure. A relatively small percentage of ALS cases (circa 10%) are familiar (fALS) and are associated with specific inherited gene mutations. We focused our attention on two mutations responsible for two distinct fASL: a mutation in a gene called Cu, Zn-superoxide dismutase-1(*SOD1*) and a mutation in *TARDBP* gene encoding a DNA-/RNA-binding protein TDP-43, respectively. In order to analyze how these mutations are involved in the pathogenesis of ALS, primary fibroblasts from two patients were used as cellular model in comparison with two healthy donors. However, given the multitude of cellular processes and molecular pathways altered in ALS patients, a model summarizing the multiplicity of phenotypes is difficult to represent. In order to overcome this limit, this study aims at using virus-free human induced Pluripotent Stem Cells (iPSCs) obtained from skin fibroblasts of ALS patients. The iPSCs represent a new *in vitro* model potentially suitable to evaluate the cellular dysfunctions in a scalable experimental format. By maintaining the genetic background of the patient, it allows to evaluate the molecular differences of two mutations causing the similar clinical features. Our preliminary data on cell growth showed notable differences in term of proliferation rate, between fibroblasts from two ALS patients and fibroblasts derived from healthy donors. No differences were manifested between iPS lines. The next goal will be to differentiate iPSCs in neural stem cells in order to investigate the disease's impact on both neuronal and glial populations.

ND59 | Amyotrophic Lateral Sclerosis: same gene, different pathways. The case of two patients carrying mutations in FUS gene

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The discovery of genes associated to Amyotrophic Lateral Sclerosis (ALS) is in constant progress and it is undoubtedly important to comprehend the mechanisms involved in the disease. However, the wide variety of functions of the proteins codified by these genes shows how complex these mechanisms are. Here, we present two cases affected by ALS with heterozygous mutations in the Fused in Sarcoma (*FUS*) gene. The first subject is a 43-years old male carrying a splice site mutation (c.1542-1g>t) which has never been described, but similar to one already reported and causing the skipping of the last exon of *FUS*. The second subject, is a 12-years old female carrying an already known frameshift mutation (c.1509_1510delAG). The two patients shared clinical features that are frequently observed in FUS-mutant ALS: early onset, prevalent lower motor neuron involvement with proximal muscle weakness, rapid course with early respiratory failure. Analysis of RNA and protein levels of FUS carried on peripheral blood mononuclear cells of the two patients revealed that the product of the c.1542-1g>t allele is not present, while the one of the c.1509_1510delAG mutation is clearly detectable, although more prone to proteolysis. Thus, different mutations in *FUS* may cause either the loss or the presence of an altered form of the protein, but result in a similar clinical phenotype. Consequently, the difficulty in comprehending the mechanisms underlying ALS is due not only to the wide variety of proteins linked to the disease, but also to the possibility that the same protein contribute to the pathogenesis in different ways. However, the study of proteins linked to ALS and their role in the pathogenic pathways could help identifying common steps among these pathways and thus classifying patients basing on their potential therapeutic targets.

ND60 | Bioink Composition for 3D Modeling of Neural Tissue using iPSCs and Neural Stem Cells

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The use of induced pluripotent stem cells (iPSCs) is increased due to their capability to be reprogrammed in a stemness state, to study different pathologies, including the neurodegenerative diseases (NDs). The powerful of iPSCs is the capability to differentiate into many cell types, in particular neuronal cells that are very difficult to obtain *ex vivo*. 3D cultures made by 3D bioplotter add a new complexity of *in vitro* models in order to create a realistic *in vitro* tissue. Our goal is to generate 3D cultures using an optimal bioink for both iPSCs and neural stem cells (NSCs) to mimic a neural tissue. We selected two biomaterials, i.e. sodium alginate (SA) and gelatin (GEL). We tested two temperatures and different printing pressure. Biocompatibility of the hydrogel was assessed using neuroblastoma cell line (SH-SY5Y), PBMCs-derived iPSCs and iPSCs-derived NSCs, using LIVE/DEAD Cell Viability Assay. We tested temperature and printing pressure using two concentration of 6% and 4%, SA, and 4% GEL. The best bioink turned out to be 6% SA and 4% GEL, printed at 25°C. SH-SY5Y cells were then encapsulated and after 5 days of culture we observed a good viability, confirming that the hydrogel is biocompatible. Reprogramming efficiency of PBMCs into iPSCs was confirmed using PCR and immunofluorescence. We also differentiated iPSCs into NSCs, and we measured the efficacy of differentiation using RT-qPCR and immunofluorescence. A good viability was maintained after 7 days of culture also with iPSCs and NSCs encapsulated into our 6% SA and 4% GEL bioink, suggesting the optimal biocompatibility of the hydrogel with these types of cells. We can conclude that our bioink, composed of 6% SA and 4% GEL is suitable for 3D bioprinting process. All cells tested show a good viability after encapsulation into our bioink. These promising results allow us to hypothesize the creation of a specific neural tissue from patients to study neurological disorders and degenerative processes.

ND61 | Iron content in Cerebrospinal Fluid as novel biomarkers for early diagnosis of Alzheimer's disease

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Iron is an essential element for normal neuronal metabolism. Iron homeostasis in the brain results from a dynamic exchange between blood and cerebral space, maintained by the brain barrier systems. Imbalance of iron metabolism is involved in several neurodegenerative disorders, such as Alzheimer's disease, leading to accumulation phenomena at tissue level and dysfunction of iron-related proteins. Excessive amounts of iron in the brain can induce oxidative stress, producing neurodegeneration and promoting neuroinflammation and neurotoxicity. Cerebrospinal Fluid (CSF) reflects the biochemical state of the central nervous system under different physiological and pathological conditions. Recent works focused on the abnormal level of metals (Fe,Cu,Al,Mn,Zn) in CSF as precursors of the clinically symptomatic dementia, suggesting their use alongside the well-established biomarkers (Aβ, t-tau and p-tau). However, the role of iron and its pathway across brain barriers in neurodegeneration remain to be elucidated. The aim of our study is the investigation of iron content in the CSF of patients affected by different form of dementia and matched controls, evaluating the interplay with other specific biomarkers. Accurate detection of iron in CSF samples is performed using Graphite-Furnace Atomic Absorption Spectrometry. CSF Fe levels in AD patients were significantly higher respect to controls. These results could highlight a key role of iron in Alzheimer's pathology, suggesting iron as possible novel specific biomarker of this disease. Future perspective will involve the evaluation of iron content in the serum of the same patients, and also advanced *in silico* models could be exploited to unravel a possible correlation with other parameters, such as oxidative and inflammatory biomarkers.

ND62 | Nose-to-brain delivery as a possible therapeutic strategy in restoring cholesterol brain levels in Huntington's disease

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Huntington's disease (HD) is a dominant inherited neurodegenerative disorder, characterized by neuronal dysfunction and cell loss, affecting mainly the cortico-striatal connectivity. Since the current pharmacological treatment for HD is the only palliative, there is a need to restore functions in patients. Exogenous cholesterol (Chol) administration to the brain of an HD (R6/2) mouse model rescues synaptic communication and protects the animals from cognitive decline, indicating Chol as a right candidate for HD treatment. Considering that the nose-to-brain drug delivery has already used in several clinical studies, we decided to verify the possibility to restore the physiological level of brain Chol through the intranasal (IN) route. During the acute trial, a group of wild-type (WT) mice were treated with a single dose of liposomes loaded D6-cholesterol (D6-Chol) (200 µg D6-Chol/dose). The delivery of D6-Chol to the brain after IN treatment was verified analysing D6-Chol concentrations in striatum, cortex and cerebellum at different time-points by LC-MS validated method. The analysis of the time-course profile of D6-Chol confirmed Chol delivery to the brain through IN route, by reaching a stable concentration at 24, 48 and 72 hours after the IN treatment (about 0.4 ng/mg). Moreover, our results suggested that Chol can use both olfactory and trigeminal pathways, leading to a homogeneous distribution in the whole brain. During the sub-chronic trial, both WT and R6/2 were treated once every two days for 19 days (200 µg D6-Chol/dose). The median amount of D6-chol at 24 hours after the last administration was more than 1.5 ng/mg in all brain areas, without difference between the two genotypes. These results confirm the accumulation of Chol after repeated administrations and noteworthy its distribution in all brain areas, strengthening the nose-to-brain delivery as a potential non-invasive and safe therapeutic strategy.

ND63 | Neural Stem Cells transplantation in pre-clinical experimental model of Parkinson's: counteraction of neuroinflammation and promotion of functional recovery

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Parkinson's disease (PD) is the second most common neurodegenerative disease, caused by midbrain dopaminergic neurons degeneration. Cell therapies have long been considered a feasible regenerative approach to compensate for cellular loss. Erythropoietin-releasing Neural Precursors (Er-NPCs) are a subclass of SVZ-derived neural progenitors with high neural differentiation features and able to survive in an unfavorable microenvironment. The aim of this work was to investigate the therapeutic potential of Er-NPCs in pre-clinical experimental model of PD, obtained by the intraperitoneal administration of MPTP in C57BL/6 mice. $2.5x10^5$ GFP-Er-NPCs were infused by stereotaxic injection in the mice left striatum. Functional recovery was assessed by two behavioral tests. The effects of Er-NPCs on neuroinflammation was assessed by measuring the expression of pro- and anti-inflammatory cytokines and evaluating by immunohistochemistry approach the expression of specific markers. Our results show that animals grafted with Er-NPCs present a behavioral improvement beginning the day 3 after transplantation and increasing in the observational period. Engrafted Er-NPCs are vital, do not form tumors, and migrate from the injection site within the striatum, reaching the SN. Furthermore, Er-NPCs administration promotes anti-inflammatory effect that was evident 24h after transplant, with a decreased expression of IL-1α, 1β, IL6, TNFα and an increased expression of IL10. Er-NPCs transplant also reduces macrophages infiltration, counteracting the M1 pro-inflammatory response the activated microglia and inducing M2 pro-regeneration traits. Moreover, we show that EPO, physiologically released by Er-NPCs, mediates these activities, since the co-injection of precursors with anti-EPO/EPOR antibodies neutralizes the effect of the treatment. We suggest that Er-NPCs represent good candidates for cellular therapy in PD for their differentiation capabilities and their anti-inflammatory properties.

ND64 | Dysregulation of astrocytic HMGB1 signaling in Amyotrophic Lateral Sclerosis

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Astrocytes have emerged as critical elements for the maintenance and function of the central nervous system. The expression on their cell membrane of RAGE and TLR4 receptors makes astrocytes susceptible to High-Mobility Group Box 1 (HMGB1), a nuclear protein typically released in the extracellular milieu by living cells experiencing physiological stress conditions or by damaged cells. Here, we show that the interaction of HMGB1 with normal astrocytes promotes neuroprotection via the production of neurotrophic factors. Multiple studies suggest a role for HMGB1 in Amyotrophic Lateral Sclerosis (ALS). Yet, no mechanistic information is available on the implication of HMGB1 signaling in this disorder. In $SOD1^{G93A}$ ALS mouse spinal cords, we found that HMGB1 is significantly released from motor neurons during disease progression, whereas non-transgenic and $SOD1^{WT}$ spinal cord tissues exhibit only a basal discharge of the protein. We postulate that extracellularly released HMGB1 can paracrinally interact with the neighbouring astrocytes to counteract the neurodegenerative process. At variance with normal cells, we found that ALS astrocytes show impaired capacity to raise trophic factor levels upon HMGB1 stimulation. Our data suggest that HMGB1 can promote neuroprotective actions in healthy astrocytes. However, this neurotrophic response is disrupted in ALS astrocytes. This suggests that diseased astroglial cells can contribute to motor neuron degeneration in ALS likely because of loss of their neurosupportive functions.

ND65 | Role of extracellular vesicles released by microglia in early synaptic dysfunction in Alzheimer's disease

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with pathogenic amyloid-β (Aβ) accumulation. Synaptic dysfunction is an early mechanism in AD, that involves increasingly larger areas of the brain overtime. However, how synaptic alterations start and propagate is largely unclear. Aim of the study is to explore the possible involvement of microglial extracellular vesicles (EV) carrying Aβ (AβEV) in the rise and propagation of early synaptic dysfunction in AD. We first analyzed the morphology and the strength of synaptic transmission in basal conditions or in response to chemical long-term potentiation (LTP) in cultured neurons exposed to AβEV or EV not carrying Aβ (ctrlEV), finding that only AβEV induce alteration of dendritic spine morphology and impair synaptic plasticity. Next, optical tweezer technique allowed us to place single EV on neuronal processes and study their dynamics in vitro. We found that AβEV are able to make contact and efficiently move along axons and that their motility is limited by AnnexinV coating. To test the hypothesis that AβEV may propagate synaptic dysfunction between connected brain regions, we injected AβEV or ctrlEV into mouse entorhinal cortex (EC) and measured LTP in the EC and its main target region, the dentate gyrus (DG), in cortico-hippocampal slices. 1h after AβEV injection, LTP was impaired only in the EC, while in 24h this effect was transferred to the DG, revealing a spreading of synaptic dysfunction between the two regions, also confirmed by patch-clamp recordings. When AβEV motility was limited by AnnexinV, no propagation was observed, suggesting that EV spread synaptic alterations by moving along projecting axons. Our data indicate that AβEV use neuronal processes as highways to propagate LTP impairment among connected brain region, providing strong evidence for EV involvement in the rise and propagation of early synaptic dysfunction in AD.

ND66 | Unraveling the role of astroglial LRRK2 in striatal glutamate homeostasis

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Mutations in Leucine rich repeat kinase 2 (LRRK2) are the most common cause of genetic Parkinson's disease (PD). In human G2019S LRRK2 post-mortem brains, we observed a significant reduction in striatal GLT-1 expression compared to age-matched control and sporadic PD. GLT-1 is the primary glutamate transporter in the adult brain that account for over 90% of synaptic glutamate clearance and it is primarily expressed in astrocytes. Growing evidence has shown that the impairment of glutamate homeostasis at the striatum might contribute to the degeneration of dopaminergic terminals. Since LRRK2 regulates intracellular vesicular trafficking of different receptors and transporters, we hypothesize that LRRK2 plays a role in GLT-1 turnover in astrocytes and that LRRK2 pathological mutations might compromise this process thus causing excitotoxicity. To this regard, we showed that Lrrk2 ablation produces a significant reduction of GLT-1 protein level in the brain. By immunohistochemistry, we also observed that Lrrk2 co-localizes with GLT-1 in mouse striatum and Lrrk2 deficiency causes an aberrant distribution of GLT-1. Specifically, GLT-1 mainly distributes in the endo-lysosomal compartment in *Lrrk2-/-* striatum. Similar results have been obtained using primary striatal astrocytes from *Lrrk2-/-* mice versus control. By pharmacologically inducing GLT-1 internalization, we noticed that GLT-1 is not correctly recycled back in *Lrrk2-/-* astrocytes suggesting that LRRK2 might be a regulator of GLT-1 fate within the cell. How the G2019S mutation impacts this pathway is under investigation. Concluding, our data suggest a functional link between the PD-linked kinase LRRK2 and GLT-1 in astrocytes opening novel hypothesis to explain the mechanism of LRRK2-mediated neurodegeneration in PD.

ND67 | Apoptosis and autophagy in the hippocampus of Krushinsky-Molodkina rats during epileptogenesis

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Krushinsky-Molodkina (KM) rats are a genetic model of heritable audiogenic reflex epilepsy. The triggers of the acute audiogenic seizure (AGS) is the auditory brainstem structures, while repetitive AGSs spread epileptiform discharges through limbic structures that can be considered as the model of epileptogenesis. Apoptosis and autophagy are processes involved in cell survival and cell death and might contribute to epileptogenesis.The purpose of our study was to determine apoptosis and autophagy in the hippocampus of KM rats at the different stages of epileptogenesis. We used Adult KM rats were required in the experiment. The hippocampi of KM rats were collected for analysis after 4, 7 or 25 AGS. Naïve KM rats were used as control. Obtained results demonstrated that after 4 AGS neither apoptosis nor autophagy was changed. However, after 7 AGS we showed an increase apoptotic cell number in the granule cell layer of the dentate gyrus that accompanied by activation of p53 and decreasing of Bcl-2 expression. In a week after 7 AGS, we observed the recovery of a granular cell population, which probably can be connected with increased neurogenesis. Additionally, we demonstrated an increase of Beclin-1 and ration of LC3B2/LC3B1, and decrease of p62 reviling activation of autophagy, that also can partly mediate survival of granular cells. 25 AGS lead to dramatically decrease of cell populations in the granule cell layer, hilus, and CA3 of the hippocampus. Although we did not see apoptotic cell death or autophagy activity that supposed cell loss at the early stages of epileptogenesis. Thus, our data demonstrated the development of neurodegeneration of the hippocampus during epileptogenesis that mainly mediated by neuronal apoptosis.

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ND68 | Emotional and social behavioral characterization of LOU/C/jall rats as an animal model of successful aging

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The LOU/C/jall rat is an inbred strain considered as a model of successful aging due to its increased longevity and absence of obesity and other metabolic diseases. It has been shown that these animals also maintain intact cognitive functions during aging, but very few studies have investigated the emotional and social aspects of this model until now. In our study young (5 months) and aged (30 months) LOU/C/jall male rats underwent a wide range of behavioral tests in order to assess cognitive, emotional and social functions in this strain. We compared their performances with those of young (4 months) and aged (24 months) Wistar male rats, considered as a pathological aging model due to their predisposition to insulin resistance and obesity. In the Elevated Plus Maze LOU/C/jall rats exhibited lower anxious behaviors than Wistar rats. In the Forced Swimming Test LOU/C/jall rats were less sensitive to stressful stimuli. With regards to social aspects, in the Social Interaction Test LOU/C/jall rats showed more social behavior (i.e., an increased amount of following and sniffing) and less non-social behavior than Wistar rats. Interestingly, age did not affect their higher social propension. Finally, we focused on the Ultrasonic Vocalizations emitted by animals in the dyadic situation of the Social Interaction Test. Since nobody has studied LOU/C/jall rats' vocalization profile, we were the first to demonstrate that in this particular strain the number of vocalizations decreases with age as in Wistar rats, while the power of emission (defined as "peak amplitude") increases with age. Such a power increase found in LOU/C/jall rats may indicate a compensatory mechanism that improves the quality of vocalizations in face of the decrease in number as a consequence of aging. Overall, we demonstrate that low anxiety levels, reduced stress response and high social interest are important aspects of LOU/C/jall rats' successful aging.

ND69 | Validation study of PBMC protein biomarkers for Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder that selectively affects motor neurons, causing gradually weakness of all voluntary muscles. There are currently no validated biomarkers for ALS, due to its clinically, genetically and neuropathologically heterogeneous characteristics, but they would be useful for early diagnosis, monitor disease progression and evaluate the efficacy of new treatments. Peripheral blood mononuclear cells (PBMCs) display pathological features mirroring those occurring in the central nervous system. In this study, we verified changes in a panel of protein biomarkers, identified in previous studies, in PBMC samples of a large cohort of ALS patients and controls. The study was done on totally 305 PBMC samples from ALS patients (n=90), healthy subjects (n=104), and patients with other neurological/neuromuscular diseases (n=111). PBMC samples were subjected to a two-step sequential protein extraction that implied low and high % of detergents, generating a soluble and an insoluble fraction. We next analysed by a slot blot immunoassay the levels of cyclophillinA (CypA), heat shock cognate protein 71kDa (HSC70), TAR DNA-binding protein 43 (TDP-43), heterogeneous nuclear ribonucleoprotein A2/B1 (hnRNPA2/B1) and superoxide dismutase 1 (SOD1) in both soluble and insoluble fractions. We confirmed changes in protein levels of PBMC proteins in ALS patients respect to controls. High levels of RNA binding proteins (TDP43, hnRNPA2/B1), proteins involved in folding process (HSC70 and CypA) and in oxidative stress (SOD1) in the insoluble fraction of ALS patients may underline alterations in RNA metabolism/stress granule formation and/or aggregation, that are key features of the disease, further confirming that PBMCs are a good source of biomarkers. The combination of some of these protein biomarkers distinguishes, with high discriminatory power, ALS patients from controls and this can be useful for early diagnosis of the disease.

ND70 | Corticostriatal synaptic plasticity alterations in the R6/1 transgenic mouse model of Huntington's disease

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Huntington disease (HD) is a neurodegenerative disorder with a progressive course that typically entails motor and cognitive decline due to abnormal dopamine (DA)-glutamate interactions and a dysregulation of the basal ganglia network. Several studies have shown that expansions of poly glutamine (polyQ) tracts at the amino-terminal fragments of a mutant form of the protein huntingtin (mHTT) cause a pathological formation of intracellular aggregates. The presence of mHTT is associated with toxicity and neurodegeneration leading to corticostriatal synapse instability and alterations of bidirectional plasticity. In R-6/2 model of disease during symptomatic phases, striatal projection neurons (SPNs) of HD mice show a loss of synaptic depotentiation, a common feature of other hyperkinetic disorders. In this work we have hypothesized that, the Long Term Depression (LTD), one of the two form of synaptic plasticity in the corticostriatal synapse, is altered in symptomatic R-6/1 mice, an HD model with gradual development of symptoms. *In vitro* patch clamp and intracellular recordings of corticostriatal slices from R6/1 mice, confirm that, similar to other HD models, once Long Term Potentiation (LTP) is induced, synaptic depotentiation is lost, in the SPNs of mutant mice. In these conditions, bath application of DA before LTD induction caused a shift in synaptic plasticity direction resulting into a LTP that can be mimicked by application of D1 receptor agonist SKF. This form of LTP also depends on NMDA receptors activation, since application of MK-801 non-competitive NMDA receptor antagonist, was able to fully block the effect of DA application. Our results demonstrate that the main forms of dopamine-dependent corticostriatal synaptic plasticity are altered in 27-week-old R6/1 mice, with different mechanisms that lead to an hyperactive synapse, with a dysregulated DA-glutamate interactions as a key player in the alterations of synaptic scaling down associated with HD symptoms.

ND71 | Generation of a prion protein (PrP)-HaloTag chimera: a novel approach to study the cellular trafficking and metabolism of PrP

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The conformational conversion of the cellular prion protein (PrP^C) into a β-sheet-rich, infectious isoform (PrP^{Sc} or prion), which replicates by inducing misfolding of native PrP^C, is the key pathogenic event in prion diseases, a group of fatal neurodegenerative disorders. To investigate PrP^C metabolism we have generated a PrP chimera containing a HaloTag (PrP-Halo) derived from a *Rhodococcus rhodochrous* haloalkane dehalogenase that can covalently bind specific fluorescent ligands. We designed two different PrP-Halo constructs by inserting the HaloTag sequence at either the N or C terminus of PrP^C, named PrP_N-Halo and PrP_C-Halo respectively. We transiently transfected HEK293 cells with these constructs. We observed that both PrP-Halo constructs were efficiently expressed and glycosylated. They could be easily detected by Western blot with anti-PrP antibodies, SDS-PAGE and typhoon imaging. We labeled PrP-Halo with a cell permeant fluorescent ligand and observed that the protein was correctly exposed on the plasma membrane like untagged PrP^C. To confirm that PrP-Halo was normally degraded through the lysosomal pathway we treated cells with the lysosomal inhibitor bafilomycin A1. We observed accumulation of PrP-Halo in lysosomes using confocal and super-resolution (SIM) microscopy. Our analysis indicates that both constructs of PrP-Halo are correctly expressed on the plasma membrane and easily detected by western blot and SDS-PAGE typhoon imaging. The possibility of specifically labeling different subsets of PrP^C molecules with cell permeant and impermeant ligands, and to analyze the protein by imaging and biochemical approaches, will allow to precisely define the cellular trafficking and metabolism of PrP^C.

ND73 | Early dysfunction of astrocytic Ca2+ signaling in Alzheimer's disease

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Alzheimer's Disease (AD) is by far the most common form of dementia. Several reports indicate that early stages of AD may be characterized by Ca^{2+} impairment and mitochondrial alterations, leading to functional dysregulations of the bidirectional relationship between astrocytes and neurons in the central nervous system. In astrocytes, neurotransmitters-induced Ca^{2+} alterations trigger the release of gliotransmitters, leading to the modulation of synaptic transmission, energy metabolism and memory processing. Interestingly, the Mitochondrial Calcium Uniporter (MCU), responsible for Ca^{2+} uptake into the mitochondrial matrix, might play a role in the above-mentioned mechanisms; however, the real connection between the MCU and AD in astrocytes is yet to be explored. The aim is to study astrocytic Ca^{2+} dyshomeostasis as an early brain dysfunction and identify new insights in Ca^{2+}-mediated pathways. Hence, we first seek to investigate Ca^{2+} dynamics in the cytosol and mitochondria of cortical and hippocampal astrocytes derived from WT mice and transgenic models, including PS2-N141I/APPswe and *MCU*-deficient mice. At present, we are optimizing primary culture conditions, experimental procedures and optimal drugs concentrations in astrocytes derived from WT mice. Preliminary data, using a mitochondria-targeted Ca^{2+} probe (4mtGCaMP6f), showed increased mitochondrial Ca^{2+} uptake when astrocytes were transfected with MCU-overexpressing plasmid and treated with 10 µM glutamate. Furthermore, exposure to stimuli, such as 10 µM ATP and 1 µM Bradykinin, resulted in a significant increase in intracellular Ca^{2+} influx (measured by Fura2-AM), Oxygen Consumption Ratio and glycolysis in cortical astrocytes. These results suggest a potential Ca^{2+}-dependent regulation of the mitochondrial respiration that might involve the mitochondrial Ca^{2+} uptake through MCU. In conclusion, we aim to provide direct evidence of the role of MCU in astrocytes and in mechanisms involved in brain injury.

ND74 | A structural approach to investigate glycation effect on protein aggregation

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Several chronic neurodegenerative diseases are characterised by deposits of misfolded or aggregated proteins, leading to the formation of neurotoxic amyloids. Intervening in this process can be a prophylactic measure for modifying the course of neurodegeneration. Increasing evidence suggests a link between diabetes and neurodegenerative processes such as Alzheimer's diseases (AD). For this reason, we study the role of glycation, a pathological process highly relevant in diabetes patients, on the structure, aggregation pathway and toxicity of the Abeta peptide (variants 1-40 and 1-42), the main component of amyloid plaques found in brain of patients affected by AD. Several biophysical techniques are employed to gain insights into the structural characteristics of the effect of glycation on the aggregation process of these peptides. Mass spectroscopy elucidated glycation reaction occurrence and identified the glycation sites of the peptides. Circular dichroism and nuclear magnetic resonance illustrated the secondary structure variation upon glycation. In addition, spectrofluorometric assays were employed to follow protein aggregation and glycation concomitantly. The first process was followed by means of thioflavin T (ThT) binding assay, employing ThT, a dye extensively used to monitor amyloid formation over time in a fluorescence-dependent way; the latter reaction was followed by monitoring formation of the fluorescent AGE (argpyriminide product). Finally, high-resolution structural studies via atomic force microscopy (AFM) evaluated the morphological effects of glycation on amyloid fibrils.

ND75 | Set up of an in vitro model of quadripartite synapse

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Synaptic dysfunction is the first common neurodegenerative event in many brain diseases, composed by an initial phase in which synaptic function is impaired but can be reverted; this phase evolves in a second irreversible stage with synaptic loss. Understanding molecular mechanisms, key-modulators and intracellular pathways governing interactions among 3 cell types, composing the quadripartite synapse, will allow inhibiting spine dysfunction offering a new therapeutic strategy to prevent neuronal death. With this aim, we set up an in-vitro model of the quadripartite synapse composed by neuronal pre and post-synaptic terminals, astrocytes and microglia. We are characterizing step-by-step the co-culture in physiological condition performing electrophysiological and biochemical studies. Starting with a co-culture composed by hippocampal astrocytes and neurons, we found that neurons alone appeared more depolarized than neurons in the co-culture showing a higher membrane resting potential. We also analysed mEPSCs: importantly, amplitude and frequency are higher in the co-culture than in neurons alone reflecting a greater number of active synapses. The co-cultures were studied also by biochemical analysis of total homogenate and TIF, representing the PSD-region. Concerning intracellular pathways JNK signalling and Casp3 were less activated in the co-culture compared to neurons alone in both total homogenate and TIF. We also found important changes in biochemical markers of the PSD-region. Now, we are setting-up the ideal condition for the co-culture in an innovative device, the 3brain chip, that allows connectivity studies using MEA approach. The next step will be to add microglia to the system and induce synaptic dysfunction with Ab oligomers. Defining and understanding critical cellular interactions and intracellular pathways regulating the quadripartite synapse in physiological/pathological conditions will allow to design new therapeutic strategies against *synaptopathy.*

ND76 | Binge-like eating in healthy and Parkinsonian rats is differently driven by endogenous and exogenous dopamine

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Foundation Santa Lucia, Neurophysiology, Rome, Italy[1] - Neurological clinic, University of Perugia, Department of medicine, Perugia, Italy[2] - IRCCS San Raffaele Pisana, University San Raffaele, Rome, Italy[3]

In Parkinson's disease, long term therapy with L-Dopa and dopamine agonists results in pulsatile dopamine receptors stimulation in both dorsal and ventral striatum causing motor fluctuations, and nonmotor side effects such as behavioral addictions. Binge eating disorder (BED) is one of the impulse control disorders, observed inpatients chronically treated with L-Dopa, that can be modeled in laboratory animals. In this study we investigated how prolonged dopaminergic replacement therapy in a 6-hydroxy-dopamine (6-OHDA) lesioned rat model, would affect binge-like consumption of palatable food. To this aim, rats were unilaterally lesioned with 6-OHDA, to generate a model of PD adapted to a modified version of Corwin's limited access protocol, with low and high food restriction schedules, in which vegetable shortening was replaced with milk chocolate bars. Electrophysiological properties and long-term potentiation of GABAergic spiny projection neurons of the nucleus accumbens core were analyzed through patch-clamp recordings from corticostriatal slices of control, parkinsonian naive and L-Dopa-treated rats to explore neuronal correlates of binge-like behavior. Our results show that sham-operated animals reliably developed food addiction-like behavior when exposed to intermittent access to a highly palatable food. Interestingly, 6-OHDA-lesioned rats were unresponsive to such food restriction regimen and did not develop preference or increased consumption. In contrast, in parkinsonian animals chronically treated with L-Dopa incremental chocolate consumption, suggestive of an addictive eating behavior, can be established but with a temporal dynamic different from the one observed in sham-operated rats, that followed that of L-Dopa administration. Our data indicate that BED requires an intact mesolimbic dopaminergic system and endogenous and exogenous dopamine drive binge-like consumption of a palatable food in healthy and parkinsonian rats with distinct temporal dynamics.

ND77 | Neuronal injury induces Connective Tissue Growth Factor expression to support peripheral neuroregeneration

Samuele Negro[1] - Marco Stazi[1] - Giorgia D'este[1] - Gabriele Sales[2] - Chiara Romualdi[2] - Cesare Montecucco[1] - Michela Rigoni[1]

University of Padova, Department of Biomedical Sciences, Padova, Italy[1] *- University of Padova, Department of Biology, Padova, Italy*[2]

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. Recently we have established an innovative experimental approach to study PNS regeneration. To confine the nerve damage to the sole motor axon terminal, thus avoiding the involvement of many cell types and inflammatory mediators, we employed an animal presynaptic neurotoxins: α-latrotoxin (α-Ltx), a pore forming toxin isolated from black widow spider venom. The neurotoxin specifically targets the presynaptic terminal and induces an acute and highly reproducible motor axon terminal degeneration, which is followed in a few days by complete regeneration. By this approach we found that: i) the transcript encoding for the Connective Tissue Growth Factor (Ctgf) is up-regulated during MAT degeneration and re-growth, ii) the encoded protein is produced and released by schwann cells (SCs), and iii) Ctgf is a strong promoter of peripheral nerve regeneration upon degeneration induced by α-latrotoxin. We have also tested the activity of Ctgf in well established forms of prolonged and severe nerve injury (compression and transection of the sciatic nerve). Even upon traumatic injuries Ctgf is strongly up regulated in SCs and promotes motor axon re-growth and peripheral neuroregeneration. In conclusion, this project defines a new actor governing regeneration of PNS, and this finding may be directly relevant to the development of new therapeutic strategies for the improvement of functional recovery after various types of nerve injuries.

ND78 | Effects of therapeutic hypothermia in an animal model of Amyotrophic Lateral Sclerosis

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Istituto di Ricerche Farmacologiche Mario Negri -IRCCS, Biochimica e Farmacologia Molecolare, Milano, Italy[1] - CIBIO, Dipartimento di Biologia Cellulare, Computazionale e Integrata, Trento, Italy[2]

Therapeutic hypothermia is a neuroprotective intervention employed routinely in a variety of clinical conditions, such as post-anoxic brain injury and hypoxic ischemic neonatal encephalopathy. Induction of hypothermia has also been shown to rescue neurological signs in experimental models of stroke, brain-spinal cord trauma and spinal muscular atrophy. Hypothermia induces the expression of a set of a cold-shock proteins and reduces neuroinflammation, leading to low free radical generation and inhibition of excitotoxicity. Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative syndrome that lead to motor neuron death and muscle atrophy, in which neuroinflammation, oxidative stress and excitotoxity are relevant pathogenic mechanisms. We therefore investigated whether therapeutic hypothermia had a protective effect on the SOD1G93A mouse model of ALS. We induced hypothermia pharmacologically in SOD1G93A mice combining the effect of 5'AMP on energy metabolism with low temperature. We tested three different schedules of treatment and selected the one with the highest neuroprotective effect to investigate the clinical outcome in the animal model in a preclinical trial. Although a clear motor neuron protection was achieved, we observed only a small delay in disease onset and a moderate increase in survival, with no motor improvement. The hypothermic treatment in SOD1G93A mice reduced oxidative stress, but increased the level of TDP-43 and its pathological forms, promoting the impairment of axonal transport, denervation and the associated muscle atrophy. In conclusion, hypothermic treatment in SOD1G93A mice showed both protective and harmful effects. Although there is a consistent protection of motor neurons, this does not seem to be sufficient to counteract the effect of the increase of TDP-43 and its neurotoxic forms, which translates only into a mild clinical efficacy.

ND79 | Mitochondrial dysfunction: a new biomarker candidate for Spinal Muscular Atrophy?

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Spinal Muscular Atrophy (SMA) is due to a mutation/deletion of the Survival Motor Neuron 1 (*SMN1*) gene which affects motor neurons (MNs) in children and young adults following a decrease in the levels of the functional SMN protein; this results in motor impairment, muscle atrophy and premature death. The experimental therapies for SMA aim at restoring SMN protein levels. Currently, only two drugs (Spinraza, Biogen and Zolgensma, AveXis/Novartis) have been approved by the Food and Drug Administration and only Spinraza is actively used; however, its long term effects are still under evaluation, especially in adults. Although the genetic cause of SMA has been identified, many aspects of its pathogenesis remain elusive and novel biological targets are investigated to develop new therapeutics and to monitor the efficacy of the existing treatment. We focus on mitochondrial proteins since already at early stages in SMA, mitochondrial function, number, area and transport are significantly altered in axons of spinal MNs. We cultured primary fibroblasts from the mouse model of SMA (SMN delta7) and of age-matched control mice. We stained mitochondria with the MitoTracker and we performed a time-lapse live imaging using a confocal microscope. By multidimensional quantitative image analysis we evaluated the differences in number, distribution and trafficking. Moreover, we isolated mitochondria from the spinal cord at postnatal day 7 of SMN delta7 and age-matched control mice. By 2D gel and MALDI-TOF mass spectrometry we identified some mitochondrial proteins which are differentially expressed and/or dysfunctional in SMA mice compared to control mice. Interestingly, mitochondrial dysfunctions are reported in other neurodegenerative diseases such as Alzheimer's and Huntington's diseases and Amyotrophic Lateral Sclerosis, and could represent a potential therapeutic target and biomarker for the early detection of a wide range of neurodegenerative diseases.

ND80 | Direct Reprogramming of Reactive Astrocytes in Neurons in Mouse Motor Cortex after Stroke

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Motor deficits caused by stroke represent one of the main causes of disability worldwide. Since rehabilitation is frequently not effective at completely re-acquire motor function, new regenerative and plasticizing treatments are strongly needed. Nowadays, one of the most promising therapeutic strategies is the replacement of the lost nervous tissue with neurons obtained by direct reprogramming of endogenous non-neural cell precursors resident in the perilesional area. In this study, we tested the effect of using "reprogramming" transcription factors to directly convert reactive astrocytes into new neurons after a focal cortical ischemic injury in the primary motor cortex. We used a genetically modified strain of C57BL6J mice, namely GFAP/CRE mice, in which the Cre recombinase protein is expressed under the control of the Glial Fibrillary Acidic Protein (GFAP) promoter. The reprogramming and reporter (GFP) genes were delivered in the mouse cortex via injection of flexed Adeno-associated virus (AAV), allowing the expression only in glial cells expressing Cre recombinase. 3 days after a photothrombotic lesion in the Caudal forelimb area (CFA), we administered the neurogenic determinants in the perilesional cortical area. 60 days after the injection, we detected a remarkable percentage of newly generated neurons among the total GFP-positive (originally astrocytes) cells. Remarkably, GFP-positive fibres were also found in cortical (e.g. contralateral motor cortex) and subcortical motor regions (e.g. internal capsula and spinal cord). Additionally, we are currently analysing data from Gridwalk and Schallert Cylinder tests to assess the effect of reprogramming on motor performance. In conclusion, direct reprogramming of resident astrocytes in the mouse motor cortex is capable to produce a new neuronal population that may acquire a motor identity, possibly aiding the motor recovery of functions of the affected forelimb.

ND81 | Quantal dopamine release and firing properties of cultured midbrain neurons: multisite recordings using diamond-based multi electrode array

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The secretory activity and the firing properties of cultured midbrain dopaminergic (DA) neurons have been investigated by combining conventional electrophysiology and diamond-based multiarrays prototypes. Micro-Graphitic Single Crystal Diamond Multi Electrode Arrays (μG-SCD-MEAs) allow a double recording: they can operate in potentiometric and amperometric configurations. When used as potentiometric biosensors, μG-SCD-MEAs resolve action potential firing of midbrain neurons (14 DIV), revealing that spontaneous firing occurs at 0.7÷7 Hz. This activity is drastically reduced (70%) by L-DOPA (20 mM), which also alters the extracellular AP waveform. Since both effects are reversed by the D_2-antagonist sulpiride, this suggest the involvement of D_2-autoreceptors. Though, within the network, a minority of neurons display either insensibility or potentiation by L-DOPA, suggesting different modulatory pathways. When used as amperometric biosensors, μG-SCD-MEAs can reveal multisite dopamine release from different DA neurons: this release occurs spontaneously at 0.11 Hz and increases 4-fold when cells are depolarized by external KCl. Because of the complexity of responses revealed by μG-SCD-MEAs, we undertook a detailed electrophysiological study on isolated DA neurons, identified by their TH-GFP positive staining, with the aim of investigating the ionic basis of cell firing and their coupling to dopamine release. Here we report that, in most cells, L-type calcium channels play a significant role in sustaining the firing activity of DA neurons: isradipine (3 μM) reduced the spontaneous firing frequency by 60-70%. The contribution of isradipine-insensitive currents in action potential generation was estimated using action potential clamp commands. Concerning the somatodendritic release of dopamine, preliminary recordings indicate that maximal recruitment of Ca^{2+} channels cause ~ 65 fF membrane capacitance increment.

ND82 | 3-Iodothyronamine ameliorates synaptic transmission after oxygen-glucose deprivation in mouse brain slices

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Abnormalities in thyroid hormone (TH) availability and/or metabolism have been hypothesized to contribute to Alzheimer's disease (AD) and to be a risk factor for stroke. Recently, 3-iodothyronamine (T1AM), an endogenous amine putatively derived from TH metabolism, gained interest for its ability to promote learning and memory in the mouse. In the present work, we investigated the effect of T1AM on ischemia-induced synaptic dysfunction in the entorhinal cortex (EC), a brain area crucially involved in learning and memory and early affected during AD. In order to study synaptic function, field excitatory post-synaptic potentials (fEPSPs) were recorded in EC/hippocampal horizontal slices obtained either from WT mice (C57bl) or mice overexpressing a human mutant form of amyloid precursor protein (mhAPP). Slices were exposed to an oxygen-glucose deprivation (OGD) protocol for 10 min and then recorded for 50 min after reperfusion with oxygenated artificial cerebrospinal fluid (ACSF). T1AM was perfused to slices at the concentration of 5 µM for 10 min during the application of OGD. A long-lasting synaptic depression was induced by OGD in WT slices. As previously reported, OGD effect was enhanced in EC slices from mhAPP mice (mean fEPSP amplitude was of 70 ± 7% of baseline, n=14 slices, 9 mice). However, T1AM perfusion was capable of preventing the long-lasting synaptic depression induced by OGD either in WT or mhAPP slices (mean fEPSP amplitude in mhAPP+T1AM was 105 ± 6%; $p < 0.01$ vs mhAPP untreated slice two way ANOVA RM, n= 10, 5 mice). A similar protective effect was achieved by the perfusion with 250 nM RO5166017 (mean fEPSP amplitude was 105 ± 10% of baseline, $p < 0.01$ vs mhAPP untreated slices two way ANOVA RM, n=6 slices, 3 mice), while T1AM-mediated protective effect was abolished by the TAAR1 selective antagonist 5nM EPPTB (mean fEPSP amplitude was 71 ± 12% of baseline, $p > 0.05$ vs mhAPP untreated slices two way ANOVA RM, n=4, 2 mice), suggesting a specific role of TAAR1 in T1AM protective effect during OGD in brain slices obtained from a mouse model of AD.

ND83 | Mechanosensitive and pharmacological properties of Piezo1 channel in embryonic mesencephalic neuron-derived cells

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Mechanical and physical cues are important components helping cells to perceive environment changes. Mechanotransduction enables cells to sense and adapt to external forces and physical constraints. Nevertheless, the way by which these mechanical stimuli are transduced into biological signals is still largely unknown. In 2010, a transmembrane protein, acting as mechanosensing ion channel, was discovered and named Piezo1. Piezo 1 is a large integral membrane protein with 24-40 transmembrane domains and an ion-conducting pore. Piezo1 protein was shown to be ubiquitously expressed in cells and tissues where it plays as one of the main actors involved in the processes controlling cellular motility. In this study, we explored the role of Piezo1 channel in mouse embryonic mesencephalic neuron-derived cell line (A1). This cell line is a good *in vitro* model because has an indefinite proliferation and possesses neuronal features that can be increased upon differentiation. We transfected A1 cells with human Piezo1-GFP plasmid to evaluate the capacity of Piezo1 to alter motility and proliferation; moreover, with the help of a nanoindenter (Piuma Chiaro) we have sought to outline mechanical properties of A1 cells that could be influenced by Piezo1 expression. Moreover, we assessed mechanosensitivity in cAMP-differentiated A1 cells using live cell calcium imaging under the stimulus of YODA1, a specific Piezo1 channel activator, and we observed reduced channel activity as compared to wild type A1 cells. These data are aimed to study the role of Piezo1 channel in Alzheimer disease setting, since neuron mechanotransduction might be affected by environment changes due to normal aging or neurodegeneration processes.

Poster Session 1 (November 14th, 17:50-20:00)

NEURAL PLASTICITY | pp. 42-53

NP01 | Emilia Conti
Generalized recovery of motor functionality after stroke by combined motor training and ipsi-lesional optogenetic stimulation.

NP02 | Virginie Sottile
Direct analysis of stem cell-derived extracellular vesicles with super resolution microscopy for live imaging in neural progenitor cultures.

NP03 | Marco Fogli
Transient neurogenic niches are generated by the sparse and asynchronous activation of striatal astrocytes after excitotoxic lesion.

NP04 | Linda Scaramuzza
Enhancement of activity rescues the early establishment of Mecp2 null neuronal features.

NP05 | Sara Bonzano
The transcriptional regulator COUP-TFI/Nr2f1 exerts an anti-astrogliogenic function in adult mouse hippocampal NSCs/progenitors enabling adult neurogenesis.

NP06 | Andrea Saul Costa
SNARE Complex Polymorphisms Associate with Alterations of Visual Selective Attention and mRNA expression in Alzheimer's Disease.

NP07 | Eleonora Vannini
Changes in neuronal activity affect vesicular positioning at cortical synapses.

NP08 | Eleonora Daini
DAT atypical inhibitors as novel antipsychotic drugs.

NP09 | Sara Cornuti
Metabolic changes influence brain plasticity in mice.

NP10 | Chiara La Rosa
Greater occurrence of "immature" neurons in mammals with expanded neocortex.

NP11 | Osvaldo Artimagnella
Post-trascriptional control of gene expression by Foxg1.

NP12 | Davide Marangon
Gas7 is a direct target of miR-125a-3p and a new player in oligodendrocyte maturation.

NEUROINFLAMMATION | pp. 77-93

NI01 | Stefano Raffaele
Effects of microglia-derived extracellular vesicles on GPR17-expressing oligodendrocyte precursor cells and post-stroke recovery.

NI02 | Annapia Vitacolonna
Activation of the MET receptor as therapeutic tool in MS: a new neuroprotective mechanism involving the glutamatergic system.

NI03 | Francesca La Rosa
Defective miRNA-223-Mediated Regulation of NLRP3 Inflammasome Activation in Alzheimer's Disease.

NI04 | Maria Velasco-Estevez
The role mechanosensation in neuroinflammation and neurodegeneration of Alzheimer's disease.

NI05 | Francesca Pischiutta
Amniotic mesenchymal stromal cell secretome protects the brain from acute injury by modulating glial activation.

NI06 | Francesca De Vito
New insights into DMF mechanism of action in experimental MS: miR-142-3p as key molecular target against inflammatory synaptopathy and motor disability.

NI07 | Laura Bellingacci
Low doses of Perampanel protect striatal and hippocampal neurons against in vitro ischemia.

NI08 | Jessica Garau
Hydroxychloroquine modulation of RNA:DNA hybrids in lymphoblasts derived from patients with Aicardi-Goutières syndrome.

NI09 | Chiara Pastori
Role of auto-antibodies and Peripheral Blood Mononuclear Cells on cerebral excitability in encephalopathies with epilepsy: a new experimental approach.

NI10 | Giorgia Moschetti
Prokineticin system as a new target to counteract experimental vincristine induced peripheral neuropathy.

NI11 | Federica Piancone
Inflammasome in the pathogenesis of Parkinson's Disease.

NI12 | Alexia Tiberi
A glial side to the neurotrophin field: studying the effects of neurotrophins on glial cells in the CNS.

NI13 | Benedetta Parodi
Monomethyl fumarate signals through the hydroxycarboxylic acid receptor-2 via cell- and environment-biased activation of different pathways.

NI14 | Andrea Bighinati
A drug combination administered via an implantable, polymeric delivery system improves the functional recovery in rat spinal cord injury.

NI15 | Paola Perin
Vascular networks of rat choroid plexus and cochlear nucleus: do they communicate?

NI16 | Katia Monsorno
Lactate metabolism in the control of microglial function.

NI17 | Gloria Vegliante
Longitudinal molecular magnetic resonance imaging of endothelial activation after severe traumatic brain injury.

NEURO-ONCOLOGY | pp. 131-134

NO01 | Roberta Azzarelli
ASCL1 phosphorylation regulates neuronal differentiation of glioma stem cells.

NO02 | Davide Ceresa
A game of clones: clonal dynamics of Glioblastoma progression suggests internal clonal competition.

NO03 | Gianmarco Pallavicini
Microcephaly gene inactivation induces Dna damage and radiosensitization in medulloblastoma.

NO04 | Ludovica Lospinoso Severini
SALL4A is a new positive regulator of Hedgehog signalling involved in medulloblastoma tumorigenesis.

PERINATAL NEUROLOGY | pp. 143-148

PN01 | Maria Serena Paladini
Prenatal stress reshapes spinal myelination affecting BDNF signaling in the experimental autoimmune encephalomyelitis model of Multiple Sclerosis.

PN02 | Melania Maria Serafini
Selenium in early life is able to promote neurodevelopment after Lead exposure.

PN03 | Michela Bassi
Early visual assessment and magnetic resonance in preterm and term infants: between structure and function.

PN04 | Simone Strano
Evolution of visual function in full term physiological newborns during the first 48 hours of life.

PN05 | Luigi Francesco Saccaro
Corpus callosum growth in complex congenital heart disease.

PN06 | Vito Antonio Baldassarro
The oxygen-glucose deprivation induced death in fetal neural stem cells-derived oligodendrocyte precursor cells is mainly driven by glucose metabolism perturbation.

NEURODEGENERATION | pp. 159-184

ND01 | Giulia Zanetti
A CXCR4 receptor agonist strongly stimulates axonal regeneration after sciatic nerve damage.

ND02 | Elena Abati
Combined RNA interference and gene therapy targeting MFN2 for the treatment of Charcot-Marie-Tooth 2A (CMT2A).

ND03 | Anna Binda
A novel HCN2 mutation associated with progressive epileptic encephalopathy.

ND04 | Alessandro Matera
Investigating the role of disease risk genes as modulators of microglial function.

ND06 | Giulia Nato
Role of Sox2 in the neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion.

ND07 | Lucia Iannotta
Deciphering the complex interplay between LRRK2 and p21-activated kinase 6 (PAK6).

ND08 | Marco Stazi
Melatonin promotes regeneration of injured motor axons.

ND09 | Elisa Pagliari
IGHMBP2 related pathological pathways in Spinal Muscular Atrophy with Respiratory Distress type 1 (SMARD1) in vitro models.

ND10 | Ilaria Palmieri
Deep RNA and DNA sequencing to support the clinical diagnosis in neurodegenerative diseases.

ND11 | Simona Schiavi
Assessing fibre specific myelin content using microstructure informed tractography.

ND12 | Giorgia D'Este
Urocortin 2 promotes functional recovery of degenerated nerve terminals.

ND13 | Elisa Pagliari
CPPs-conjugated antisense nucleotides: a new therapeutic strategy for Spinal Muscular Atrophy symptomatic patients.

ND14 | Sevda Boyanova
The antipsychotic amisulpride utilises plasma membrane monoamine transporter at the blood-brain barrier: implications to the heightened sensitivity to antipsychotics observed in Alzheimer's disease.

ND15 | Paola Fabbrizio
P2X7 activation enhances skeletal muscle metabolism and regeneration in SOD1G93A mouse model of amyotrophic lateral sclerosis.

ND16 | Estibaliz Santiago Mujika
Posttranslational modifications of tubulin in dementia.

ND17 | Amaya Urdánoz-Casado
Circular RNAs from Alzheimer´s disease-related genes in entorhinal cortex.

ND18 | Chiara Magliaro
A Smart Region Growing algorithm for isolating single neurons in confocal datasets.

ND19 | Simone Agostini
Herpes simplex virus-1 (HSV-1) infection induces a potent but ineffective IFN-lambda production in immune cells of AD and PD patients.

ND20 | Chiara Begliuomini
VARS2-linked mitochondrial encephalopathy: two case reports enlarging the clinical phenotype.

ND21 | Maria Garofalo
Whole transcriptome analysis comparison of Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis patients.

ND22 | Giada Lavigna
Doxycycline treatment in transgenic fatal familial insomnia mice.

ND23 | Ilaria Piano
Photoreceptors rescue in Retinitis Pigmentosa. Emerging treatments exploiting knowledge of pathogenetic mechanisms by using MicroRNA therapeutics.

ND24 | Giulia Frumento
Function and expression of spinal cord metabotropic glutamate receptors 1 and 5 are enhanced in the SOD1G93A mouse model of amyotrophic lateral sclerosis during disease progression.

ND25 | Marco Milanese
Glutamate mGlu5 receptor as a target to modulate the reactive phenotype of astrocytes in the SOD1G93A mouse model of amyotrophic lateral sclerosis.

ND26 | Chiara Begliuomini
Tourette Syndrome in Wilson's disease: causal or casual association? A case report.

ND27 | Matteo Bordoni
The role of HDAC6 in ALS pathogenesis and its interaction with TDP43.

Poster Session 2 (November 15th, 12:00-14:00)

NEURAL PLASTICITY | pp. 54-64

NP13 | Maria Fernanda Veloz
The effect of ageing on the spatial distribution of glycogen in Layer I somatosensory cortex of mice.

NP14 | Magdalena Martínez García
Neural Plasticity in First-Time Mothers: a neuroimaging perspective.

NP15 | Anna Panuccio
Neuromorphological and hormonal correlates of paternal behavior.

NP16 | Letizia Manca
Role of GPR83 in stress response, cocaine addiction and motivation for food.

NP17 | Sara Mazzoleni
Characterization of a new conditional PCDH19 KO mouse model to understand the pathophysiology of PCDH19-related epilepsy.

NP18 | Lucia Caffino
Alteration of cognitive function and cortical glutamatergic mechanisms in an experimental model of anorexia nervosa.

NP19 | Francesca Balsamo
The neuroanatomo-functional correlates of paternal behavior.

NP20 | Valeria Calabrese
Electrophysiological and biochemical characterization of Tph2 transgenic mouse model.

NP21 | Arianna De Rosa
Anticipated expression of D-aspartate oxidase since embryonic stage drastically reduces D-aspartate levels in the mouse brain and influences spatial memory at adulthood.

NP22 | Toniella Giallongo
HuR's interaction with lincBRN1a and lincBRN1b is implicated in neuronal stem cells differentiation.

NP23 | Kyllian Ginggen
Investigation of microglia-mediated synapse remodeling.

NEUROINFLAMMATION | pp. 94-110

NI18 | Ginevra Toma
The intra and extra cranial veins in relationship with chronic migraine.

NI19 | Ilenia Savinetti
Gene Expression Profiling Identifies Inflammatory Signatures in Peripheral Blood Monocytes of Primary Progressive Multiple Sclerosis Patients.

NI20 | Roberta Parolisi
Effects of Extracellular Vesicles on Myelin Repair.

NI21 | Margherita Prosperi
Correlations of inflammatory biomarkers with clinical features and onset patterns in an Italian sample of preschoolers with Autism Spectrum Disorders.

NEURO-ONCOLOGY | pp. 135-138

PERINATAL NEUROLOGY | pp. 149-153

NEURODEGENERATION | pp. 185-211

ND35 | Ilaria Rosa
Assessment of amyloid pathology and anti-amyloid treatment in the 5xFAD mouse model of Alzheimer's disease.

ND36 | Maria Giovanna Rizzo
Innovative approach to discover new markers of Alzheimer's Disease for state/stage diagnosis by Phage Display technology.

ND37 | Silvia Penati
Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline.

ND38 | Carmina Natale
A new cellular system to uncover the prion-like properties of Tau P301L.

ND39 | Silvia Strocchi
Hippo and necroptosis pathways as possible players in the neuronopathic Gaucher.

ND40 | Antonella Borreca
Translation efficiency is upregulated in hAPP mice before and immediately after the onset of cognitive impairments: insights for anticipating Alzheimer Disease diagnosis and treatment.

ND41 | Maria Nicol Colombo
VAPB or not VAPB? Looking for a correlation between VAPB and neuronal excitability.

ND42 | Cecilia Pandini
LncRNAs and ALS: the role of MYC-induced Non-Coding RNA MINCR.

ND43 | Maria Chiara Lionetti
Tackling complexity in neurodegenerative disease.

ND44 | Federica Rey
Study of the oncogenic lncRNA ZEB1-AS1 in sporadic ALS: identification of a new deregulated pathway.

ND45 | Francesca Fagiani
New nature-inspired hybrids modulating BDNF: a novel multi-target pharmacological approach to counteract neurodegeneration.

ND46 | Graziella Agrò
The specific JNK inhibitor peptide (D-JNKI1) prevents motor deficits and dendritic spine dysfunction in Angelman Syndrome mouse model.

ND47 | Silvia Animali
Indeexing arousal with pupillometry and EEG: implication for normal vs. pathological aging.

ND48 | Antonella Cardinale
The involvement of serotoninergic system in Parkinson's Disease: a morphologicalcharacterization of Tph2 mouse model.

ND49 | James Ashley
A New Mechanism of Action for Saffron Repron®: The Importance of an Orderly Visual Cycle.

ND50 | Ilaria Balbo
Myelin alterations in Elovl5 knock-out mice, murine model of Spinocerebellar Ataxia 38 (SCA38).

ND51 | Sara Bagnoli
Establishing the turquoise killifish Nothobranchius furzeri as a model for neurodegeneration.

ND52 | Daria Belan
Assessment of the neuroprotective potential of the new HSP70 inducer in the aging brain of rats in the model of Parkinson's disease.

ND53 | Alice Belloni
Multisensory Temporal Binding Window in multiple sclerosis.

ND54 | Fabio Biella
Investigation of molecular pathological hallmarks and therapeutic strategies in C9orf72 human lines.

ND63 | Toniella Giallongo
Neural Stem Cells transplantation in pre-clinical experimental model of Parkinson's: counteraction of neuroinflammation and promotion of functional recovery. [page 220]

Poster Session 3 (November 15th, 16:15-18:30)

NEURAL PLASTICITY | pp. 65-76

NP24 | Yauheniya Harbachova
Audiogenic kindling alters functional activity of the hypothalamic-pituitary-adrenal axis in Krushinsky-Molodkina rats.

NP25 | Clara Cambria
Novel Role of ATR in the Central Nervous System.

NP26 | Marie Pronot
Characterization of a synaptic SUMO2/3-ylome.

NP28 | Rosalba Olga Proce
Investigating brain development and neuronal circuit assembly in primary immunodeficiency WHIM syndrome models.

NP29 | Annalisa Savardi
Discovery and Characterization of Novel Selective NKCC1 Inhibitors for Down Syndrome, Autism and Brain Disorders with Depolarizing GABAergic Transmission.

NP30 | Vittoria Spero
Molecular mechanisms underlying stress resilience and vulnerability: a role for neuroplasticity and redox balance.

NP31 | Cecilia Steinwurzel
Inter-individual variability of short-term ocular dominance plasticity in human adults.

NP32 | Andrea Termine
Optogenetic stimulations to promote the extinction of fear memories.

NP33 | Francesca Tinelli
Modelling Moyamoya Angiopathy in vitro: from Italian patient's PBMCS to endothelial cells.

NP34 | Claudia Torelli
Active training promotes recovery of visual functions in adult amblyopic rats.

NP35 | Chiara Tortelli
Pupillometry reveals perceptual differences that are tightly linked to autistic traits.

NEUROINFLAMMATION | pp. 111-130

NI35 | Roberta Di Giacomo
Predictive value of high titer of GAD65 antibodies in a case of limbic encephalitis.

NI36 | Rossana Di Martino
Mitochondrial dysfunctions trigger the onset of neuroinflammation in animal models of Parkinson's disease.

NI37 | Rossella Di Sapia
Cholesterol 24-hydroxylase inhibition during epileptogenesis is neuroprotective, delays epilepsy onset and blocks seizures progression in a murine model of temporal lobe epilepsy.

NI38 | Natalia Cappoli
The mTOR kinase inhibitor rapamycin enhances the release of the pro-inflammatory cytokine IL-6 modulating the activation of human microglial cells.

NI39 | Federico Carlini
Modulation of REST and galectin expression induced by fingolimod treatment during experimental autoimmune encephalomyelitis.

NI40 | Livia Guadalupi
Voluntary running wheel protects against brain damage and motor defects induced by cuprizone (CPZ).

NI42 | Chiara Marini
miRNA shuttled by mesenchymal stem cell-derived exosomes downregulate the activated phenotype of primary astrocytes from end stage SOD1G93A mice.

NI43 | Pasquale Marrazzo
Different extracts from chestnut tree wastes downmodulate inflammation markers in a microglia cell model.

NI44 | Domenico Mercurio
Mannan-binding lectin-associated serine protease-2 (MASP-2) depleted mice show a better outcome after traumatic brain injury.

NI45 | Giorgio Corsi
Role of microglia in the regulation of sleep and circadian behavior.

NI46 | Erica Costantini
Age-dependant changes of nAChRs and TNFa in learning- and memory-related brain regions in APPswe/PS1dE9 mice.

NI47 | Giulia Musso
Clinical relevance and laboratory management of MOG antibody testing in children presenting with acute neurological symptoms.

NI48 | Giulia Musso
Paraneoplastic polyneuropathy with multiple antibody detection, a case report.

NI49 | Laura Neglia
Mannose-binding lectin elicits a direct toxic effect on ischemic endothelial cells from human brain vessels.

NI50 | Francesca Sironi
C9orf72 deletion anticipates motor onset, exacerbates denervation and increases immune response in SOD1G93A mouse model.

NI51 | Eliana Sammali
Infusion of human amniotic mesenchymal stromal cells improve functional recovery ofaged traumatic brain injured mice promoting protective astrocytic polarization.

NI52 | Maria Cristina Mariani
Sympathetic nervous system signals to beta-3 adrenergic receptor-expressing bone marrow cells promote lymphoid hematopoiesis in experimental autoimmune encephalomyelitis.

NI53 | Martina Di Nunzio
Microglia depletion in a murine model of epilepsy: effects on seizures and neuropathology.

NI54 | Fabio Perrucci
Soluble-TREM2 exerts a role on neurons independently of microglia interplay.

NI55 | Alessia Zotta
Altered expression of the Cerebral Cavernous Malformation gene CCM1 upon exposure to proinflammatory stimuli and aging: a study in animal and cellular models.

NEURO-ONCOLOGY | pp. 139-142

NO09 | Giacomo Milletti
AMBRA1 controls cerebellar morphogenesis through the regulation of N-Myc stability.

NO10 | Simona Paglia
A neurogenic model of adult brain cancer in Drosophila.

NO11 | Leire Pedrosa Eguílaz
Identification and functional characterization of long non-coding RNAs involved in the pathogenesis of glioblastoma.

NO12 | Marco Pizzocri
Engineered drug-loaded PLGA-PEG nanoparticles functionalized with an MMP-cleavable peptide for glioblastoma treatment.

PERINATAL NEUROLOGY | pp. 154-158

PN12 | Valeria Marchetti
In search for druggable mechanisms in MCPH17 primary microcephaly: a preliminary study on the effects of a postnatal antioxidant treatment.

PN13 | Denis Zosen
Cracking Brain Development: Chick Embryo Model and Neuropharmacology Studies.

PN14 | Ganna Balagura
SINEUPs: a novel molecular therapeutic strategy for the treatment of developmental and epileptic encephalopathies caused by pre-synaptic genes haploinsufficiency – preliminary in vitro results.

PN15 | Sara Mancinelli
Unravelling the role of electrical activity on cerebral cortex development and disease.

PN16 | Roberta Pintus
When perinatal programming goes wrong: a case of intra-ventricular hemorrhage.

NEURODEGENERATION | pp. 212-239

ND55 | Marina Boido
Chitosan-based hydrogel as a promising tool to support the paracrine activity of mesenchymal stem cells in spinal cord injury.

ND56 | Shoeb Anwar Ansari
Nano metal chelators: A new therapeutic approach in neurological disorder associated with metal imbalance.

ND57 | Federica Cordella
Dysbiosis induces an alteration of microglial function and synaptic signaling.

ND58 | Angela D'Anzi
Human induced pluripotent stem cells as model to study two forms of familiar Amyotrophic Lateral Sclerosis.

ND59 | Giovanni De Marco
Amyotrophic Lateral Sclerosis: same gene, different pathways. The case of two patients carrying mutations in FUS gene.

ND60 | Valentina Fantini
Bioink Composition for 3D Modeling of Neural Tissue using iPSCs and Neural Stem Cells.

ND61 | Eleonora Ficiarà
Iron content in Cerebrospinal Fluid as novel biomarkers for early diagnosis of Alzheimer's disease.

ND62 | Monica Favagrossa
Nose-to-brain delivery as a possible therapeutic strategy in restoring cholesterol brain levels in Huntington's disease.

ND64 | Giulia Guidotti
Dysregulation of astrocytic HMGB1 signaling in amyotrophic lateral sclerosis.

ND65 | Martina Gabrielli
Role of extracellular vesicles released by microglia in early synaptic dysfunction in Alzheimer's disease.

ND66 | Ludovica Iovino
Unraveling the role of astroglial LRRK2 in striatal glutamate homeostasis.

ND67 | Aleksei Kulikov
Apoptosis and autophagy in the hippocampus of Krushinsky-Molodkina rats during epileptogenesis.

ND68 | Silvia Giuditta Giulia La Marca
Emotional and social behavioral characterization of LOU/C/jall rats as an animal model of successful aging.

ND69 | Silvia Luotti
Validation study of PBMC protein biomarkers for amyotrophic lateral sclerosis.

ND70 | Gioia Marino
Corticostriatal synaptic plasticity alterations in the R6/1 transgenic mouse model of Huntington's disease.

ND71 | Antonio Masone
Generation of a prion protein (PrP)-HaloTag chimera: a novel approach to study the cellular trafficking and metabolism of PrP.

ND73 | Chiara Mazzola
Early dysfunction of astrocytic Ca2+ signaling in Alzheimer's disease.

ND74 | Giulia Milordini
A structural approach to investigate glycation effect on protein aggregation.

ND75 | Clara Alice Musi
Set up of an in vitro model of quadripartite synapse.

ND76 | Giuseppina Natale
Binge-like eating in healthy and Parkinsonian rats is differently driven by endogenous and exogenous dopamine.

ND77 | Samuele Negro
Neuronal injury induces Connective Tissue Growth Factor expression to support peripheral neuroregeneration.

ND78 | Laura Pasetto
Effects of therapeutic hypothermia in an animal model of Amyotrophic Lateral Sclerosis.

ND79 | Serena Stanga
Mitochondrial dysfunction: a new biomarker candidate for Spinal Muscular Atrophy?

ND80 | Marco Salluzzo
Direct Reprogramming of Reactive Astrocytes in Neurons in Mouse Motor Cortex after Stroke.

ND81 | Giulia Tomagra
Quantal dopamine release and firing properties of cultured midbrain neurons: multisite recordings using diamond-based multi electrode array.

ND82 | Francesca Tozzi
3-Iodothyronamine ameliorates synaptic transmission after oxygen-glucose deprivation in mouse brain slices.

ND83 | Martina Zambito
Mechanosensitive and pharmacological properties of Piezo1 channel in embryonic mesencephalic neuron-derived cells.

Notes