OSPEDALE POLICLINICO SAN MARTINO
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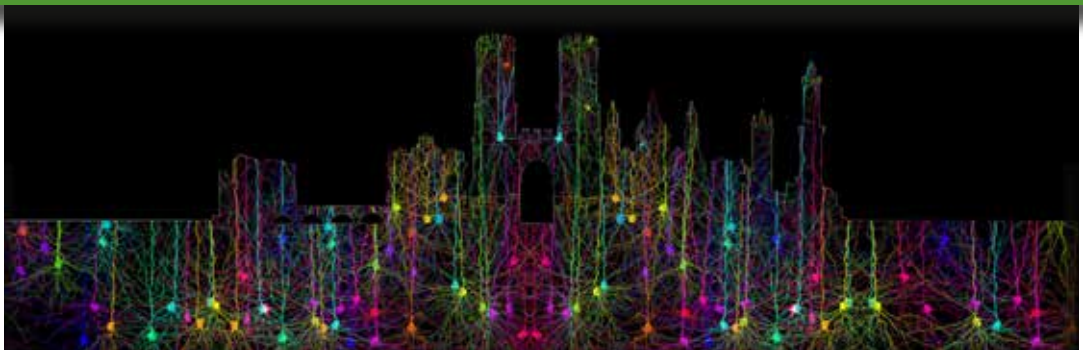
Istituto di Ricovero e Cura a Carattere Scientifico

29th 30th JUNE 2018 GENOA·ITALY

Brayn

1st BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS



Ospedale Policlinico San Martino - Auditorium IST Nord
Largo Rosanna Benzi 10, Genoa, Italy

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Marco Ferrazzoli Italian National Research Council, Rome (Italy)
Nunzio Iraci BIOMETEC University of Catania (Italy)
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1st BRAINSTORMING RESEARCH ASSEMBLY FOR YOUNG NEUROSCIENTISTS

JUNE 29st-30st 2018
GENOA - ITALY

Congress Venue:
Ospedale Policlinico San Martino – Auditorium IST Nord
Largo Rosanna Benzi 10, Genoa, Italy

www.braynconference.com

Dear Young Neuroscientists,

We are delighted to introduce you to the 1st 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. 310 delegates have registered to the conference. 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, 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 1st BraYn conference. We hope that you will enjoy the meeting and the beautiful city of Genoa!

The BraYn Staff

1st Brainstorming Research Assembly for Young Neuroscientists
June 29st-30st, 2018
www.braynconference.com

Congress Venue: Ospedale Policlinico San Martino - Auditorium IST Nord
Largo Rosanna Benzi 10, Genoa, Italy

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JUNE 29th

14:00 Registration

15:25 Opening ceremony

15:30 LECTIO MAGISTRALIS | **Marco Ferrazzoli** (CNR, Rome)
The lay scientist: scientific knowledge has become an opinion.
Chairman: Elisabetta Volpe

16:00 LECTURE | **Rosa C. Paolicelli** (IREM, Zurich)
Microglia-mediated synapse loss in neurodegeneration.
Chairmen: Giuseppina D'Alessandro and Matteo Tamborini

16:30 LECTURE | **Matthew Campbell** (Trinity College, Dublin)
Regulation of the blood-brain barrier in health and neurodegenerative disease. *Chairman*: Stefano Angiari

17:00 Coffee break and POSTER SESSION 1

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Session I - Neurodegeneration • ORAL COMMUNICATIONS
Chairmen: Manuela Medelin, Barbara Bettegazzi, Giovanni Nardo, Giulia Santamaria

18:15 **Eoin O'Keeffe** (Trinity College, Dublin) | *Dynamic blood brain barrier regulation in sub-concussive brain injuries.*

18:30 **Samuele Negro** (Biomed. Sci. Dept. UniPD) | *CXCL12/SDF-1 from perisynaptic Schwann cells promotes regeneration after motor axon terminal injury.*

18:45 **Marina Boido** (NICO, UniTO) | *Increasing agrin function antagonizes muscle atrophy and motor impairment in a murine model of SMA.*

19:00 **Irma Vismara** (IRCCS M. Negri) | *Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine in situ preserving cytoarchitecture and promoting functional recovery in spinal cord injury.*

19:15 **Natalie Hudson** (Trinity College, Dublin) | *Circadian regulation of the inner retinal vasculature; a paradigm for Geographic Atrophy development.*

19:30 **Deborah Ferrara** (CIBIO, UniTN) | *RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis.*

19:45 Closing remarks

20:30 Social dinner at Castello Simon Boccanegra (walking distance 50 mt).

JUNE 30th

8:55 Opening

Session II - Neuroinflammation • ORAL COMMUNICATIONS
Chairmen: Ilaria Prada, Alessandra Musella, Elisabetta Volpe, Stefano Angiari, Maria Velasco

9:00 **Valerio Chiurchiù** (IRCCS S. Lucia, UniRM) | *Pro-resolving lipid mediators and neuroinflammation: a novel strategy of resolution.*

9:15 **Giovanna Capodivento** (DINOGMI, UniGE) | *CSF Sphingomyelin concentration: a myelin biomarker for acquired demyelinating neuropathies.*

9:30 **Nicola Mattugini** (BMC-LMU, Germany) | *Region- and layer-specific differences in astrocytes reprogramming to neurons after brain injury.*

9:45 **Maria Rosito** (Kocher Inst. UniBE - IIT Rome) | *A silicon nanomembrane-based in vitro platform to visualize immune cell trafficking across the live human blood-brain barrier.*

10:00 Coffee break and POSTER SESSION 2

11:00 LECTURE | **Nunzio Iraci** (BIOMETEC, University of Catania).
Extracellular vesicles as a novel strategy of cell-to-cell communication.
Chairman: Giovanna Calabrese

* * *

Session III - Neural Plasticity • ORAL COMMUNICATIONS
Chairmen: Giovanni Calabrese, Alessandra Aldinucci, Giulia Nato

11:30 **Corrado Calì** (BESE, Thuwal) | *Neuroanatomical basis of Energy-Dependent synaptic plasticity investigated using virtual reality tools.*

11:45 **Matteo Spinelli** (IHPh UniCat Rome) | *Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a.*

12:00 **Antonio Falace** (Meyer Hospital, Florence) | *Filamin-A regulates morphological development and functional maturation of the cortical networks by modulating the ARHGAP24/RAC1 pathway.*

12:15 **Brunno Rocha Levone** (UniCol. Cork) | *Specific sub-regions along the longitudinal axis of the hippocampus mediate chronic stress effects on behaviour and corticosterone effects on neurogenesis.*

12:30 Lunch box with sponsor symposium
• **Gianluca Rotta** (BD Biosciences Italia) | *A new protocol for the detection and absolute count of microvesicles in untouched body fluids.*
• **Enrico Ghersi** (Miltenyi Biotec) | *Improving scientific reproducibility through REAfinity recombinant antibodies.*
• **Diego Muzzini** (Carlo Erba Reagents) | *10x Genomics: Changing the definition of sequencing.*

14:00 Coffee break and POSTER SESSION 3

15:30 Sponsor symposium
• **Francesco Moneta** (Bruker) | *Preclinical imaging solutions.*
• **Savino Lacerenza** (Fujifilm) | *High Frequency Ultrasound and Photoacoustic imaging for Small Animals.*
• **Paolo Boschi** (ZEISS) | *Microscopy Widefield Product Manager: 3D Volume Microscopy in Neuroscience.*
• **T. Claudio Bencivenga** (Sartorius Group) | *IncuCyte S3: Live-cell analysis System.*

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Session IV - Neuro-Oncology • ORAL COMMUNICATIONS
Chairmen: Giuseppina D'Alessandro, Matteo Tamborini, Paola Infante

16:30 **Eleonora Vannini** (CNR Pisa) | *Halting glioma growth and sparing neuronal function via Rho GTPase activation.*

16:45 **Marta Vuozzo** (IBFM-CNR) | *Translational study of hypoxia with [18F] FAZA-PET imaging in high-grade glioma: comparison with MRI and immunohistochemical biomarkers.*

17:00 **Francesco Alessandrini** (IRCCS, San Martino Hospital, Genoa) | *IL-12 armed retargeted herpes simplex virus as therapy for preclinical model of high-grade glioma.*

17:30 **Round table with invited discussants**
Giambattista Bonanno, **Matthew Campbell, Marco Ferrazzoli, Nunzio Iraci, Nicole Kerlero de Rosbo, Rosa C. Paolicelli**, **Antonio Uccelli**.
Translation of animal models to clinic: the new way forward.

18:30 BRAYN Awards Ceremony

18:40 Closing Ceremony

ORAL COMMUNICATIONS

Dynamic blood brain barrier regulation in sub-concussive brain injuries

Eoin O'Keefe[1§], E. Kelly[2§], E. Wallace[2], C. Greene[1], S. Hughes[3], J. Kealy[1], N. Doyle[3], M.M. Humphries[1], M. Farrell[4], G.A. Grant[5], A. Friedman[6,7], R. Veksler[6], M. G. Molloy[8], J.F. Meaney[9], N. Pender[3], C.P. Doherty[2*#] and M. Campbell[1*#]

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Traumatic brain injury (TBI) is large health issue on a global scale, contributing to the deaths of thousands and the debilitation of many more each year. While moderate and severe TBI may be recognised by overt symptoms or abnormalities on conventional imaging modalities (MRI or CT scans), mild TBI, or concussion, rely on individual neuropsychological assessments. While undetected by current neuroimaging paradigms, subtle changes in the gliovascular unit that may following mild or sub-mild TBI may contribute to development of neurodegenerative conditions later in life, as suggested by findings of Blood Brain Barrier disruption (BBBD) in an individual diagnosed with Chronic Traumatic Encephalopathy, a condition primarily associated with repeated mild TBI. Here we present evidence of changes at the Blood Brain Barrier in a sub-set of children following a season of Rugby Union, measured by Gadolinium extraversion signal. Paired with this were findings of increased levels of BDNF and MCP-1 in plasma samples, in addition to increased pro-inflammatory response by Peripheral Blood Mononuclear Cells post-season, as measured by IL-1β production following necrotic brain tissue stimulation. In addition, examining a young adult cohort produced similar finding when examined within hours of engaged in a Rugby Union match. These finding suggest that a combination of analytical methods are required in order to gain an understanding of potential neural damage occurring due to mild TBI or sub-concussive blows.

Keywords: Brain injury, Inflammation, Biomarkers, Degeneration

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CXCL12/SDF-1 from perisynaptic Schwann cells promotes regeneration after motor axon terminal injury

Samuele Negro[1], F. Lessi[2], E. Duregotti[1], P. Aretini[2], M. La Ferla[2], S. Franceschi[2], M. Menicagli[2], M. Pirazzini[1], C.M. Mazzanti[2], M. Rigoni[1] and C. Montecucco[1,3]

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The neuromuscular junction (NMJ) is a tripartite synapse composed of the motor axon terminal, covered by perisynaptic Schwann cells (PSCs), and the muscle fibre, separated by a basal lamina. It is exposed to different kind of injures such as mechanical traumas, pathogens, including neurotoxins and neuromuscular diseases. The NMJ, at variance from central synapses, has retained through evolution the capacity to regenerate after damage, but little is known on the inter-cellular signals involved in its functional recovery from trauma, autoimmune attacks, or neurotoxins. We demonstrated that the chemokine CXCL12 is produced specifically by PSCs following axon terminal degeneration induced by alpha-latrotoxin. CXCL12 acts via binding to the neuronal CXCR4 receptor. A CXCL12 neutralizing antibody or a specific CXCR4 inhibitor strongly delays recovery from motor neuron degeneration in vivo. Recombinant CXCL12 in vivo accelerates neurotransmission rescue upon damage and very effectively stimulates the axon growth of spinal cord motor neurons in vitro. These findings indicate that the CXCL12-CXCR4 axis plays an important role in the regeneration of the neuromuscular junction after motor axon injury. The present results have important implications in the effort to find therapeutics and protocols to improve recovery of function after different forms of motor axon terminal damage.

Keywords: Degeneration, Regeneration, Neuron-glia communication

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Increasing agrin function antagonizes muscle atrophy and motor impairment in a murine model of SMA

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Spinal muscular atrophy (SMA) is a recessive autosomal neuromuscular disease, characterized by motor impairment, muscle atrophy and premature death following motor neuron (MN) degeneration. Neuromuscular junction (NMJ) abnormalities have been reported in SMA, including neurofilament (NF) accumulation, immature and smaller than normal endplates, and muscle denervation. We have studied the role of agrin (a synaptic organizer responsible for NMJ development) in SMNdelta7 mice, an experimental model of SMA. We observed a 50% reduction in agrin expression levels in quadriceps of P10 SMA mice compared to age-matched WT. We then treated SMA pups from birth onwards with therapeutic agrin biological NT-1654, an active splice variant of agrin retaining synaptogenic properties and resistant to proteolytic cleavage by neurotrypsin. The treatment significantly delayed the motor performance decrease and extended the murine survival. Moreover H/E-stained sections of the quadriceps showed that NT-1654 treatment strongly prevented the size decrease of muscle fibers. Finally, evaluating the diaphragm, we observed that NT-1654-treated SMA mice had more mature NMJs and reduced NF accumulation, compared to vehicle-treated SMA mice. We conclude that increasing agrin function in SMA has beneficial outcomes on muscle fibers and NMJs as the agrin biological NT-1654 can restore the crosstalk between muscle and MNs.

Keywords: Animal model, Degeneration

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Mesenchymal stem cells encapsulated into biomimetic hydrogel scaffold gradually release CCL2 chemokine in situ preserving cytoarchitecture and promoting functional recovery in spinal cord injury

Irma Vismara[1], S. Papa[1], A. Mariani[1], M. Barilani[2,3], S. Rimondo[4], M. De Paola[1], N. Panini[1], E. Erba[1], E. Mauri[4], F. Rossi[4], G. Forloni[1], L. Lazzari[3], P. Veglianese[1]

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Spinal cord injury (SCI) is an acute neurodegenerative disorder caused by traumatic damage of the spinal cord. The neuropathological evolution of the primary trauma involves multifactorial processes that exacerbate the pathology. This complexity suggests that multi-therapeutic approaches, rather than any single treatment, might be more effective. Encouraging preclinical results indicate that stem cell-based treatments may improve the disease outcome due to their multi-therapeutic ability. Mesenchymal Stem Cells (MSCs) are currently considered one of the most promising approaches. Significant improvement in the behavioral outcome after MSC treatment sustained by hydrogel has been demonstrated. However, it is still not known how hydrogel contribute to the delivery of factors secreted from MSCs. Among different mediators secreted by MSCs after seeding into hydrogel, we have found CCL2 chemokine, which could account for the neuroprotective mechanisms of these cells. CCL2 secreted from human MSCs is delivered efficaciously in the lesioned spinal cord acting not only on recruitment of macrophages, but driving also their conversion to an M2 neuroprotective phenotype. Surprisingly, human CCL2 delivered also plays a key role in preventing motor neuron degeneration in vitro and after spinal cord trauma in vivo, with a significant improvement of the motor performance of the rodent SCI models.

Keywords: Spinal cord injury, Inflammation, Degeneration, Stem cells

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Circadian regulation of the inner retinal vasculature: a paradigm for Geographic Atrophy development

Natalie Hudson[1], L. Celkova[1], E. Fahey[2], E. Ozaki[2], S. Doyle[2], M. Campbell[1].

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The phagocytosis and renewal of photoreceptor outer segments (POS) is a process that occurs daily upon onset of light. The involvement of circadian rhythms in retinal function however, are still not fully understood. Here, we examined the role of circadian clock components in the regulation of inner blood retina barrier (iBRB) function that we show is directly related to the replenishment of shed POS'. We found that TJ component claudin-5 cycled in the retinal vasculature throughout the day in a circadian-dependent, rather than diurnal manner, with lower levels at 8PM compared to 8AM. These changes phenotypically led to more permeable retinal vessels in the evening compared to morning as observed by fundus fluorescein angiography (FFA) and dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) analyses. Circadian regulated changes in retinal vascular permeability was not evident in BMAL1FL/FL-Tie-2 mice, where the clock gene BMAL1 was lacking in endothelial cells. Mice exposed to a high fat diet in tandem with persistent suppression of claudin-5 developed rapid onset of a geographic atrophy (GA) like phenotype, which is the end stage of the condition dry age related macular degeneration (AMD). We have discovered that the iBRB is highly dynamic and plays a critical role in replenishing POS. Circadian regulation of claudin-5 facilitates the exchange of material between blood and the neural retina. Therefore, regulating claudin-5 or circadian clock components may represent a novel therapeutic target for treating GA.

Keywords: Animal model, Degeneration, Visual perception

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RNA-mediated intercellular miscommunication: role of extracellular vesicle cargos in Amyotrophic Lateral Sclerosis

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Amyotrophic Lateral Sclerosis (ALS) is a rare neurodegenerative disorder that primarily targets upper and lower motor neurons. The progression of the disease is mediated by altered intercellular communication in the spinal cord between neurons and glial cells. Intercellular communication, mainly happening through extracellular vesicles (EVs) is responsible for the horizontal transfer of proteins and RNAs to recipient cells. Previously, we proved that EVs released from ALS mutant SOD-1 astrocytes selectively induced toxicity in wild type motor neuron, suggesting EVs as mediators of toxicity. Although, multiple factors are implicated in motor neuron degeneration, recent evidences point towards a fundamental role for RNA and RNA-binding protein dys-homeostasis as crucial players in ALS pathogenesis. Our hypothesis is that EVs constitute a mechanism for local and systemic intercellular transfer not only of proteins but also of RNA, responsible for driving disease progression and contribute to motor neuron degeneration. We isolated EVs from spinal and cortical astrocytes with a novel methodology, called NBI (under patent). We observed that EVs, derived from a transgenic mouse model of ALS overexpressing mutant TDP-43, were able to induce cell death in wild type motor neurons, confirming our previous findings. Our future goals will be to analyze EVs cargo.

Keywords: Neuroimaging, Degeneration, Neuron-glia communication

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Pro-resolving lipid mediators and neuroinflammation: a novel strategy of resolution

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Neuroinflammation is a chronic process involving sustained activation of brain glia and recurrent infiltrations of peripheral leukocytes. When persistent and uncontrolled, it ultimately leads to neurodegeneration, which is typical of several disorders of the central nervous system, from brain injuries to multiple sclerosis and Parkinson disease, for which a conceptual change in diagnostic and therapeutic direction is needed. Such conceptual change is offered by the recent concept of "resolution of inflammation" which is orchestrated by the previously unrecognized ω-3-derived lipids termed specialized pro-resolving mediators (SPMs) that include resolvins and protectins. Recent evidence indicates that altered SPM metabolism and function can contribute to several chronic inflammatory diseases. On the basis of this scenario, our research group has shown for the first time the existence of a dysfunctional pro-resolution pathway in 3 different paradigms of neuroinflammation: a rat model of focal brain injury, a transgenic α-synuclein-overexpressing rat model of Parkinson's disease and in multiple sclerosis. The in vitro and/or in vivo SPM administration promoted functional recovery, neuroprotection and attenuation of peripheral/brain inflammation, suggesting that neuroinflammation might be ascribed to an impairment of the SPM-dependent resolution machinery and that boosting this endogenous pathway could represent a novel therapeutic strategy against several neuroinflammatory diseases.

Keywords: Molecular biology, Animal model, Electrophysiology, Brain injury, Inflammation, Biomarkers, Regeneration, Immune system, Neuron-glia communication

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CSF Sphingomyelin concentration: a myelin biomarker for acquired demyelinating neuropathies

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Fast, accurate and reliable methods to quantify the amount of myelin still lack, both in humans and experimental models. The overall objective of the present study was to demonstrate that sphingomyelin (SM) in the cerebrospinal fluid (CSF) of patients affected by demyelinating neuropathies is a myelin biomarker. We found that SM levels mirror both peripheral myelination during development and small myelin rearrangements in experimental models. As in acquired demyelinating peripheral neuropathies myelin breakdown occurs, SM amount in the CSF of these patients might detect the myelin loss. Indeed, quantification of SM in 262 neurological patients showed a significant increase in patients with peripheral demyelination compared to subjects affected by non-demyelinating disorders. Interestingly, SM alone was able to distinguish demyelinating from axonal neuropathies and differs from the principal CSF indexes, confirming the novelty of this potential CSF index. In conclusion, SM is a specific and sensitive biomarker to monitor myelin pathology in the CSF of peripheral neuropathies. Most importantly, SM assay is simple, fast, inexpensive, and promising to be used in clinical practice and drug development.

Keywords: Inflammation, Biomarkers, Remyelination

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Region-and layer-specific differences in astrocytes reprogramming to neurons after brain injury

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Direct reprogramming of local glial cells into neurons is a promising approach for brain repair. A key question is which glial cells to target. Astrocytes are promising candidates as the retain expression of patterning transcription factors from their ancestors in development, the radial glial cells, suggesting that they are best specified to generate neuronal subtypes appropriate for the respective brain region when forced to turn into neurons. However, proliferating astrocytes perform beneficial functions after brain injury. We therefore decided to target non-proliferating astrocytes using AAVs where the neurogenic factors were cloned in flexed orientation, so they are reverted only in GFAP-Cre expressing astrocytes. Using this system we compared direct neuronal reprogramming in different positions in the Grey Matter (GM) and White Matter (WM) of the cerebral cortex after injury. We discovered a combination of proneural factors (Neurog2 or Ascl1) with another transcription factor allows highly efficient neuronal reprogramming in GM astrocytes, but not in WM astrocytes. We further show that these neurons acquire different identities at different layer positions, demonstrating for the first time the profound influence that the region- and layer-specific identity of astrocytes has on the neuronal subtype generated in direct reprogramming in vivo.

Keywords: Brain injury, Regeneration, Stem cells

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A silicon nanomembrane-based in vitro platform to visualize immune cell trafficking across the live human blood-brain barrier

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Here we report on the development of a novel microfluidic human in vitro cerebrovascular barrier (CVB) model featuring stem cell derived brain like endothelial cells (BLECs) and nanoporous silicon nitride (NPN) membranes (µSiM-CVB). The nanoscale thinness of NPN membranes combined with their high permeability and mechanical stability make them an ideal scaffold for the assembly of an in vitro model of the blood-brain barrier (BBB) featuring cellular elements of the neurovascular unit (NVU). Dual-chamber devices divided by NPN membranes yield tight barrier properties in BLECs, and allow an abluminal pericyte-co-culture to be replaced with pericyte-conditioned media. With the benefit of physiological flow and superior imaging quality, our µSiM-CVB captures each phase of the multi-step T-cell migration across the BBB. The small volume of < 100 µL of the µSiM-CVB will enable in vitro investigations of rare patient derived immune cells with the human BBB by live cell imaging under flow.

Keywords: Nanomaterials/nanoparticles, Inflammation, Immune system

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Neuroanatomical basis of Energy-Dependent synaptic plasticity investigated using virtual reality tools

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Long-term memory formation is an energy-expensive process, which is accompanied by structural changes at synapses, such as an increase in spines' volume and density. To directly investigate such changes, and their dependence on brain energy metabolism, we perform morphometric analysis on segmented 3D Electron Microscopy (EM) imaged volumes. To this aim, we developed a set of custom-made tools tailored for our needs and pioneered the use of 3D analysis using virtual reality (VR). We focused our analysis on glycogen, a precursor of lactate and an energy storage molecule specifically expressed in astrocytes and inferred its spatial relationship with synaptic contacts. We compared the neuropil of mice undergoing a novel object recognition (NOR), in presence or absence of a potent inhibitor of glycogenolysis, 1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride (DAB), already known to cause amnesia in rats. We found that the impairment in long-term memory formation correlated with failing to form new synapses, together with a decrease in the levels of glycogen, supposedly the source of lactate whose exogenous application was able to rescue the structural and behavioral phenotype of mice. VR analysis highlighted the presence of hotspots correlated with structural plasticity.

Keywords: Plasticity, Imaging, Neuron-glia communication

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Brain insulin resistance impairs hippocampal synaptic plasticity and memory by increasing GluA1 palmitoylation through FoxO3a

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High-fat diet (HFD) causes metabolic alterations and impacts on hippocampal synaptic plasticity, learning, and memory through poorly understood mechanisms. We observed that HFD increases palmitic acid deposition and alters insulin signaling in the hippocampus. These events cause FoxO3a-mediated overexpression of the palmitoyltransferase zDHHC3 leading to hyper-palmitoylation and hypo-phosphorylation of AMPA glutamate receptor subunit GluA1. Accordingly, hippocampal neurons stimulated with palmitic acid and insulin (IPA) showed an imbalance of GluA1 palmitoylation/phosphorylation ratio, alteration of GluA1 trafficking to the synaptic membrane and inhibition of AMPA currents. The critical role of aberrant GluA1 palmitoylation in HFD-related alteration of synaptic plasticity was confirmed by overexpression of palmitoylation-deficient GluA1 and hippocampus-specific silencing of Zdhhc3. Both experimental models restored, respectively, the impairment of synaptic plasticity in organotypic slices and cognitive deficit in HFD mice. Finally, intranasal delivery of palmitoyltransferase inhibitor, 2-bromopalmitate, restored normal levels of GluA1 palmitoylation in the hippocampus and counteracted the impairment of learning and memory in HFD mice. Our data reveal a key role of FoxO3a/Zdhhc3/GluA1 axis in the HFD-dependent impairment of cognitive functions and identify a novel mechanism underlying the crosstalk between metabolic and cognitive disorders.

Keywords: Molecular biology, Electrophysiology, Cognitive

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Filamin-A regulates morphological development and functional maturation of the cortical networks by modulating the ARHGAP24/RAC1 pathway

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Mutations in the FilaminA (FlnA) are the main cause of Periventricular Nodular Heterotopia syndrome, which combines cortical malformations, epilepsy and intellectual disability. However, the physiopathological basis for this is still largely unknown. Here, to investigate the neurobiological role of FlnA we selectively inactivated FlnA in the mouse forebrain neuroprogenitors. Loss of FlnA recapitulates the brain alterations reported in patients, however cortical structure and layering are preserved suggesting that primary causes of seizures and cognitive deficits have to be delved in a pure dysfunction of the neuronal networks. Then we investigated the function of FlnA at single cell level in vivo and found that Flna plays a major role in dendritogenesis and synaptogenesis via ARHGAP24/Rac1 signaling pathway. Furthermore, Flna KO cortical pyramidal neurons displayed a net increase in the frequency of inhibitory postsynaptic currents associated with a significant amplitude reduction suggesting an unbalance between excitatory and inhibitory currents in FlnA KO cortical networks.

Keywords: Animal model, Electrophysiology

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Specific sub-regions along the longitudinal axis of the hippocampus mediate chronic stress effects on behaviour and corticosterone effects on neurogenesis

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Accumulating evidence suggests that the hippocampus is functionally segregated along its longitudinal axis whereby the dorsal hippocampus (dHi) plays a predominant role in spatial learning and memory, while the ventral hippocampus (vHi) plays a predominant role in the regulation of anxiety. Gene expression studies have also suggested that the intermediate hippocampus (iHi) might be functionally independent. Here, using ibotenic-induced lesions of these specific areas of the mouse hippocampus, we assessed the roles of the dHi, iHi and vHi in the behavioural responses to chronic psychosocial stress. Moreover, we isolated neuroprogenitor cells (NPCs) from each hippocampal sub-region and assessed the effects of chronic exposure to corticosterone on neuronal differentiation and maturation. Lesions of any sub-regions prevented stress-induced increases in anxiety and lesions of dHi and vHi prevented stress-induced anhedonia. Lesion of iHi exacerbated stress-induced social avoidance and of vHi promoted antidepressant-like behaviour and active coping behaviour. Chronic exposure to corticosterone reduced neuronal differentiation and maturation, preferentially in vHi-derived cells. Taken together, we suggest that future studies of the hippocampus aimed at identification of novel drug development targets should consider the differential roles of hippocampal sub-regions in stress-induced changes in behaviour and neurogenesis.

Keywords: Animal model, Stem cells

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Halting glioma growth and sparing neuronal function via Rho GTPase activation

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The most aggressive form of gliomas is represented by Glioblastoma Multiforme (GBM), which has a rapid evolution, neoplastic infiltration of adjacent normal brain tissue and solid proliferating tumor at the periphery. The standard of care consists in surgical resection of tumor mass followed by cycles of radiotherapy and chemotherapy. However, this intervention protocol is only partly effective. Thus, there is an urgent need to find innovative and efficient approaches for the treatment of GBM patients. Novel strategies should aim not only at targeting glioma growth, but also at preventing functional deterioration of spared brain networks. In the last years I assessed the potential of CNF1 (Cytotoxic Necrotizing Factor 1), a bacterial protein toxin, as an anti-neoplastic drug in GBM. CNF1 enters cells close to the site of delivery and induces a long-lasting activation of Rho GTPases, enhancing neuronal plasticity and blocking cytodieresis in proliferating cells. Specifically, CNF1 leads glioma cells to death, increasing survival of glioma-bearing mice, reducing glioma mass volume and sparing neuronal responses and architecture in the healthy tissue surrounding GBM mass. Studies with a CNF1 derivative capable of crossing the BBB are currently ongoing in our lab.

Keywords: Animal model, Electrophysiology, Plasticity, Cancer

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Translational study of hypoxia with [18F]FAZA-PET imaging in high-grade glioma: comparison with MRI and immunohistochemical biomarkers

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The correlation between [18F]FAZA-PET/CT imaging data and hypoxia markers in a mouse model of glioblastoma (GBM) and in patients with high-grade glioma (HGG) was investigated. Orthotopic GBM mouse models were obtained by injecting temozolomide (TMZ)-sensitive and –resistant cell lines. Animals were categorized in vehicle and TMZ-treated groups. All animals underwent MRI and [18F]FAZA-PET before and 7 days after treatment to assess therapy efficacy. Eighteen patients underwent pre-therapy MRI and [18F]FAZA-PET/CT; maximum and mean standardized uptake value (SUV), Metabolic Tumour Volume and Hypoxic Volume were considered as PET-derived parameters. Patients underwent biopsy or surgical resection. Immunohistochemical analysis for hypoxic (HIF-1α, CA-IX), glucose transporter (GLUT-1), tumour angiogenesis (CD31) and proliferation (ki-67) markers was performed. In GBM animal models, TMZ-sensitive treated tumour displayed a significant decrease in [18F]FAZA uptake compared to control after treatment. [18F]FAZA-PET/CT detected lesions in 17/18 patients. In surgical subgroup (n=8), CA-IX significantly correlated to all PET-derived parameters. In bioptic subgroup (n=10), CD31 was significantly correlated with all SUV parameters. Hypoxia imaging using [18F]FAZA-PET/CT is able to assess therapy efficacy in GBM mouse model and it can be a reliable method to evaluate tumour hypoxia in HGG patients as supported by the correlation between imaging parameters and CA-IX and CD31.

Keywords: Animal model, Neuroimaging, Cancer

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IL-12 armed retargeted herpes simplex virus as therapy for preclinical model of high-grade glioma

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High grade gliomas (HGG) are the main malignant tumors of central nervous system and, so far, almost incurable because of the radio- and chemo-resistance. A novel therapeutic approach, based on recombinant oncolytic herpes simplex viruses (oHSV) that target cells expressing specific receptors, combined with immunomodulation, fits in the field of new promising strategies aimed at enhancing a targeted and efficient therapeutic response. We evaluated the effects of a retargeted fully virulent HSV-1 (named R-115) armed with mIL12 on established syngeneic hHER2 expressing HGG. R-115 exhibited a specific hHER2 tropism. Retargeted viruses are intrinsically safer than attenuated ones and it is effective in counteract tumor growth, doubling the median survival after a single injection of viral preparation into fully established gliomas. Noteworthy, we observed for the first time in our studies a substantial percentage of long survivors associated with the development of resistance to recurrence of the same neoplasia, that could potentially improve with earlier or repeated treatments. Moreover, we pointed out that mIL12 expressing R-115 enhanced the production of antibodies targeting transplanted glioma cells and it makes the tumor tissue accessible to infiltration of T-lymphocytes. Results obtained represent a step forward towards the possibility to treat HGGs with retargeted oHSV.

Keywords: Animal model, Immune system, Cancer

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POSTER SESSIONS

ND1 | 12 Red Squares App-Coo-Test. A valid touch screen application for the quantitative assessment of the upper limb movement disorders in patients with Friedreich's ataxia

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Objective scales to quantitatively evaluate movement disorders in patients with Friedreich's ataxia (FRDA) are very important for the use in clinical trials of newly developed medications and rehabilitation. Our study was aimed at developing a touch screen application, freely downloadable from the Internet, able to carry out quantitative measurements of the upper limb coordinative capabilities in patients with FRDA. The App, that we named "12 Red Squares App-Coo-Test" (12-RSACT), assesses these skills by measuring the test execution time. We investigated 80 controls and 34 patients with FRDA. A linear regression model and the Pearson's correlation coefficient were used to explain the correlation between the measurements obtained using the 12-RSACT and those obtained with the Scale for the Assessment and Rating of Ataxia (SARA), the Nine Hole Peg Test (9HPT) and the Click Test. We observed a high linear correlation between the results obtained with the 12-RSACT and those obtained with the SARA, the 9HPT and the Click Test. These correlations were statistically significant, with a P-Value p <0.00001. Therefore, the 12-RSACT is a new and easy-to-use tool that can reliably identify the upper limb movement disorder in patients with FRDA.

Keywords: Degeneration

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ND2 | App-Coo-Balance-Test. A new application to assess static and dynamic balance in patients with cerebellar ataxia

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Unstable gait, lack of coordination, tremor and speech disorders are the main characteristics of ataxia syndrome. This study was aimed at developing an easy and reliable application, freely downloadable from the Internet, able to assess static and dynamic balance in patients with cerebellar ataxias (CA) and to produce quantitative measurements that can be used in clinical trials. We investigated 40 controls and 23 patients with CA. As the balance abilities of the patients with CA worsen with the worsening of the ataxic symptomatology, the results obtained using the new application have been correlated with the Scale for the Assessment and Rating of Ataxia (SARA) and the Cerebellar Composite Severity Score (CCFSS). A linear regression modeling and the Pearson's correlation coefficient were used to explain the correlation between the measurements obtained using the App-Coo-Balance-Test and those obtained with SARA and CCFSS. We observed a high linear correlation between the results obtained with the new application and those obtained with the SARA and the CCFSS ($p<0.01$). The App-Coo-Balance-Test is therefore a valid system to quantitatively measure the level of oscillations both when the patient is in a static position with their feet together or on a broad base and during the gait.

Keywords: Degeneration

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ND3 | The biophotonic challenge in neuroscience: development of innovative methods to explore the neurodegeneration mechanisms and discover new biomarkers

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Surface Plasmon Resonance imaging (SPRi) and Raman spectroscopy are well-known photonic-based technologies that are experiencing increasing application in biomedical research, thanks to their sensitivity, label-free and high-throughput features. Herein, we propose biophotonics as innovative and valuable tool for the characterization of brain derived exosomes (EXO) with the specific aim of discovering new biomarkers of neurodegenerative pathologies. EXO are nanoscaled vesicles that stem from all body cells to vehicle bioactive molecules. We developed an antibody SPRi-array to separate different neurally-derived EXO from plasma: those from neurons, oligodendrocytes, astrocytes and microglia were successfully identified with good sensitivity and specificity. The analysis of the relative amount of proteins and lipids present on the EXO membrane was also performed, demonstrating their heterogeneous composition and the potentiality of SPRi-chip to study their interaction with other molecules, i.e. amyloid. In parallel, Raman spectroscopy was applied to serum EXO from patients with Parkinson's disease (PD). Our preliminary data demonstrate the presence of vesicles associated/loaded with atypical cargoes when compared to healthy controls, providing support to the proposed role of EXO in PD pathogenesis and suggesting the possibility to evaluate the Raman spectrum of circulating EXO as a PD biomarker itself, complementary to other specific molecular markers.

Keywords: Biomarkers, Degeneration, Biophysics

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ND4 | Inhibition of miR-125a-3p promotes OPC maturation following lysolecithin induced demyelination

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Multiple sclerosis 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. 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 (Lecca et al., Sci Rep, 2016). Here, by using a combined transcriptomic and bioinformatic approach we identified new miR-125a-3p targets, and demonstrated that their silencing contributed to the reduction of MBP expression, suggesting a new regulatory mechanism in OPC maturation. Moreover, to evaluate whether miR-125a-3p modulation could influence remyelination in a pathological context, we silenced it by lentiviral infection in a lysolecithin-induced demyelinating model. Interestingly, we found an up-regulation of some of its targets coupled to increased levels of markers of mature/myelinating oligodendrocytes, indicating that miR-125a-3p silencing accelerated lesion repair. Based on these results, we postulate that an antago-miRNA specific for miR-125a-3p may help to promote remyelination in demyelinating diseases. Sponsored by Fondazione Cariplo, grant n° 2014-1207 to DL.

Keywords: Molecular biology, Animal model, Remyelination

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ND5 | Non-coding RNAs (ncRNAs) content of Plasma Neural Derived Exosomes (NDEs): new potential biomarkers for Alzheimer's Disease (AD) diagnosis

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The aim of our study was to analyze the content in terms of ncRNAs (microRNAs and long non coding RNAs) in plasma NDEs of Prodromal AD, AD patients and healthy controls in order to validate the detection of these molecules in NDEs as non-invasive and easy- to-access AD biomarkers. We isolated plasma NDEs from 20 prodromal AD, 20 AD patients and 20 healthy controls by using ExoQuick exosome precipitation solution (SBI). Total exosomes were enriched for neural sources by immunoprecipitation with anti-human L1CAM antibody and analyzed by flow cytometry (FACS Aria, BD) and Transmission Electron Microscopy (TEM). NDEs miRNAs levels were determined by RT-qPCR using TaqMan OpenArray technology in a QuantStudio 12K system (Thermos Fischer Scientific) while NDEs lncRNAs expression has been tested by syber-green commercial available arrays (Qiagen). We tested the expression of 754 miRNAs and a preliminary analysis led us to individuate the expression of 50 miRNAs that reached the optimal quality score. Among those, there are some, as miR-146, miR-223, miR-15b already found to be associated with neurological diseases. Moreover the expression profile of 164 lncRNAs showed an overall dysregulation in NDEs lncRNAs expression levels between groups of subjects. The overall significance of these preliminary results are that NDEs are easy detectable in biological fluid and are an enriched source of ncRNAs. They could likely represent reliable early peripheral biomarkers for AD diagnosis.

Keywords: Molecular biology, Ageing, Biomarkers

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ND6 | Understanding the role of VAPB in peripheral blood mononuclear cells of patients affected by sALS

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A point mutation, P56S, in the gene encoding VAPB (vesicle-associated membrane protein-associated protein B), an endoplasmic reticulum (ER)-integrated membrane protein, leads to autosomal-dominant form of Amyotrophic Lateral Sclerosis (ALS), classified as ALS-8. Mutant VAPB forms ER-associated aggregates, leading to a complete reorganisation of ER structures, activation of cellular stress and ultimately to neurodegeneration. Numerous studies demonstrated VAPB involvement also in sporadic ALS (sALS), although definite pathogenic molecular mechanisms are still unclear. ALS-8 form is infrequent and patients' biopsies are rarely available, thus careful analysis of VAPB in peripheral blood mononuclear cells (PBMCs) could represent a good not-invasive option for studying ALS. We isolated PBMC from sporadic ALS patients, Parkinson's disease (PD) patients and healthy control (HCs), to evaluate VAPB expression through flow cytometry assay and its localisation by immunofluorescence analysis. Immunofluorescence assay revealed the presence of VAPB misfolding evident as specific ER alteration in all ALS patients. Furthermore, flow cytometry analysis showed a reduction of VAPB expression in ALS patients. In conclusion the data obtained support the possibility that VAPB could serve as a novel candidate biomarker for sporadic ALS diagnosis and that PBMCs could serve as an important tool for studying ALS and for early diagnosis of this pathology

Keywords: Biomarkers, Degeneration, Protein aggregation

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ND7 | Mesenchymal stem cells conditioned secretome: a new frontier therapeutic strategy in the treatment of Alzheimer's disease

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The multiplicity of systems affected in Alzheimer's disease (AD) brains encourages the development of multi-target therapies. Mesenchymal stem cells (MSC) represent a promising candidate being endowed with neuro-regenerative/reparative, immunomodulatory and anti-amyloidogenic abilities, but their clinical application is still limited by several risks related to their direct implantation inside the host. We herein exploited the paracrine action theory, which states that MSC repair damaged environments by releasing multiple bioactive molecules in their "secretome", rather than through their physical engraftment. We verified in vivo that one intravenous injection of MSC-derived conditioned secretome (MSC-CS), collected from MSC exposed in vitro to AD stimuli, fully restored memory in 12- and 22-month-old APP/PS1dE9 mice 7-day post-injection. In older mice, brain repair was achieved under a repeated intranasal treatment. Plaques were greatly reduced in both the cortex and the hippocampus. Gliosis and the phagocytic marker CD68 were significantly decreased. Neuro-regeneration was observed and life span of APP/PS1dE9-treated mice increased significantly. Our data prove that MSC-CS can substantially recapitulate multiple neuro-reparative/regenerative actions of MSC in AD mice. Future identification of the therapeutic secretome factors would overcome MSC implantation, avoiding those risks related to their unknown fate once inside the host.

Keywords: Cognitive, Degeneration, Stem cells

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ND8 | Morpholino conjugated with Cell Penetrating Peptides: a promising therapeutic strategy for Spinal Muscular Atrophy symptomatic cases

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Spinal muscular atrophy is a childhood motor neuron disease caused by mutations in Survival Motor neuron 1 gene. Currently, antisense oligonucleotides targeting the paralogous Survival Motor neuron 2 gene is the only approved treatment. However, to date, there are still several hurdles to overcome such as biodistribution, site and number of injections, and treatment efficacy in symptomatic cases. In this study, we evaluated the therapeutic efficacy of the oligonucleotide antisense variant Morpholino conjugated with cell-penetrating peptides that can improve cellular and tissue uptake. We have already demonstrated that Morpholino is able rescue the phenotype in pre-symptomatic affected mice. We linked four different peptides to our already validated Morpholino sequence. First, we evaluated the best peptides able to deliver Morpholino to the central nervous system after a systemic injection performed in symptomatic mice. Results demonstrated the efficiency of all conjugates and the superiority of r6 and (RXRRBR)2XB. These conjugates were tested in a larger cohort of mice to evaluate the effect on the pathological phenotype. Survival and functional analysis of treated mice strikingly confirmed the superiority of the conjugates on unconjugated Morpholino. Our experiments achieved for the first time the disease rescue with a treatment administered in the symptomatic phase.

Keywords: Molecular biology, Animal model, Degeneration, Regeneration

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ND9 | MicroRNA-125a-3p negatively regulates oligodendroglial maturation and re-myelination: molecular mechanisms and clinical implications in multiple sclerosis

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In the mature central nervous system (CNS), oligodendrocytes provide support and insulation to axons thanks to the production of a myelin sheath. During their maturation to myelinating cells, oligodendroglial precursors (OPCs) follow a very precise differentiation program, finely orchestrated by transcription factors, epigenetic factors and microRNAs (miRNAs), a class of small non-coding RNAs involved in post-transcriptional regulation. Any alterations in this program can contribute to dysregulated myelination, impaired remyelination and neurodegenerative conditions, as it happens in multiple sclerosis (MS). Here, we identify miR-125a-3p, as a new actor in oligodendroglial maturation. Indeed, over-expression of this miRNA by mimic treatment impaired while its inhibition with an antago-miR stimulated oligodendroglial maturation. Moreover, its expression was significantly up-regulated after lysolecithin and cuprizone-induced de-myelination in mice, and also in white matter lesions of MS patients. In silico analysis demonstrated that the negative regulation of miR-125a-3p on myelin genes is due to its simultaneous action on several targets including kinases, adhesion molecules, and cytoskeletal proteins. Globally our data suggest that miR-125a-3p could represent a new pathogenetic mechanism that negatively regulate re-myelination and that an antago-miRNA specific for miR-125a-3p may help in promoting oligodendrocyte maturation in diseases characterized by impaired myelin repair. Sponsored by Fondazione Cariplo, grant n° 2014-1207 to DL.

Keywords: Animal model, Biomarkers, Remyelination

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ND10 | Exosome-shuttled miRNAs derived from mesenchymal stem cells affect the phenotype of spinal cord astrocytes isolated from late disease phase SOD1G93A mice

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Amyotrophic lateral sclerosis affects motoneurons but involves different cell types, including astrocytes, which promote an inflammatory environment contributing to motoneuron death. Administration of mesenchymal stem cells (MSCs) in SOD-1G93A mice ameliorated disease course and inflammation in spinal cord by paracrine mechanisms. We have speculated that MSCs exert their action by transfering miRNA trough their released exosomes. We studied here the effect of MSC-derived exosomes and exosome-shuttled miRNAs on cultured astrocytes from adult SOD1G93A mice. GFAP and vimentin, markers of astrocytic reactive phenotype, increased in SOD1G93A astrocytes and this increase was reduced after exosome treatment. Also the pro-inflammatory cytokines IL1β, TNF-α, IL-6 augmented in SOD1G93A astrocytes and were reduced by exosomes. In contrast, the anti-inflammatory cytokine, IL-10, decreased in SOD1G93A astrocytes and this reduction was reverted by exosomes. NLRP3, involved in neuroinflammation-induced necroptosis, increased in SOD1G93A astrocytes and was reduced by exosomes. Furthermore, we transfected SOD1G93A astrocytes with miR-466q and miR-467f, which are up-regulated in MSCs and in their derived exosomes. Both miRNAs decreased IL1β and TNFα expression in SOD1G93A astrocytes. Our data suggest that exosome-shuttled miRNAs ameliorate the inflammatory state and promote the shift of astrocytes to a neuroprotective phenotype and are promising for translational pre-clinical trials in SOD1G93A mice.

Keywords: Spinal cord injury, Inflammation, Stem cells

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ND11 | TBC1D24 regulates axonal outgrowth and membrane trafficking at the growth cone in rodent and human neurons

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Mutations in TBC1D24 are described in patients with a spectrum of neurological diseases, from mild to severe and syndromic epilepsies. Most of the patients exhibit early onset neurological phenotypes, but no phenotype/genotype correlation is emerging. Aim of this project is to study the role of TBC1D24 during the first stages of neuronal development.We used rat primary cortical neurons, silenced for TBC1D24 expression to investigate the involvement of this protein during the first stages of neuronal differentiation. Our model revealed a significant defect in axonal specification and axonal initial segment formation, leading to an impairment of action potential firing. TBC1D24 exerts its role by modulating membrane trafficking at the axonal growth cone as assessed by imaging of endocytic recycling and finely tunes the activity of the small GTPase Arf6 as showed by using biochemical assay for Arf6 activation state and rescue of the phenotype obtained by dominant-negative Arf6. The axonal formation defect was recapitulated in induced pluripotent stem cells (iPSCs)-derived neurons from a TBC1D24 patient with severe encephalopathy and not in neurons derived from a patient affected by familiar infantile myoclonic epilepsy. In conclusion, this axonal specification defect is relevant for human neuron differentiation and correlates with disease severity.

Keywords: Molecular biology, Electrophysiology, Imaging, Stem cells

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ND12 | Investigating anticonvulsant drugs as potential treatments for Alzheimer's disease

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Release of amyloid-beta and tau, the two pathological proteins characteristic of Alzheimer's disease, is known to be correlated with neuronal firing. We investigated the effects of two anticonvulsant drugs, phenytoin and GS967, in two mouse models of Alzheimer's disease, the triple knock-in APP NL-G-F (Swedish/Arctic/Beyreuther-Iberian), and a transgenic tau model (P301L mutation). Using electrophysiological techniques, we analysed release probability and LTP in the hippocampus of NL-G-F mice, at the stage at which plaques first appear (3-3.5 months). Despite other models showing changes in synaptic transmission at this stage, NL-G-F mice showed no changes in synaptic transmission. This may be due to the Arctic mutation shifting the equilibrium towards deposition of amyloid-beta- indeed increasing evidence suggests the oligomeric form to be the toxic species at the synapse. Plaque and tangle abundance in the hippocampus of both models was determined immunohistochemically. Chronic treatment with phenytoin significantly reduced plaques in NL-G-F mice ($p<0.001$). There was also a trend for the treatments to reduce tangle load in the hippocampus of tau mice (2-way ANOVA, effect of treatment $p=0.08$). In conclusion, our study shows preliminary evidence that phenytoin may reduce plaque and tangle load in a model of Alzheimer's disease.

Keywords: Animal model, Electrophysiology, Degeneration

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ND13 | Direct Reprogramming of Human Fibroblasts to Explore Neurodegeneration in Amyotrophic Lateral Sclerosis

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An important limitation to study the pathology of the CNS is that living human neural cells are not easy to obtain. To overcome this problem, techniques based on iPSCs reprogramming have recently emerged. However, the acquisition of pluripotency implies a resetting of cellular age, which is particularly adverse to model late onset neurodegenerative diseases. A promising route to circumvent this limitation is the access to neural cells through direct reprogramming strategies that do not imply cell rejuvenation. In this study, we use direct reprogramming approaches to model human amyotrophic lateral sclerosis, a fatal neurodegenerative condition, typically characterized by selective degeneration of motor-neurons, but also affecting glial and muscular components. For neuronal reprogramming, we use a method based on the retroviral-mediated expression of Neurog2, Bcl-2 and Isl1. This approach yielded a pure population of motor-neurons (β-III-tubulin+, ChAT+, Hb9+ and Isl2+) from human fibroblasts. In addition, reprogramming of fibroblasts into myoblasts and astrocytes was accomplished by co-expression of Bcl-2 and MyoD or NFIA/B and SOX9, respectively. These approaches allowed us to establish multicellular cultures, where the three cell types coexist and establish functional connections. This model will be an ideal platform to study the molecular basis of this devastating disease.

Keywords: Degeneration

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ND14 | Role of proNGF and effects of electroacupuncture in the septo-hippocampal system of diabetic rats

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Diabetic encephalopathy (DE) is a mild cognitive impairment associated with neurodegeneration of the cholinergic septo-hippocampal system (SHS), impaired hippocampal (HP) neurogenesis and dysregulated proNGF/NGF activity. The sensory stimulation elicited by electroacupuncture (EA) affects HP physiology and SHS neurotransmission, conceivably modulating the proNGF expression, release and activity. Here we investigated the effects of experimental type-1 diabetes and of EA on the SHS, focusing on the role of proNGF in modulating the cholinergic system and HP physiology. Twice-a-week EA treatment counteracted the diabetes-induced loss of cholinergic markers in septal neurons and their fibers; impairment in NGF/proNGF ratio; unbalance in proNGF-A/proNGF-B gene expression and protein content; impairment of hippocampal cell proliferation and differentiation into neuronal precursors. To investigate the involvement of proNGF-A and proNGF-B in the pathogenesis of DE, we delivered the two proteins intranasally in control and diabetic rats. ProNGF-B administration negatively affected the cholinergic phenotype and the hippocampal neurogenesis, while proNGF-A promoted a recovery of the diabetes-impaired cholinergic neurotransmission and improved the hippocampal neurogenesis. Our data indicate that EA could restore the diabetes-induced dysfunctions in the SHS, probably acting on the balance between the proNGF variants, which could differentially affect the development and progression of DE.

Keywords: Animal model, Degeneration, Imaging

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ND15 | Roles of Elovl5 on neuronal function; new insights on spinocerebellar ataxia type 38

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Mutations in the very long chain fatty acid elongase 5 gene, ELOVL5 cause spinocerebellar ataxia type 38 (SCA38). ELOVL5 is highly expressed by cerebellar Purkinje cells frequently involved in ataxia. The ELOVL5 gene encodes for an enzyme involved in omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) biosynthesis. Downstream products of ELOVL5 include arachidonic acid and docosahexaenoic acid, from which endocannabinoids and other molecules with biological effects on the brain are produced. Deletion of Elovl5 in mice is associated with ataxia and cerebellar atrophy, in line with clinical findings in patients with SCA38. Patch-clamp recordings of Purkinje cells, revealed an impairment of an endocannabinoid-dependent form of short-term plasticity. The lipidomic profile of nervous tissue Elovl5 null mice showed a profound disruption in the phospholipids pattern, with a strong reduction of the long chain polyunsaturated fatty acids and an up regulation of the saturated forms. As a consequence of such alteration in the phospholipids profile, the conduction velocity of peripheral nerves was significantly reduced in Elovl5 null mice, similarly to findings in patients with SCA38 disease. In conclusion, these results indicate a crucial role of Elovl5 in maintaining specific neuronal functions, which might be in part responsible of motor symptoms.

Keywords: Animal model, Electrophysiology

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ND16 | Cerebrovascular contributions to seizure development and epilepsy

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The blood-brain barrier (BBB) positioned along blood vessels of the central nervous system is one of the most selective and tightly regulated barriers, reflecting the brain's critical roles in cognitive function, maintaining homeostasis and strictly coordinating the functions of peripheral organs. The BBB is important in regulating the exchange of ions and nutrients between blood and brain but also to protect delicate neural tissue from potentially damaging blood-borne agents such as pathogens, immune cells and anaphylatoxins. BBB dysfunction is a hallmark pathology of epilepsy with recent evidence pointing to a crucial role of the BBB-associated tight junction protein claudin-5 in maintaining normal neurological function. The goal of this research was to investigate changes in the expression of BBB enriched components by immunohistochemistry, Q-RT-PCR and western blot analysis in surgically resected epileptic brain tissue. In addition, the effect of drug/genetic-induced seizures on BBB function was investigated in vivo in rodents. Tight junction deficits and BBB dysfunction were evident in human epileptic brains with deficits in tight junctions and scaffolding proteins and increases in markers for neuroinflammation and gliosis. Knockdown of claudin-5 in mice was associated with tonic-clonic seizures and ictal activity by electroencephalography as well as astrocytic gliosis and microglial activation.

Keywords: Molecular biology, Animal model, Brain injury

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ND17 | P2X7 Receptor Activation Modulates Autophagy in SOD1-G93A Mouse Microglia

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Autophagy and inflammation play determinant roles in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS), an adult-onset neurodegenerative disease characterized by deterioration and final loss of upper and lower motor neurons priming microglia to sustain neuroinflammation and a vicious cycle of neurodegeneration. Given that extracellular ATP through P2X7 receptor constitutes a neuron-to-microglia alarm signal implicated in ALS, and that P2X7 affects autophagy in immune cells, we have investigated if autophagy can be directly triggered by P2X7 activation in primary microglia from superoxide dismutase 1 (SOD1)-G93A mice. We report that P2X7 enhances the expression of the autophagic marker LC3-II, via mTOR pathway and concomitantly with modulation of anti-inflammatory M2 microglia markers. We also demonstrate that the autophagic target SQSTM1/p62 is decreased in SOD1-G93A microglia after a short stimulation of P2X7, but increased after a sustained challenge. These effects are prevented by the P2X7 antagonist A-804598, and the autophagy/phosphoinositide-3-kinase inhibitor wortmannin. Finally, a chronic in vivo treatment with A-804598 in SOD1-G93A mice decreases the expression of SQSTM1/p62 in lumbar spinal cord at end stage of disease. These data identify the modulation of the autophagic flux as a novel mechanism by which P2X7 activates ALS-microglia, to be considered for further investigations in ALS.

Keywords: Animal model, Inflammation, Degeneration

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ND18 | Modulation of the intrinsic neuronal excitability by multifunctional liposomes tailored for treatment of Alzheimer's disease

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Nanotechnologies turned out to be promising in the development of diagnostic and therapeutic approaches toward neurodegenerative disorders. We utilized the patch-clamp technique on primary cultures of cortical neural cells isolated from neonatal rats, aiming to evaluate their electrical properties after the incubation with liposomes (mApoE-PA-LIPs), previously proved able to cross the blood brain barrier and to be effective on mouse models of Alzheimer's disease (AD), in absence and in presence of β-amyloid peptide oligomers. Data show a high degree of biocompatibility, evaluated by LDH release and MTT assay, and the lack of cellular internalization. After the incubation with mApoE-PA-LIPs neuronal membranes show an increase in the input resistance (from 724,14±76 MOhms in untreated population to 886,06±86 MOhms in the treated one) a reduction in the rheobase current (from 29.6±3 pA to 24.2±3 pA in untreated and treated, respectively), and an increase of the firing frequency, consistent with an ultimately increase in intrinsic excitability. Data obtained after co-incubation of mApoE-PA-LIPs with β-amyloid peptide oligomers suggested a retention of liposome efficacy. These data suggest the ability of liposomes to modulate neuronal electrical properties and are compatible with the previously demonstrated amelioration of cognitive functions induced by treatment of AD mice with liposomes.

Keywords: Nanomaterials/nanoparticles, Electrophysiology

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ND19 | Neurodegeneration and behavioural and cognitive alterations in mouse model of Autosomal Dominant Osteopetrosis type-2 (ADO2)

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ADO2 is a genetic bone disease caused by missense mutations of the ubiquitously-expressed chloride channel type-7 (CLCN7) gene. We previously demonstrated that the ADO2-causing heterozygous Clcn7-G213R mutation affects not only the bone but also different organs in a mouse model of ADO2. Widespread expression of CLCN7 also in the brain led us to hypothesize that behavioral and cognitive abilities may be affected in the ADO2 mouse. Indeed, anxiety was increased in 3- and 12-month-old ADO2-mice vs WT littermates in the open field test (time spent in the centre -48%;p=0.0004), elevated plus maze test (latency and time in open arms +3.6fold;p=0.02, -42%;p=0.01), light-dark box test (-46%;p=0.02) and forced swimming test (immobilisation +1.4;p=0.001). Thioflavin-T staining exhibited prevalent beta-amyloid accumulation in many brain regions (amygdala:+2.28fold;p=0.006, hippocampus:+3.6fold;p=0.01, thalamus:+4.6fold;p=0.02) of ADO2 mice vs WT. Prominent penetration of GFAP+ glial cells in the ADO2 hippocampus was seen alongside increased GFAP+ cell number (+2.1fold;p=0.01) vs WT. Furthermore, in the cerebellum, GFAP+ cells were redistributed from the white matter to the cell layer (+1.5fold;p=0.04). Additionally, supporting our analysis of other ClC7-expressing organs, we found increased gamma-adaptin in the midbrain (+2.4fold;p=0.005) and LC3b in the cerebellum. Together, our results indicate that ADO2 induces brain alterations causing neurological deficits.

Keywords: Animal model, Degeneration, Protein aggregation

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ND20 | Tight Junctions of the Blood-brain Barrier as a Therapeutic Target in Epilepsy

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Epilepsy is a group of disorders of the brain characterised by recurrent seizures, the cause of which is often unknown, but recent interest has focused on the role of blood-brain barrier (BBB) dysfunction in seizures. The BBB is formed by endothelial cells of the central nervous system (CNS) which are held together by tight junctions (TJ) composed of a number of protein components including claudins and occludin. BBB leakage has been demonstrated as an early characteristic following seizures and has also been suggested to have a direct role in seizure induction with suppression of claudin-5 inducing seizures in adult mice. The modulation of these TJ proteins represents a possible therapeutic target for epilepsy. Here, we have characterised TJ protein expression and show evidence of BBB leakage in surgically resected human epileptic brain tissue. Additionally, we have shown the effect of anti-epileptic drugs (AED) on TJ protein expression in vitro. Immunohistochemical analysis of surgically resected human epileptic brain sections revealed breakdown of TJ proteins and extravasation of IgG and Fibrinogen. Additionally, there was widespread accumulation of phosphorylated tau protein and astrocytic gliosis supporting the loss of BBB integrity in epileptic brain tissue. In vitro analysis investigating the effect of common AEDs on TJ protein expression levels suggests an upregulation of claudin-5. These data support the potential of therapeutic modulation of TJ proteins for the treatment of epilepsy.

Keywords: Molecular biology, Biomarkers, Imaging

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ND21 | White matter Structural asymmetries and language impairment: MR-DTI and fMRI study

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PURPOSE. To investigate the potential association between structural organization of language-related white matter pathways with both language disability and atypical language lateralization in non-lesional epilepsy.
METHODS. Thirty right-handed epileptic patients (mean age 15±8 years, range 3-40) underwent a 3T MRI study including DTI and task-based fMRI sequences. Language lateralization index (LI) was determined by fMRI using a verb generation paradigm. Constrained spherical deconvolution analysis and probabilistic tractography were used to reconstruct the language-related bundles. Fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AD) were considered as indicators of microstructural organization. Track volume, track count, voxel count, and fiber length were also evaluated. Language disability was investigated by Griffith Mental Development Scale (GMDS), the WISC-III/IV scale, or the WAIS scale depending on patient age.
RESULTS. Generalized linear model revealed that patients have reduced volumetric parameters in many of the white-matter bundles in the left hemisphere compared to controls ($p<.05$). Patients with language disability have white-matter alterations of language fasciculi compared to those whose language performance is normal ($p<.05$). fMRI analysis confirmed that epileptic patients have an atypical pattern of language dominance (mean LI= 0.193) and revealed its association with both language impairment ($p=0,011$) and microstructural abnormalities ($p<.05$).

Keywords: Neuroimaging, Cognitive

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ND22 | Failure of nuclear mRNA export in a cellular model of C9orf72-linked ALS

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Introduction: A GGGGCC (G4C2) repeat expansion in C9orf72 gene is the most frequent cause of Amyotrophic Lateral Sclerosis (ALS). Although several mechanisms have been involved in C9orf72 toxicity, their pathogenic relevance is still unclear. We have previously shown that expanded repeat causes translational repression and nuclear retention of poly-adenylated mRNA, suggesting that defects in nuclear mRNA export might underlie these effects. The general aim of this study was to verify this hypothesis.
Methods: Cultured cells expressing G4C2 repeats were used as cellular models. Fluorescence in situ hybridization (FISH) and immunofluorescence analysis were used to assess RNA foci formation, mRNA distribution and protein localization. Nuclear and cytoplasmic RNA were isolated from transfected cells and analysed through RNA-sequencing.
Results: G4C2 RNA binds and sequesters two essential factors of nuclear mRNA export machinery, NXF1 and ALY/REF. Moreover, functional modulation of NXF1 affects key phenotypes characterizing G4C2 cells. Finally, RNA-seq analysis shows that G4C2 affects the nuclear/cytosolic distribution of mRNAs with a role in membrane trafficking and RNA metabolism.
Conclusions: G4C2 repeat expansion affects NXF1-mediated mRNA export pathway, leading to the nuclear retention of specific mRNAs, and this might in turn contribute to the pathogenesis of C9orf72-ALS.

Keywords: Molecular biology

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ND23 | The Mitopark mouse suggests a causal role for HCN loss of function in the progression of Parkinson's disease

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AIMS. Differential vulnerability between Substantia Nigra pars compacta (SNpc) and Ventral Tegmental Area (VTA) dopaminergic (DAergic) neurons is a hallmark of Parkinson's disease (PD).We previously demonstrate that Hyperpolarization-activated current (Ih) is suppressed by the by 1-methyl-4-phenylpyridinium (MPP+). Furthermore, reduced Ih function was previously observed in Mitopark mice, a genetic mitochondrial model. So, we tested the hypothesis that Ih loss of function is causally linked to differential dopaminergic degeneration by (1) selective blockade of Ih in TH-GFP mice and (2) a pharmacological rescue of Ih function with the Ih enhancer lamotrigine (LTG) in presymptomatic Mitopark mice.
METHODS. Inactivation of Ih in vivo was obtained by stereotaxic injection of ZD7288 or ivabradine in TH-GFP mice. Mitopark at 6 weeks old mice received an intra-peritoneal dose of LTG 15 mg/kg daily or saline. Locomotor activity such as Open Field (OFT), rotarod test and apomorphine-induced rotations.
RESULTS. Our results demonstrate that pharmacological Ih suppression in vivo causes selective SNpc-specific DAergic loss leading to parkinsonian motor phenotype. After LTG treatment, mitopark mice show a better locomotion activity in OFT and a decrease in number of falls compared to vehicle in rotarod test.
CONCLUSION. Pharmacological manipulation of Ih function in vivo suggest a possible link with DAergic degeneration in PD. Further study will be required to determine whether this mechanism may be of general relevance in the progression of disease in other PD models.

Keywords: Animal model, Electrophysiology,Degeneration

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ND24 | Protein Arginine Methyltransferase 6 is a novel modifier of Huntington's disease pathogenesis

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Huntington's disease (HD) is a fatal neurodegenerative disorder characterized by progressive atrophy of the striatum and cerebral cortex. HD is caused by the expansion of a polyglutamine tract in huntingtin (HTT). Several post-translational modifications (PTMs) of HTT have been identified which modify the toxicity of mutant HTT. Arginine methylation is one of the most abundant PTMs and is catalyzed by protein arginine methyltransferases (PRMTs). Arginine methylation has been recently implicated in the pathogenesis of polyglutamine diseases, yet its role in HD remains to be fully elucidated. By mass spectrometry, we discovered that HTT is methylated at specific arginine residues. Next, we found that HTT interacts with two members of the PRMTs family and gets methylated by one specific enzyme (PRMT6). Importantly, the interaction with PRMTs is reduced when the polyglutamine stretch of HTT is expanded. PRMT6 downregulation in striatal cells expressing mutant HTT exacerbates toxicity and the expression of a methylation-defective mutant of HTT decreases the survival of mouse primary cortical neurons, suggesting that PRMT6-mediated arginine methylation is protective in HD. Accordingly, overexpression of PRMT6 attenuates striatal cell death and suppresses mutant HTT-mediated lethality in HD flies. Altogether, these results suggest that PRMT6 is a novel modifier of HD pathogenesis.

Keywords: Molecular biology, Degeneration

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ND25 | A neuronal triggers specific RNAs, local translation of Annexin A2 and cytoskeletal remodeling in Schwann cells

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Schwann cells are key players in neuro-regeneration: they sense alarm signals released by degenerating nerve terminals and differentiate toward a pro-regenerative phenotype, with phagocytosis of nerve debris and nerve guidance. At the murine neuromuscular junction, hydrogen peroxide (H2O2) is a key signal of Schwann cells activation in response to a variety of nerve injuries. Here we report that Schwann cells exposed to low doses of H2O2 rewire the expression of several RNAs at both transcriptional and translational levels. Among the genes positively regulated at both levels, we identified an enriched cluster involved in cytoskeleton remodeling and cell migration, with the Annexin (Anxa) proteins being the most represented family. We show that both Annexin A2 (Anxa2) transcript and protein accumulate at the tips of long pseudopods that Schwann cells extend upon H2O2 exposure. Interestingly, Schwann cells reply to this signal and to nerve injury by locally translating Anxa2 in pseudopods, and undergo an extensive cytoskeleton remodeling. Our results show that, similarly to neurons, Schwann cells take advantage of local protein synthesis to change shape and move towards damaged axonal terminals to facilitate axonal regeneration.

Keywords: Degeneration, Regeneration, Imaging, Neuron-glia communication

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ND26 | Glucocorticoid receptor modulation alters spine plasticity, inflammation and behavior performance in 3xTg-AD mice

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High levels of glucocorticoids, through activation of glucocorticoid receptors (GR), induce in brain alterations comparable to those occurring in Alzheimer's disease (AD). The aim of this project is to assess the effects of GR modulation in the pathogenesis of AD in 3xTg-AD mouse model. Following GR agonist (dexamethasone) or antagonist (mifepristone) administration, 3xTg male mice were analyzed for combined Golgi Cox staining and immunofluorescence to assess dendritic spines density and microglia activation. Glucocorticoid direct effects were studied on mouse microglial primary culture, while in vivo spine turnover was investigated through two-photon laser microscopy in AAV-GFP infected neurons. Changes in cognitive performance were monitored via behavioral tests. The results showed that dexamethasone reduced spine density and induced microglia proliferation in hippocampal CA1 region, whereas mifepristone significantly increased the spine density. M1-marker CD68 expression was reduced after glucocorticoids treatment, indicating a direct effect on microglia cultures. In conclusion, GR hyper-activation induced degeneration of dendritic spines in the hippocampus CA1 region, through neuron intracellular mechanisms and microglia activation, suggesting that glucocorticoids may act in a synergic manner with the mechanisms underlining inflammation and neurodegeneration present in AD. Therefore, the blockade of GR might represent a therapeutic target to slow AD progression.

Keywords: Plasticity, Two photons, Neuron-glia communication

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NI1 | Monitoring Neuroinflammation with the TSPO tracer [18F] VC701, after LPS systemic administration in male/female adult and aged mice

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Neuroinflammation is widely studied in many neurodegenerative diseases, including Alzheimer's and Parkinson's disease, in which age play a crucial role. Aim of the study is monitoring of brain inflammation and its modulation by gender and age after systemic administration of Lipopolysaccharide by ex vivo biodistribution with specific Translocator protein (TSPO) radiotracer 18F-VC701 and by inflammatory cytokines expression. 18F-VC701 biodistribution was measured at 6 hours after systemic injection of in adult and aged C57BL/6 male and female mice. Mice were sacrificed and specific brain regions collected for gamma counting and RT-PCR analysis of different markers of inflammation. LPS induced a significant increase of tracer uptake in cortex and cerebellum of aged male mice. Measurements of cytokines transcripts showed higher IL-1beta and TNF-alfa mRNAs expression in both male and female aged mice treated with LPS. Aged male mice showed a greater IL-1beta mRNAs expression compared to aged female mice. LPS induce decrease of TREM-2 expression level in almost all LPS treated brain areas. The results of this study confirmed an exaggerated response of the aged brain and different response in males and females mice to a peripheral inflammatory challenge that could be detected in vivo by PET imaging with TSPO ligands.

Keywords: Animal model, Neuroimaging, Brain injury, Ageing, Inflammation, Biomarkers

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NI2 | Pathogenic role of inflammation in Retinitis Pigmentosa: a target for preventing daylight vision loss

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In Retinitis Pigmentosa (RP), a mutation causes the primary degeneration of rods, followed by secondary cone death, culminating into blindness. We showed that in rd10 mice, a RP mouse model, the peak of cone death (P45) is associated with a strong retinal inflammatory response prevailing over any other biological process. We hypothesise that the breakdown of the eye "immune privilege" leads to the accumulation of inflammatory mediators detrimental to cones and contributing to their degeneration. To test this hypothesis, we treated rd10 mice with a well-known anti-inflammatory drug, Dexamethasone (4mg/kg), from P23 to P60, encompassing the period of maximum rod and cone death. At P45, Dexamethasone administration reduced retinal microglia/macrophages activation concomitantly increasing cone survival and improving visual acuity. At P60 these effects were still present, as a higher number of cones and lower level of inflammatory species were found in the retina of treated mice compared to controls. The role of inflammatory response in the worsening of retinal phenotype in RP has been investigated only recently. Our study reveals a link between retinal inflammation and cone degeneration, showing cones rescue with a commonly employed drug. This opens the possibilities to extend steroid treatment to slow down human RP. – Funding sources: Macula Vision Research Foundation, USA; Fondazione Roma, Italy; European Project H2020-MSCA-ITN-2014

Keywords: Inflammation, Degeneration, Immune system

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NI3 | Role of IL-1 signaling in controlling synaptic function

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Interleukin-1 has been described as one of the main pro-inflammatory cytokines found to be elevated in several neurological disorders and associated with cognitive deficits. Despite the established role of the cytokine in different brain diseases, its possible physiological function during neurodevelopment is not completely known. Trying to clarify if, and how, Interleukin-1 signalling participates in the maintaining of synapse homeostasis by means of IL-1R KO mouse model, we found that the genetic lack of IL-1R affects excitatory inputs, leading to a significant increase in the expression of excitatory synaptic markers in both cortex and hippocampus. Consistently, electrophysiological recordings from hippocampal acute slices demonstrated enhanced frequency and amplitude of miniature excitatory postsynaptic currents in IL-1R-/- mice. We also observed a transient increase in microglia number in IL-1R-/- mice during the first weeks of postnatal life. Our results seem to suggest a key role of IL-1R signaling in modulating glutamatergic synapses and its absence leads to a general potentiation of excitatory inputs. Since microglia contribute to postnatal synaptic pruning and network refinement, the higher number of microglia in IL-1R-/- mice may be involved in this process, eventually leading to short-term effects on postnatal synaptic maturation and long-term effects on adult brain networks.

Keywords: Electrophysiology, Inflammation, Immune system, Imaging, Neuron-glia communication

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NI4 | Activity assays for evaluation of clinical grade MSC-EV anti-inflammatory properties for use in treatment of drug-resistant epilepsy in children

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Introduction: MSCs can release extracellular vesicles (EVs). Several studies have shown immunomodulatory effects of MSCs. However, to date knowledge about mechanisms underpinning this immunomodulation is limited. Here we investigate the immunoregulatory properties of clinical grade MSC-EVs (CG-EVs) on lymphocytes and natural killer cells (NK) compared to research grade counterparts (RG-EVs). – Methods: Human umbilical cord MSC-derived RG- or CG-EVs were co-cultured alongside human PBMC from healthy donors in presence of CD3/CD28 beads (T cells stimulation) or CpG (B cells activation). Meanwhile, MSCs or EVs were cultured with PBMC in presence of IL-2 or IL-15 to assess NK degranulation and IFN-g production or proliferation, respectively. – Results: Following incubation with RG- or CG-EVs, T potency assays revealed similar Treg increase, counteracting Teff expansion, while B potency assays showed reduction of B cells proliferation and plasma cell differentiation. Exposure of RG- or CG-EVs to K562-stimulated PBMC induced a significant decrease of producing IFN-g and degranulating NK compared to parental MSCs. Similarly, percentage of proliferating NK was reduced following both MSCs and EVs co-culture. – Conclusion: Our data suggest the utility of CG-EVs for treatment of several immunological diseases. We aim to use MSC-derived EVs for clinical tests in treatment of epilepsy resistant to antiepileptic drugs.
Funding: The Cell Factory BVBA.

Keywords: Molecular biology, Immune system, Stem cells

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NI5 | Biomarkers to monitor myelin loss and remodeling

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The identification of de-remyelination biomarkers specific and sensitive to monitor disease progression and myelin remodeling, to test putative pro-remyelinating compounds and thereby select specific treatments is a prime goal within the Multiple Sclerosis (MS) community. Sphingolipids are major components of the myelin membrane and they are essential and rate limiting for its correct development, arrangement and function. Even more, sphingomyelin pathway is triggered by different stimulants and participates in pathogenic processes of MS. Therefore, we performed sphingolipid-targeted analysis by LC-MS/MS on tissue, cerebrospinal fluid (CSF) and serum of different CNS demyelinating experimental models to verify whether the levels of these lipids correlate with the amount of myelin damage and disease status. In particular, the cuprizone-fed model in which de- and remyelination can be exactly and reliably monitored was used to verify the specificity and sensitivity of sphingolipids as putative myelin biomarkers, while experimental autoimmune encephalomyelitis (EAE) mice were used to correlate biochemical data with disease scoring. As we found altered expression pattern of sphingolipids either in tissue homogenates and biological fluids of both the animal models, we are confident to finalize the identification of biomarker/s to monitor demyelination and remyelination that can be readily translated to human patients.

Keywords: Animal model, Biomarkers, Remyelination

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NI6 | LPS-induced inflammation affects biosynthesis of neurosteroids in the chicken pineal gland

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Bidirectional communication between the pineal gland and immune system becomes a better-understood process. Peripheral inflammation affects biosynthesis of melatonin, a major pineal hormone, whereas melatonin itself modulates the development of inflammation. The pineal gland is also major organ responsible for neurosteroidogenesis. At least 11 different neurosteroids have been demonstrated to be synthetized de novo in the avian pineal gland. Our aim was to examine whether the transcription of genes encoding enzymes of the neurosteroid biosynthesis pathway may be influenced by peripheral inflammation. 16-day-old male chicken kept under L:D 12:12 conditions were LPS-injected intramuscularly two hours before the end of the light phase. Four hours later the pineal glands were isolated and the mRNA levels of Cyp11a1, Cyp7b1, Hsd3b2, Akr1d, Srd5a1 and Srd5a3 genes were measured. We found that inflammation increased transcription of Cyp11a1 and Cyp7b1 genes, whereas the transcription of Hsd3b2, Akr1d, Srd5a1 and Srd5a3 decreased. Moreover, in vitro investigations conducted on pinealocytes transfected with reporter vector indicated that LPS added to culture medium activate NF-kappaB signaling pathway. To conclude LPS-induced inflammation seams to influence neurosteroidogenesis in the pineal gland via activation of NF-kappaB signaling pathway. – Supported by National Science Centre grant UMO-2016/21/B/NZ3/00364

Keywords: Molecular biology, Inflammation, Immune system

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NI7 | Hydroxychloroquine treatment on lymphoblasts derived from patients with Aicardi-Goutières syndrome

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Aicardi-Goutières syndrome (AGS) is a rare genetic disorder with recessive and dominant inheritance. Mutations in the 7 AGS genes determine accumulation of endogenous DNA or RNA:DNA hybrids, which are recognized as foreign nucleic acids. RNA:DNA hybrids trigger the cGAS-STING pathway which induces an interferon-alpha mediated immune response. Hydroxychloroquine inhibits the cGAS-STING pathway and facilitates autophagosome formation, though it accumulates within lysosomes blocking autophagy. In this study we assessed the effectiveness of hydroxychloroquine in modulating interferon-alpha response and evaluated a possible involvement of autophagy in RNA:DNA hybrids discard. After a 24 hours treatment with 25uM hydroxychloroquine we saw a significant decrease of expression of interferon stimulated genes (IFIT1, IFI44). Regarding autophagy, there were no significant differences in the expression of autophagic markers between healthy controls and AGS derived lymphoblasts. However, after hydroxychloroquine treatments we identified increasing levels of LC3 and p62. Colocalization of RNA:DNA hybrids and p62/LC3 and decrease of RNA:DNA hybrids in AGS derived lymphoblasts suggested removal of toxic nucleic acids by hydroxychloroquine. Hydroxychloroquine seems able to stop interferon-alpha activation and release, determining improvements in patients' condition. According to our data, this treatment could represent an effective method to decrease RNA:DNA hybrids, main cause of autoimmune symptoms in AGS patients.

Keywords: Inflammation, Immune system

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NI8 | Cell activation, death and motility of human Th17 cells are finely regulated by specific transcription factors

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T helper (Th) 17 cells are implicated in neuroinflammatory diseases, where the pathogenicity is associated to their intrinsic features, like resistance to apoptosis, and high expression of inflammatory cytokines, co-stimulatory and adhesion molecules. However, the mechanisms leading to these pathogenic functions are largely unknown. Given the central role of the differentiation process in the acquisition of typical Th17 cell features, we performed high-throughput transcriptome profiling of Th17 cells differentiated from naïve CD4 T cells. In this context, we compared the transcriptional signature of Th17 cells generated in presence of all Th17-promoting cytokines (IL-1beta, IL-6, IL-23 and TGF-beta) and Th cells generated in Th17 suboptimal conditions (IL-6, IL-23 and TGF-beta). We identified 644 Th17-specific genes, differentially expressed between Th17 and Th0 cells, and in suboptimal versus optimal Th17 condition. Then, we used a predictive bioinformatics approach to highlight transcription factors (TFs) that potentially bind their promoter regions. We identified new TFs regulating Th17 cells, such as SOX2, and their potential target genes, which segregate into classes implicated in Th17 features of relevance for neuroinflammation: regulation of cell death, cell migration and activation of immune response. These results revealed promising new targets for the control of the pathogenic features of Th17 cells.

Keywords: Molecular biology, Inflammation, Immune system

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NI9 | Anti-inflammatory effect of IL-9 in multiple sclerosis as achieved by modulation of macrophage activation

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Multiple sclerosis (MS) is a neuroinflammatory disease of the central nervous system (CNS). Given the immunoregulatory role exerted by IL-9 in MS, we studied the mechanisms of IL-9 effect on CNS resident and infiltrating immune cells. Firstly, we found that human macrophages express IL-9 receptor and potentially respond to IL-9. This finding was supported by immunohistochemistry of post-mortem brain sections of MS patients. We thus focused on the involvement of macrophages in IL-9 mediated protection in MS. We found that IL-9 induces activation of STAT-1, 3 and 5, it reduces the expression of activation markers, and it increases the secretion of the anti-inflammatory cytokine TGF-beta, in human macrophages differentiated in vitro from blood monocytes. Furthermore, IL-9 mRNA expression significantly correlates with TGF-beta1 inducer 1, involved in TGF-beta signaling pathways, in post-mortem brain sections of MS patients whose brains lack inflamed follicles and express highest levels of IL-9. These results reveal a new role of IL-9 in modulating the activation phenotype of human macrophages toward an anti-inflammatory phenotype and suggest that this mechanism can contribute to the beneficial effects of IL-9 observed in MS. Further studies are needed to fully describe the mechanisms underlying IL-9 mediated neuroprotective effect in MS.

Keywords: Inflammation, Immune system

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NI10 | Amyloid plaque pathology triggers expression of mechanosensing Piezo1 channels in astrocytes

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A hallmark of Alzheimer's disease is the formation of amyloid plaques. These aggregates of Aβ1-42 are hard structures that cause neurodegeneration and change the physical properties of the extracellular matrix, turning it stiffer. Astrocytes surrounding amyloid plaques are highly mechanosensitive cells that likely sense this change via mechanosensing ion channels. Here, we used a transgenic rat model of Alzheimer's disease (TgF344-AD) that overexpress human genes APPSwe and PSEN1ΔE9 to study the expression of the mechanosensing ion channel Piezo1 in reactive astrocytes around the plaques. We found that Piezo1 is overexpressed on the astrocytes surrounding plaques. Moreover, TgF344-AD rats that received repeated UTI of E.Coli displayed further elevations of Piezo1 expression. We then investigated the expression and potential role of Piezo1 in primary mouse astrocytes. We found Piezo1 was overexpressed in LPS-stimulated astrocytes and treatment with its agonist, Yoda-1, led to decreased release of proinflammatory cytokines TNF-α, IL-1β and IL-6 and decreased migration. In contrast, blocking Piezo1 with GsMTx4 led to higher levels of IL-1β and IL-6 and enhanced migration. Taken together, upregulation of Piezo1 in reactive astrocytes may be an innate neuroprotective mechanism to control gliosis and neuroinflammation, suggesting Piezo1 may be an interesting target for the regulation of neuroinflammation

Keywords: Animal model, Ageing, Inflammation

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NI11 | Moving from systemic to central nervous system inflammation: the role of A20 in the neuropathology of Multiple Sclerosis

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Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). The anti-inflammatory enzyme A20 is considered a central gatekeeper in systemic inflammation and immunity through NF-kB inhibition. High-powered studies identified A20 as a new risk gene for MS and demonstrated that A20 expression levels were reduced in MS patients immune cells compared to healthy controls (HC) and negatively correlated with a worst clinical course. Based on recent evidences on mice which suggest a role for A20 in the CNS, we aimed to unveil the contribution of A20 to the CNS MS pathology, studying the A20 protein and gene expression in human post-mortem MS brain tissues. We demonstrated that A20 is present in human HC brain tissues in both white matter (WM), mainly in astrocytes and in grey matter (GM) in some neurons. In MS brain, A20 protein and transcript were massively expressed in all the active demyelinated lesions in WM and in GM by both infiltrating macrophages and by resident CNS cells including some microglial cells and all the reactive astrocytes. The huge A20 activation in the active plaques could represent a defensive mechanism contributing to the inflammation resolution and the regeneration processes.

Keywords: Brain injury, Inflammation, Degeneration, Immune system

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NI12 | In vitro and in vivo characterization of REST activity and expression under neuroinflammatory conditions

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The transcriptional repressor RE1-Silencing Transcription Factor (REST) regulates neurogenesis and neuronal identity through cell-specific gene repression, allowing expression of its targets in mature neurons where REST is quiescent. REST dysregulation has been implicated in several neurodegenerative disorders, including Alzheimer and Huntington diseases, tumors of the nervous system, and epilepsy. We found that REST is overexpressed in the spinal cord of mice with experimental autoimmune encephalomyelitis (EAE), suggesting that its dysregulation might be an important factor in the pathogenesis of the disease. Moreover, specific targeting of REST-dependent transcription and/or molecular pathways could be an alternative therapeutic strategy towards therapy for Multiple Sclerosis (MS). Starting from these observations, we have firstly analyzed the expression of REST target genes in EAE and characterized the cell-specificity of REST overexpression, investigating the differential contribution of neuronal and glial cell populations to REST upregulation. Moreover, we have analyzed REST activity in primary neuron cultures treated with different proinflammatory cytokines, in order to study the cellular and molecular pathways involved under inflammatory conditions mimicking the EAE environment. These studies will establish whether REST upregulation represents a new pathogenetic mechanism in EAE, and will identify REST-dependent pathways that can be potential therapeutic targets for EAE and potentially MS.

Keywords: Inflammation, Degeneration, Neuron-glia communication

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NI13 | Novel approaches to target neuroinflammation: the role of microglia during epileptogenesis

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There is increasing evidence that reactive astrocytes contribute to the onset and progression of epilepsy but the role of microglia is not yet well understood. Our hypothesis is that targeting microglia will effectively block epileptogenesis, or result in drastic seizure reduction, since microglia activation during epileptogenesis results in the expression of inflammatory molecules with ictogenic properties. We aimed to specifically block the Colony Stimulator Factor 1(CSF1) receptor which regulates proliferation, differentiation and survival of microglia, using PLX3397 which completely depletes microglia after 3 weeks of treatment with drug-in-food pellet. Microglia was depleted at the time of epileptogenic injury (status epilepticus, SE) or during the chronic disease phase in a murine model of epileptogenesis evoked by intra-amygdala kainic acid injection. No significant differences in SE severity, spontaneous seizure onset or disease development occurred in both depleting conditions. These results show that ablation of microglia in the preventive or chronic disease phases does not affect the disease itself. We are now depleting microglia right after the onset of the disease to assess the consequences on disease progression. Moreover, we will study drugs interfering with specific microglia functions to determine if a specific functional state is implicated in epileptogenesis.

Keywords: Animal model, Inflammation

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NI14 | Microglia-derived extracellular vesicles regulate the recruitment, proliferation and differentiation of oligodendrocyte precursor cells

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Microglia have an enormous plasticity in response to CNS injury and acquire different activated phenotypes, participating not only in mechanisms of injury but also in tissue repair. However, the mode(s) of action of microglia in exerting their functions is unclear. Here, we explored the action of Extracellular Vesicles (EVs) released from differently activated microglia on Oligodendrocyte Precursor Cells (OPCs), the glial cells more strongly damaged in Multiple Sclerosis. We first injected EVs produced in vitro by pro-inflammatory microglia (inflammatory EVs) or pro-regenerative microglia (pro-regenerative EVs) in the lysolecithin mouse model of focal demyelination. Immunofluorescence analysis revealed that inflammatory EVs tend to limit OPC proliferation and block re-myelination at lesion site, whereas pro-regenerative EVs promote OPC proliferation and myelin repair. We next investigated the effects of microglia derived-EVs on OPC proliferation, differentiation and migration in vitro. Immunofluorescence analysis revealed that inflammatory EVs limit OPC proliferation, while pro-regenerative EVs tend to increase it. However, pro-regenerative EVs and, to a lesser extent, inflammatory EVs enhance the differentiation of cultured OPCs into mature oligodendrocytes and myelin deposition. In addition, all type of microglia-derived EVs are able to act as chemoattractants for OPCs. Collectively, these results indicate a relevant role for EVs in microglia-oligodendrocyte crosstalk.

Keywords: Brain injury, Inflammation, Remyelination

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NP1 | Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion

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In the adult brain astrocytes in the sub-ventricular zone and in the hippocampal dentate gyrus produce neurons throughout life. We recently demonstrated that after Quinolinic Acid lesion in mouse, parenchymal astrocytes in the striatum also undergo a neurogenic activation and generate neuroblasts locally. Yet the mechanisms that drive this response are unclear. Here we show through genetic lineage tracing that, as in canonical adult niches, 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 to the neurogenic state depends on the expression of 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 activate the execution of the neurogenic program. Mechanisms implicated in Sox2-dependent priming or in activating the execution of the neurogenic competence are currently under investigation. 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.

Keywords: Stem cells

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NP2 | Proline-rich Transmembrane protein 2 (PRRT2) controls neuronal excitability by negatively modulating Na+ channel activity

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Mutations in PRRT2, a neuron-specific protein involved in neurotrasmitter release, have been identified in a group of paroxysmal syndromes of infancy, including epilepsy, paroxysmal dyskinesia and migraine. To model the disease and dissect out the physiological role of PRRT2 we studied the phenotype of human (iPSC-derived neurons) and mouse neurons lacking PRRT2. At four weeks of differentiation iPSC-derived neurons from PRRT2 homozygous patients showed neuronal-like morphologies and expression of neuronal differentiation markers. Electrophysiological analysis showed increased Na+ currents and hyperexcitability that were fully rescued by expression of wild-type PRRT2. Similar electrophysiological features were observed in primary mouse KO neurons. By expressing PRRT2 in HEK-293 cells stably expressing NaV channel subtypes, we found a specific interaction between PRRT2 and NaV1.2/NaV1.6 but not NaV1.1 channels. This interaction causes a reduction in the membrane exposure of NaV1.2/NaV1.6 subtypes leading to a decrease of Na+ currents. The study demonstrates that the lack of PRRT2 leads to a hyperactivity of voltage-dependent Na+ channels in homozygous PRRT2 KO human and mouse neurons and that, in addition to the reported synaptic functions, PRRT2 is an important negative modulator of neuronal excitability, providing a new basis for the pathogenesis of the PRRT2-linked paroxysmal disorders.

Keywords: Molecular biology, Electrophysiology, Plasticity, Stem cells

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NP3 | Hyperactivity of Rac1-GTPase pathway impairs neuritogenesis of cortical neurons by altering actin dynamics

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The small-GTPase Rac1 is a key molecular regulator linking extracellular signals to actin cytoskeleton dynamics. Loss-of-function mutations in RAC1 and other genes of the Rac signaling pathway have been implicated in the pathogenesis of Intellectual Disability (ID). The Rac1 activity is negatively controlled by GAP proteins, however the effect of Rac1 hyperactivity on neuronal networking in vivo has been poorly studied. ArhGAP15 is a Rac-specific negative regulator, expressed in the main subtypes of pyramidal cortical neurons. In the absence of ArhGAP15, cortical pyramidal neurons show defective neuritogenesis, delayed axonal elongation, reduced dendritic branching, both in vitro and in vivo. These phenotypes are associated with altered actin dynamics at the growth cone due to increased activity of the PAK-LIMK pathway and hyperphosphorylation of ADF/cofilin. These results can be explained by shootin1 hypo-phosphorylation and uncoupling with the adhesion system. Functionally, ArhGAP15-/- mice exhibit decreased synaptic density, altered electroencephalographic rhythms and cognitive deficits. These data suggest that both hypo- and hyperactivation of the Rac pathway due to mutations in Rac1 regulators can result in conditions of ID, and that a tight regulation of Rac1 activity is required to attain the full complexity of the cortical networks.

Keywords: Molecular biology, Animal model, Plasticity

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NP4 | Neurodevelopment disorders linked to ARX mutations in different genetic models: how to compensate the damage

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The X-linked ARX gene encodes the Aristaless-related homeobox protein, ARX, a transcription factor (TF) with a role in GABAergic interneuron migration and maturation. ARX mutations cause a spectrum of X-linked neurodevelopmental disorders including lissencephaly (XLAG), a severe cortical malformation, and a catastrophic epileptic encephalopathy. Here we describe the conservation of an ARX-dependent disease pathway among human, mouse and worm establishing a gene-phenotype association from one organism to another. Starting from the homologous gene relationships between ARX and its murine (Arx) and worm (alr-1) counterparts, we established a neuronal phenolog relationship among the human phenotypes associated to ARX mutations, Arx disease models in mouse, and alr-1 mutants in C. elegans. We proved that the homologous counterparts of KDM5C in mouse (Kdm5C) and worm (rbr-2) are under the control of ARX/Alr-1 transcriptional activity and we established a robust downregulation of KDM5C/rbr-2 transcript in human, murine and worm mutants defective for ARX/alr-1. We also present results on molecular and phenotypic rescue obtained by drug repositioning in each model tested upon in vivo epi-treatment. Finally, our data allow us to define the ARX/alr-1 phenolog-disease pathway and constitute a valuable basis for applying mechanism-based therapies to treat neurodevelopmental diseases caused by defects in transcriptional regulators.

Keywords: Molecular biology, Animal model

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NP5 | Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders

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The gut microbiome may influence brain development and behavior, mainly through the modulation of physiological metabolism and the immune system. Recent studies have determined that the microbiome has direct effects on behavior and may be dysregulated in neurodevelopmental conditions. Considering that these disorders, such as autism, have a strong genetic etiology, it is necessary to understand if genes associated can influence the gut microbiome, and if probiotics can be a therapeutic tool. Using 16S high-throughput sequencing, we have determined the gut microbiome community of mice and human autism models, and its relative controls. We have identified dysbiosis of several genera and species of bacteria in the gut, a sex-dependent dysregulation of the immune system. L. reuteri, a species with decreased relative abundance in our models, positively correlated with the expression of GABR subunits in the brain. Treatment of mice with L. reuteri induced an attenuation of unsocial behavior and a decrease in repetitive behaviors, without affecting anxiety, and induced an increase in GABR expression in multiple brain regions and affected serum immune system markers. This study has confirmed that genetic differences associated with autism can induce changes in the microbiota profile and further suggests a therapeutic potential for probiotic treatment.

Keywords: Molecular biology, Biomarkers, Plasticity

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NP6 | GSK3β in somatosensory Cortex: modulation of timing-dependent long-term depression through direct phosphorylation of Kv4.2 channels

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Spike Timing Dependent Plasticity (STDP) is a temporally asymmetric form of Hebbian learning induced by tight temporal correlations between the spikes of pre and postsynaptic neurons. Despite its key role in dictating learning rules and information storage during development and refinement of neuronal circuits, the molecular mechanisms mediating STDP are not completely understood. Here, we identified glycogen-synthase kinase 3 beta (GSK3β) and the voltage-gated K+ channel Kv 4.2 as novel molecular determinants of spike timing-dependent long-term depression (tLTD) in the mouse somatosensory cortex. We found that tLTD and A-Type K+ currents are bi-directionally modulated by pharmacological agents affecting the levels of active GSK3 and by GSK3β knockdown. Moreover, we revealed that blocking A-type K+ currents with a selective inhibitor of Kv4.2 channels AmmTX3, reflects the effects of GSK3 up-regulation on tLTD and prevents additional changes in synaptic strength. Collectively pharmacological, immunohistochemical and biochemical experiments showed that GSK3β influence in tLTD induction is mediated by direct phosphorylation at Ser-616 of the Kv4.2 subunit and identify the functional interaction between GSK3β and Kv4.2 channel as a novel mechanism for tLTD modulation concerning GSK3β role in synaptic plasticity.

Keywords: Animal model, Electrophysiology, Plasticity

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NP7 | TTC3: a Down syndrome gene involved in neuronal migration

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Down syndrome (DS) is caused by trisomy of Human Chromosome 21 (HSA21). The clinical manifestations of DS vary in both penetrance and intensity among affected individuals, but the only hallmark common to all patients is intellectual disability (ID) (Rachidi et al., 2008). DS-associated ID is the result of developmental brain abnormalities, leading to altered neuronal migration and connections within the cortex (Imaia et al., 2014, Golden et al., 1994). One likely candidate for these phenotypes is TTC3, a HSA21 protein, whose expression is increased in cells derived from DS experimental models (Saran et al., 2003) and from DS patients (Suizu et al., 2009). Moreover, upregulation of TTC3 inhibits neuronal differentiation by modulating actin cytoskeleton (Berto et al., 2007, 2014). Here, we study the effect of TTC3 overdosage in cortical development, upregulating the protein using in utero electroporation and we demonstrated that TTC3 levels are critical for correct neuronal positioning within the cortex. These data support the hypothesis that DS-cortical phenotypes can be ascribed, at least in part, to the increased dosage of TTC3.

Keywords: Molecular biology, Animal model, Cognitive

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NP8 | Identification and functional investigation of mammalian piRNA-pathway in adult hippocampal neurogenesis

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In mammals, P-element-induced wimpy testis (PIWI) proteins such as MILI and MIWI, and their products PIWI-interacting RNAs (piRNAs, 26–32 nt single-stranded non coding RNAs), are mostly expressed in germline cells, where have been shown to repress transposable elements (TE). Few studies have reported the expression of piRNAs in the adult nervous system, suggesting their potential involvement in regulating TE-dependent somatic mosaicism and/or plasticity in mature neurons. However, functional evidences of PIWI proteins/piRNAs in the mammalian nervous system have not been provided yet. Here we report that in the nervous system expression of PIWI proteins and of piRNAs is particularly prominent in the adult neural stem cell of the mouse hippocampus, and that their levels change during the process of adult neurogenesis, both in vivo and in vitro. Importantly, manipulation of PIWI proteins in adult neural stem cells alters neurogenesis. To the best of our knowledge, our results provide the first evidence of a functional role for the PIWI-pathway proteins in the adult mammalian nervous system.

Keywords: Molecular biology, Animal model, Plasticity, Imaging, Stem cells

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NP9 | Excitotoxic lesion-induced striatal neuroblasts have a transient life but receive both local and long-range connections

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We and others have recently demonstrated that, after Quinolinic Acid lesion or stroke, subsets of striatal astrocytes undergo a spontaneous neurogenic activation leading to the local generation of a huge amount of neuroblasts. Yet the fate and functional integration of these newborn cells remains largely unexplored. Similar to other models of physiological and lesion-induced striatal neurogenesis, QA-induced newborn neurons live transiently and fail to express molecular markers of fully differentiated neurons. For many of these cells immature features are confirmed by an electrophysiological profile typical of immature neuroblasts. Nevertheless, 3D reconstructions support an ongoing maturation process in which neuroblasts gradually attain complex morphologies and often show dendritic spines. Accordingly, through retro-rabies virus mediated retrograde tracing of newborn neuron first afferents, we show that striatal neuroblasts receive local connections from other neuroblasts and spared striatal neurons. Strikingly, newborn cells also receive synaptic contacts from cortical and thalamic neurons residing in areas physiologically projecting to the striatal sub-region where neurogenesis is found. These results indicate that striatal immature neurons integrate transiently in pre-existing circuits. It is intriguing to speculate that these neurons may be involved in transient forms of compensatory plasticity after lesion.

Keywords: Brain injury, Plasticity, Stem cells

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NP10 | Preliminary characterization of a knock-in mouse model for the hyperglycosylating P0D61N Myelin Protein Zero mutation

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Mutations in the Myelin Protein Zero gene (MPZ), encoding P0, the major structural glycoprotein of peripheral nerve myelin, are found in 5% of Charcot-Marie-Tooth (CMT) patients. P0 variants may cause different gain of function including misglycosylation (either gain-of-glycosylation or loss-of-glycosylation), a novel pathomechanism occurring in several genetic disorders that has never been fully elucidated. Therefore, we decided to establish a mouse model for the P0D61N mutation, a MPZ variant causing hyperglycosylation of P0. The knock-in mouse model was generated by the Core Facility for Conditional Mutagenesis of the San Raffaele in Milan using the Crispr/Cas9 system. The phenotype was characterized by behavioral, electrophysiological and neuropathological tests, at different time points. All evaluations showed significant differences in the heterozygous P0D61N mice as compared to wild type animals. The tremor was evident in all P0D61N mice and correlated with a significant motor impairment on the accelerating Rotarod. Electrophysiological parameters also differed between the two groups; further, the preliminary pathological analysis confirmed a demyelinating phenotype. In conclusion, we present here the preliminary phenotypical characterization which recapitulates the human phenotype of this variant. Since this is the first animal model of a hyperglycosilating variant, it could represent an useful tool for testing therapeutic approaches.

Keywords: Animal model, Electrophysiology

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NP11 | Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity

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The morphological, molecular and functional heterogeneity of astrocytes is under intense scrutiny, but how this diversity is ontogenetically achieved remains largely unknown. The cerebellum, with its variety of morphologically distinct astroglial subtypes allocated in different layers, is an excellent model to address this issue. Here, by quantitative in vivo clonal analyses and proliferation studies we demonstrate that the major cerebellar astrocyte subtypes emerge according to an unprecedented and remarkably orderly developmental program comprising i) a time-dependent decline in both clone size and progenitor multipotency, associated with clone allocation first to the hemispheres and then to the vermis; ii) distinctive clonal relationships among astrocyte subtypes, revealing diverse lineage potentials of embryonic and postnatal progenitors; iii) stereotyped clone architectures and recurrent modularities that correlate to layer-specific dynamics of postnatal proliferation/differentiation. Eventually, in silico simulations suggest the presence of deterministic components in the astrogliogenic program and do not support a purely stochastic model.

Keywords: Stem cells

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NP12 | Xlr gene as a new candidate for susceptibility to cocaine addiction

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Interacting with genes, environmental conditions can exert profound and long-lasting influence on newborn's brain development, increasing vulnerability to different psychopathologies, including substance use disorder. We have previously reported that exposure to early unstable environment (Repeated Cross Fostering- RCF) increases in C57BL/6J (C57) and reduces in DBA2/J mice susceptibility to develop an addiction-like phenotype in adulthood. Microarray experiments followed by RT-PCR validation identified two target genes, Xlr4a and Xlr4b, already suggested to be involved in neuronal plasticity, oppositely regulated in the Nucleus Accumbens (NAc) of the two strains (down in C57 and up in DBA). Here we hypothesize that Xlr4 contributes to the behavioral addiction-like phenotype shown by RCF C57 animals. To test this hypothesis, AAV selective Xlr4 down-regulation (KD) in the NAc of adult Control C57 animals was performed to model RCF manipulation. Xlr4-KD Control mice show cocaine-induced CPP similarly to the RCF mice. In addition, Xlr4-KD mice also show a priming-induced CPP reinstatement, thus supporting a cocaine addiction-like phenotype. Morphological changes as well as expression of genes linked to neuronal plasticity were investigated in Xlr4-KD mice to better characterize the role for Xlr4 gene in susceptibility to cocaine addiction in a mouse model of early unstable environment.

Keywords: Molecular biology, Animal model, Plasticity

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NP13 | Brain Metabolic Response to Metformin: the role of Endoplasmic Reticulum

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Imaging of brain FDG uptake relies on the assumption that this tracer accurately delineates overall glucose consumption (MRGlu) of the central nervous system. We recently documented in cancer that the link between FDG retention and glucose disposal is relatively loose and profoundly dependent upon the activity of a specific glucose processing machinery located in the endoplasmic reticulum (ER) and triggered by the MTF-sensitive enzyme hexose-6-phosphate-dehydrogenase (H6PD). MTF can actually cross the blood-brain barrier and to inhibit mitochondrial respiration leading to an accelerated glycolytic flux and lactate release of both neurons and astrocytes. Testing the hypothesis that MTF metabolic effect actually extends to the central nervous tissue, here we show that brain FDG retention is loosely linked to overall glucose consumption. Rather, it reflects a specific H6PD-dependent metabolism located within the ER. This pathway is mostly represented in neurons and much less in astrocytes. It is associated with an OXPHOS activation, characterized by both oxygen consumption and ATP synthesis, in response to 2DG. MTF profoundly hampers this effect in neurons while it is ineffective in astrocytes. These data represent a paradigm-shift in current understanding of glucose consumption in the central nervous system.

Keywords: Molecular biology, Neuroimaging, Plasticity

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NP14 | Fate mapping of adult hippocampal neural stem/progenitor cells in a model of neuroinflammation

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In the adult mouse hippocampus, newborn neurons and astrocytes arise throughout life from multipotent neural stem cells (NSCs) located in the subgranular zone of the dentate gyrus (DG). Neuroinflammation severely affects adult neurogenesis and increases astrocytes in the DG. However, few in vivo data are available concerning the effects of neuroinflammation on adult DG NSC/progenitor cell fate. In this study, we used two experimental approaches for fate mapping of DG NSCs and/or neuronal progenitors in an acute lipopolysaccharide (LPS)-induced neuroinflammation model. First, we exploited the tamoxifen (TAM)-inducible Cre-LoxP system by using the Glast-CreERT2;Rosa26-floxed-stop-YFP mouse line. Interestingly enough, we found TAM treatment attenuated the neuroinflammatory response upon LPS treatment, indicating that this system, widely used in the field of adult neurogenesis, seems not suitable to study the early effects of neuroinflammation in adult neurogenic niches. Next, as alternative strategy we stereotaxically injected a retrovirus expressing the Cre-recombinase (RV-Cre) in the DG of Rosa26-floxed-stop-YFP mice followed by LPS treatment. RV-Cre efficiently targeted mitotic progenitors in the DG. Notably, in LPS-treated mice we observed a significant increase in newborn YFP+ astrocytes and reduced YFP+ neurons, compared to Saline-injected mice, providing direct in vivo evidence for neuron-to-astroglia shift in DG progenitor fate upon inflammation.

Keywords: Inflammation, Plasticity, Stem cells

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NP15 | Transgenerational long lasting effects on motivational system of adolescent exposure to nicotine, alcohol and cannabinoids in rats

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AIM. This novel study focused on the transgenerational long-term effects of adolescent binge-like exposure to alcohol, marijuana and nicotine in adult rats of both genders, with respect to motivation system, in adult F1 offspring generation. – METHODS. Subjects included 80 Long-Evans rats, half male and half female, coming from a previous generation of treated/control rats (F0), making up 8 groups in total, control-control parents (CCP), treated-control parents (TCP), control-treated parents (CTP), and treated-treated parents (TTP). Treatment lasted for 28 days, and started on P30, the drugs/vehicles were given as follows; daily intraperitoneal injections of nicotine (1 mg/kg), intraperitoneal injection of WIN55,212-2 (1.2 mg/kg) and ethanol (3 g/kg) via gavage were instead administered on two consecutive days per week of treatment. To test for the effects of the drugs/vehicles on the motivation system, rats at P90 were tested in operant conditioning chambers, by learning to press the correct lever and being awarded banana flavored pallets, at different schedules, namely, Fixed Ratio (FR1 and FR5), Progressive Ratio (PR), extinction and reinstatement. – RESULTS. A gender effect was observed in all the evaluated behavioral parameters. Within females, during extinction and reinstatement, the TCP and CCP rats showed an increase of active lever presses compared to TTP and CTP rats. Within males, during reinstatement, the TTP and CTP rats showed an increase of presses on the active lever compared to TCP and TCP rats. In general, during both extinction and reinstatement, females clicked more than male rats. – CONCLUSIONS. The results show that F0 adolescent polydrug abuse affected the behavior and the response to adolescent exposure to the same mixture of drugs of the F1 offspring, in both genders. This implies that further studies should be done focusing on epigenetics.

Keywords: Animal model, Plasticity, Statistics

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NO1 | Citron kinase inactivation inhibits medulloblastoma progression by inducing apoptosis and cell senescence

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BACKGROUND. 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. – METHODS. We tested this hypothesis using transient siRNA transfection and an inducible stable shRNA system in MB cell lines DAOY and ONS-76. We also performed xenografts assay with inducible cell lines. Finally, we resorted to a temporally controlled CITK ko strategy in the NeuroD-SmoA1 model; so that we was able to inactivate CITK in primary MB similar to arise in patients. – RESULTS AND CONCLUSION. In MB cell lines 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 arising in 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. These results suggest that CITK can be a useful therapeutic molecular target for MB treatment.

Keywords: Molecular biology, Animal model, Cancer

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NO2 | Isolation and characterization of putative stem cells from human meningiomas

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Meningiomas, the most common primary intracranial tumors, are stratified into three grades of malignancy reflecting increasing recurrence risk. Generally, surgery is the definitive therapy; however, almost 20% of tumors recur, representing a serious therapeutic challenge. In malignant tumors recurrence is due to cancer stem cells (CSCs), responsible for drug resistance and metastasis. CSCs are characterized by self-renewal, stem marker expression and ability to differentiate into tumor-specific cell types. Recently, the expression of stem markers (Sox2, Nanog and Oct4) have been identified in meningioma, suggesting the presence of "stem cell-like" cells possibly involved tumor aggressiveness. The expression of CD105 (endoglin) in a subset of meningioma cells has been associated with CSC-phenotype and poor outcome. We isolated CD105-positive cells from 30 primary cultures of human low-grade meningioma by immunomagnetic separation. Compared to bulk meningioma cells, CD105-positive cells express stem markers, showing high proliferation rate, ability to form spheroids and differentiate into adipocytes and osteocytes. Moreover, we assayed the functional properties of CD105-positive cells observing that the activation of their chemokine receptors by CXCL11/CXCL12 promotes in vitro angiogenesis and migration. In conclusion, benign meningiomas contain a CD105-positive cell subset, which, at odd to the CD105-negative counterpart, shows stem-like features likely sustaining aggressive phenotype.

Keywords: Molecular biology, Stem cells, Cancer

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NO3 | Itch/βarrestin2-dependent non-proteolytic ubiquitylation of SuFu controls Hedgehog signalling and medulloblastoma tumourigenesis

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Suppressor of Fused (SuFu), a tumour suppressor mutated in medulloblastoma, is a central player of Hh signalling, a pathway crucial for development and deregulated in cancer. Although the control of Gli transcription factors by SuFu is critical in Hh signalling, our understanding of the mechanism regulating this key event remains limited. Here, we show that the Itch/b-arrestin2 complex binds SuFu and induces its Lys63-linked polyubiquitylation without affecting its stability. This process increases the association of SuFu with Gli3, promoting the conversion of Gli3 into a repressor, which keeps Hh signalling off. Activation of Hh signalling antagonizes the Itch-dependent polyubiquitylation of SuFu. Notably, different SuFu mutations occurring in medulloblastoma patients are insensitive to Itch activity thus leading to deregulated Hh signalling and enhancing medulloblastoma cell growth. Our findings uncover new mechanisms controlling the tumour suppressive functions of SuFu and reveal that their alterations are implicated in medulloblastoma tumourigenesis.

Keywords: Molecular biology, Protein aggregation, Cancer

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NO4 | mApoE-Functionalized Liposomes: a dual-task strategy to Cross the Blood Brain Barrier and to Target Glioblastoma Stem-Cells

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Glioblastoma (GBM) is among the deadliest of all human cancers with less than 5 % of patients surviving beyond 5 years. To achieve long-term survival and ultimately cure the patients, it is essential to develop novel treatments to selectively kill therapy-resistant GBM stem-like cells (GSCs). Indeed, their innate tumor- initiating aptitude makes GSCs a crucial target for effective therapeutic strategies. Due to the presence of the Blood-Brain-Barrier (BBB), reaching GSC dispersed into the brain parenchyma is hardly difficult and complex. Using mApoE-functionalized liposomes as doxorubicin carriers (mApoE-DOXO-LIPs), we provide the proof of concept for a dual-targeting strategy able to improve BBB crossing and to ensure GSCs targeting. In vitro experiments using a BBB cellular model indicate that encapsulated-DOXO preserves endothelial cells viability while retaining, after BBB crossing, a significant cytotoxic activity against GBM cells. Experiments in patient- derived GSCs showed that cell targeting and uptake are sustained by specific receptor-mediated endocytosis involving mApoE and Low Density Lipoprotein Receptor (LDLR). Experiments in GSCs transplanted mice show that mApoE-DOXO-LIPs inhibit in vivo tumor growth, confirming mApoE-DOXO-LIPs double function ability to cross BBB and to interfere with GSCs viability.

Keywords: Molecular biology, Nanomaterials/nanoparticles, Animal model, Stem cells, Cancer

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NO5 | Oligodendroglioma cells are dependent on extracellular glutamine but do not exhibit glutamine anaplerosis

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A MYC-dependent transcriptional program drives uptake and consumption of glutamine (Gln) in several types of human cancer cells. These cells usually exploit high Gln availability for anaplerosis and exhibit a metabolic addiction for the amino acid. Glutamine addicted cancers are often characterized by low levels of Glutamine Synthetase (GS), the only enzyme that catalyzes de novo Gln synthesis. GS is usually low or absent in human oligodendrogliomas (OD). We have confirmed GS negativity in HOG and Hs683 cells, two lines derived from human ODs. While complete Gln depletion or the glutaminolytic enzyme L-Asparaginase cause massive cell death in both HOG and Hs683 cultures, small amounts of extracellular Gln are sufficient for OD cell growth. Gln starvation does not significantly affect the cell content of 2-oxoglutarate or pyruvate, which are not able to rescue cell growth, but down-regulates the WNT/Beta-catenin pathway, lowers MYC expression, and lowers protein synthesis. Gln transport inhibitors cause a significant, but not complete, depletion of intracellular Gln and cell growth inhibition, but do not cause cell death. Thus, human OD cells are GS-negative and Gln-auxotrophic but do not exhibit Gln-dependent anaplerosis.

Keywords: Cancer

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ND27 | A novel versatile and automated tracking software (TrAQ) for the characterization of behavioural rodent models

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Quantitative metrics of laboratory animals' locomotion from video footage are crucial data in behavioural and neuroscience studies. Commercial tracking software are stable and versatile but quite expensive, requiring various degrees of users interaction. Open source tracking software exist but they are often task specific and/or cumbersome to use. TrAQ (Tracking rodents in L'Aquila) is a new MATLAB-based tracking software for off-line analysis, developed to minimize the user interventions and allow single-click multiple-videos processing. TrAQ allows automatic recognition of the animal and arena, providing the position of the animal's centroid, head and tail and other quantitative two-dimensional behavioural parameters among which the number of body-centred turns. We tested our TrAQ with smartphone-recorded videos and the 6-hidroxydopamine unilaterally injected rats in the Substantia Nigra pars compacta. In this model the number of body-centred turns performed after the administration of dopaminergic agonists correlates with the neurodegeneration degree and represents a worst-case condition, were other software fail, because of the compulsory animal pivoting. Even in this condition TrAQ counting of body-centred turns is reliable and results are validated against manual annotation. The MATLAB environment allows any kind of data analysis and we plan to freely distribute TrAQ as an open-source software.

Keywords: Animal model

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ND28 | Pharmacological JNK-pathway inhibition reduces severity of spinal muscular atrophy disease in a mouse model of SMAII

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Spinal muscular atrophy (SMA) is a severe neurodegenerative disorder of the early childhood, characterized by a selective degeneration of lower motor neurons (MNs), resulting in progressive skeletal muscle denervation and atrophy. Currently, the cellular/molecular mechanisms underlying MN death are mostly unknown. Recently, it has been shown that the JNK-signalling pathway might be involved in SMA pathogenesis. We tested a synthetic JNK-inhibitor peptide (D-JNKI), by chronic administration to SMA mice and WT littermates from P1 to P10. During D-JNKI administration, we evaluated motor function in treated and untreated mice; then, at P12, spinal cord and quadriceps muscle were histologically analysed. JNK inhibition has a positive effect on SMA mice behaviour, improving motor performances and the trophism of muscular fibers. Indeed, D-JNKI administration ameliorates the size of the neuromuscular junctions, leading to a better muscle innervation. Moreover, in the spinal cord, JNK-pathway inhibition delays MN death and decreases astrogliosis. Finally, administration of D-JNKI significantly increases SMA mice lifespan. Notably the inhibition of JNK does not affect the development of WT mice. Together, our findings identify JNK inhibition as a way to reduce neurodegeneration and muscle denervation/atrophy, and highlight JNK-pathway as a target for developing alternative pharmacological strategies for SMA treatment.

Keywords: Molecular biology, Spinal cord injury, Degeneration

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ND29 | Nuclear receptor related 1 protein (Nurr1) in attention deficit hyperactivity disorder (ADHD): searching for a disease murine model

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The transcription factor nuclear receptor related 1 protein (Nurr1) has been found to play a critical role in the development and the maintenance of midbrain dopaminergic neurons, regulating the tyrosine hydrolase and the dopamine transporter expression. Hence, Nurr1 has been implicated in dopamine-associated brain disorder, including the attention deficit hyperactivity disorder (ADHD). This neurodevelopmental disease affects 5–8% of school aged children and often leads to adverse consequences in adulthood including drug abuse, delinquency, anxiety, depression and social rejection. ADHD is characterized by three main symptoms represented by hyperactivity, impulsivity and inattention. Here, we characterized the heterozygous Nurr1 knock-out (Nurr1-KO) mice in order to evaluate its behavioral phenotype related to their locomotor activity, impulsivity, attention, anxious like behavior, spatial and social memories. We highlighted that Nurr1-KO male mice shows hyperactivity and impulsivity. Conversely, only female shows attention defect. Furthermore, Nurr1-KO mice do not display anxious-like behavior and memories defects. Our RT Real time PCR analysis in the dissected brain regions revealed a gene expression down-regulation of Nurr1 specifically in the dopaminergic mesolimbic regions. These results represent a promising starting point to considered the Nurr1-KO mouse as a murine model of ADHD useful to evaluate therapeutic approaches in psychiatric disorder.

Keywords: Molecular biology, Animal model

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ND30 | Atrial Natriuretic Peptides protects dopaminergic neuron-like cells from neurotoxin-induced damage via Up-Regulation of the Wnt/beta-Catenin Pathway

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Increasing evidence indicated a crucial role of the Wnt/beta-catenin signaling in mDA neuron development. Recently, dysregulation of this pathway has been proposed as a novel pathomechanism leading to Parkinson's Disease (PD) and some molecular components of the signaling have been evaluated as potential therapeutic targets for PD. Atrial natriuretic peptide (ANP) and its receptors are widely expressed in mammalian CNS where they are involved in neural development, synaptic transmission, and neuroprotection. We previously demonstrated that, in colorectal cancer cells, ANP affects the Wnt/beta-catenin signaling through a Wnt receptor-mediated mechanism. Using an in vitro model of PD, we assessed if ANP possesses neuroprotective ability by up-regulating the Wnt/beta-catenin signaling. As cellular model of DA neurons, we used the human neuroblastoma SH-SY5Y cells, that were subjected to neurotoxin insult for mimicking the neurodegeneration of PD. Through confocal microscopy and molecular analyses, we demonstrated that ANP positively affect the Wnt/beta-catenin signaling, by inducing beta-catenin stabilization and nuclear translocation, and that the ANP-induced activation of Wnt pathway significantly attenuates the neurotoxin-produced damage of the DA neuron-like cells. Thus, exogenous ANP could be an innovative neuroprotective molecule for midbrain and, more in general, for brain diseases for which aberrant Wnt signaling seems to be involved.

Keywords: Molecular biology, Brain injury, Degeneration

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ND31 | Effect of C9orf72 deletion on the pathology progression in a familial amyotrophic lateral sclerosis (ALS) mouse model

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Intronic expansion of G4C2 in C9orf72 gene is the most common genetic alteration in ALS. Although the C9orf72 haploinsufficiency has been proposed as toxic mechanisms, C9orf72-/- mice show no neurodegeneration, suggesting that both loss and gain of function are required for motoneuron pathology. However, C9orf72-/- mice exhibit reduced macrophagic/microglial function and increased inflammatory state, mechanisms overall involved in ALS progression. To determine if a reduction of C9orf72 may impact on the disease course independently of its trigger, we investigated the effect of the C9orf72 gene deletion on the disease progression of SOD1-G93A mice, the best-characterized model of ALS. Transgenic SOD1-G93A and C9orf72-/- mice were crossbred and behavioral and histopathological analyses were performed. We found that C9orf72 deletion did not modify the neuromuscular deficit, but delayed the weight loss of SOD1-G93A mice. Furthermore, neither motoneuron loss nor neuromuscular junction denervation was modified. C9orf72-/- mice showed splenomegaly and lymphadenopathy, which were reduced in SOD1-G93A/C9ORF72-/- mice. In addition, the overexpression of CD11b and CD68 microglia in the spinal cord of SOD1-G93A mice was reduced by the deletion of C9orf72, while no difference was observed for astrocytosis. In conclusion, interfering with these effects is not sufficient to change the progression of ALS pathology.

Keywords: Animal model, Degeneration

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ND32 | Investigating the involvement of coding and long non coding proto-oncogene c-MYC in ALS

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease caused by the selective loss of upper and lower motor neurons. Deep transcriptome studies on ALS highlighted deregulation also in genes associated with other diseases, such as cancer. Therefore, the aim of the work was to evaluate the role of the oncogene c-MYC and its lnc-RNA MINCR in ALS. C-MYC binds the E-box sequence on the DNA in complex with Max and MYCBP, which stabilizes the complex. RNA-seq data, confirmed by qRT-PCR, showed a down-regulation in ALS of MINCR, MYCBP and GADD45a, a c-MYC target protein implicated in DNA repair mechanism. MYCBP protein levels were reduced in ALS patients, whereas protein levels of c-MYC didn't show difference. Max immunoprecipitation revealed that c-MYC binds Max more in control subjects than in ALS patients. These data show that c-MYC pathway could be implicated in ALS through the down-regulation of MYCBP by MINCR: it may prevent the c-MYC binding to DNA. The deregulation in c-MYC transcriptional activity may alter different pathways, including apoptosis and DNA repair. Concerning this point, the decrease of GADD45a in ALS patients seems to indicate a deregulation in DNA repair mechanism resulting in DNA damage, one of the ALS hallmark.

Keywords: Molecular biology, Degeneration

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ND33 | Chronic Treatment with Antioxidant Nutraceutical Molecules Slow Down the Degenarative Process in an Animal Model of Retinitis Pigmentosa

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The term Retinitis Pigmentosa (RP) defines a group of inherited dystrophies characterized by progressive degeneration of the visual cells and abnormalities in retinal pigment epithelium. The vision is lost slowly but inexorably, the outcome is a total blindness. The primary mutation responsible for RP is in rods but the biological mechanisms of damage remain still elusive. Apoptosis is generally the main modality of photoreceptor cell death but also necrosis and autophagy are involved. Similarly, the biological mechanisms linking the primary degeneration of rods to the secondary death of cones, are still poorly understood. Possible causes of cone secondary death are mainly oxidative stress and release of toxic factors from dying rods. Here we show that chronic treatment per os with a flavanone (Naringenin) abundant in citrus fruits such as grapefruits (Citrus paradisi) and oranges (Citrus sinensis), and a flavonol (Quercetin) abundant in grapes, green tea and apple, produce an improve of retinal fuction (elettroretinogram recording), of retinal morphology (immunohistochemistry) and a reduction of stress-oxidative markers (western blot analysis). In summary, treatment with Naringenin and Quercetin, induce a significant benefits on the life span of retinal neurons in an animal model of RP, the rd10 mouse. – Founding: PRA2017 (University of Pisa)

Keywords: Animal model, Electrophysiology, Degeneration

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ND34 | Modelling autosomal dominant lateral temporal epilepsy (ADLTE) with Human iPSC-derived Neurons

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Mutations of LGI1 and REELIN genes are responsible for autosomal dominant lateral temporal epilepsy (ADLTE), a neurological condition characterized by lateral temporal seizures with prominent auditory or aphasic auras. Lgi1 protein is secreted at the synapses and interacts with synaptic proteins ADAM23 and PSD95. In mouse LG1 plays a role in synaptic transmission and neurodevelopment. Our aim is to define alterations of neuronal maturation and synaptic transmission in human neurons carrying different LGl1mutations. We studied the phenotype of neurons differentiated from induced pluripotent stem cells (iPSCs) of 3 patients. Human neurons reached a mature state with neuronal-like morphology and expression of neuronal markers. Electrophysiological analysis revealed that these cells show both outward and inward macroscopic currents and generate action potentials in response to depolarizing current pulses. To evaluate synaptic transmission, spontaneous and evoked excitatory and inhibitory postsynaptic currents (PSCs) were recorded, as well as PSCs evoked in neighbouring neurons by stimulation of iPSC-derived neurons. To characterize neuronal maturation abnormalities, we performed analysis of soma area, neurite length and arborisation. Moreover, the role of LGI1 in synaptic maturation and function in human neurons were elucidated by counting the number of dendritic protrusions with pre- and post-synaptic boutons.

Keywords: Molecular biology, Electrophysiology, Stem cells

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ND35| Modeling fragile X syndromw with human IPSC-derived neurons

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Fragile X syndrome (FXS) is the most common inherited form of human mental retardation and it is caused by expansion of CGG repeat within the FMR1 gene. The resulting epigenetic silencing causes the loss of the fragile X mental retardation protein (FMRP) with defects in the regulation of dendritic spine morphology and synaptogenesis. Growing evidence demostrates aspects of human development and disease can be acurately modelled in vitro using human induced pluripotent stem cells (hiPSCs). The aim of our study is to create an in vitro model based on hiPSCs and test new potential drugs for FXS. We developed a culture system for cortical neuron differentiation and we obtained TUJ1-positive cells in order to study electrophysiological properties and axon growth dynamics of FXS-iPSCs derived neurons. In particular, we are analyzing voltage gated channels and firing properties of FXS-iPSCs derived neurons and synaptic activity on neuronal networks in vitro, using patchclamp and ion imaging recordings. Therefore, the creation of a robust in vitro model based on hiPSCs can be used to study FXS in a time frame that is relevant to the disease, understand its mechanisms and allow for therapeutic testing, all in cells carrying the genetic background of individual patients.

Keywords: Electrophysiology, Degeneration, Stem cells

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ND36 | A role for Colony Stimulating Factor 1 Receptor Signalling in the generation of cerebrovascular and BBB pathology

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Hereditary Diffuse Leukoencephalopathy with Spheroids (HDLS) is a rare neurodegenerative leukoencephalopathy presenting clinically as cognitive dysfunction, memory deficit and motor impairment. Neuropathological examination reveals axonal swellings (spheroids), neurofilament and Ab positivity within the white matter of affected individuals. The causative gene for this monogenic dominant disorder encodes the colony stimulating factor 1 receptor (CSF1R); previously known to be involved in the differentiation, maturation and regulation of immune cells in the myeloid lineage and brain microglia, this receptor tyrosine kinase and binds two ligands CSF1 and interleukin-34 the latter of which is enriched in nervous tissue. Classically associated with macrophage function, the CSF1R gene has been revealed to have a critical role in microglial viability as well as the response of the central nervous system (CNS) to injury and stress revealing a new regulator in central nervous system (CNS) homeostasis. Recently we have identified two familial cohorts of HDLS with perivascular pathologies including amyloid beta accumulation like identical to that observed in Alzheimer's disease, astroglial and microglial activation, and an accompanying increase in BBB permeability. Functional analyses of HDLS-variant CSF1R in vitro revealed conserved but reduced translation and membrane localisation, indicating that CSF1R haploinsufficiency alone may not be required for HDLS pathogenesis. We have identified aberrant post-translational processing and functionality of mutant-CSF1R and propose these altered characteristics and subsequent BBB dysfunction, rather than loss of function, may drive HDLS pathology.

Keywords: Molecular biology, Ageing, Degeneration, Immune system, Protein aggregation

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ND37 | Pharmacological treatment with CTEP, an mGluR5 negative allosteric modulator, in SOD1G93A mice

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The etiology of Amyotrophic lateral sclerosis (ALS) is not completely understood, but one of the major cause is glutamate (Glu)-mediated excitotoxicity. In particular, Group I metabotropic glutamate receptors (mGluR1 and mGluR5) may represent a potential target for the treatment of ALS. In previous studies, we generated SOD-1G93A mice with a partial mGluR5 deletion and we observed a shift of the pathology onset, a prolonged survival probability, and an amelioration of clinical symptoms. We investigated here the effect of the pharmacological blockade of mGluR5 in SOD1G93A mice administering by gavage 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy)phenyl)-1H-imidazol-4-yl)ethynyl)pyridine (CTEP), an orally bioavailable mGluR5 negative allosteric modulator (NAM), starting at 90 days of life until euthanasia. Clinical onset, survival probability, progression of the pathology, and disease cellular hallmarks were analyzed in drug-treated vs. vehicle-treated SOD-1G93A mice. CTEP, concentration dependently, delayed significantly the disease onset, augmented survival probability, ameliorated motor clinical symptoms, and reduced astrocyte and microglia activation in SOD1G93A mice. In conclusion, our previous and present results suggest that mGluR5 may represent a promising target in ALS and the present pharmacological data predict translational perspectives for CTEP or other mGluR5 NAMs, already under clinical study, for the cure.

Keywords: Animal model, Degeneration

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ND38 | Features of Frontotemporal Lobar Degeneration in the Cyclophilin a Knock-Out Mice

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Cyclophilin-A (PPIA) is a multifunctional protein that has been associated with different human diseases. We first associated PPIA with nervous system degeneration, identifying it as a translational biomarker of ALS. We recently demonstrated that PPIA is a functional interacting partner of TDP-43 and regulates its nuclear-cytoplasmic trafficking. TDP-43 is the major component of neuronal-cytoplasmic inclusions in frontotemporal-lobar degeneration patients (FTLD-TDP). The molecular mechanisms at the basis of TDP-43 pathology have not been elucidated yet. Here we proposed PPIA as a major player of this process. We show that PPIA knock-out (PPIA-/-) recapitulate major features of FTLD-TDP, such as TDP-43 aggregation and mislocalization in the brain. PPIA-/- mice show also atrophy of cortex and hippocampus, regions affected by the disease in the patients. In absence of PPIA, mice exhibited an increased disinhibition and an altered social behavior with no memory and motor impairment as FTLD patients. However, unlike patients, PPIA-/- mice displayed neuronal loss with no glial activation. In conclusion, our findings indicate that PPIA has an important effect on neuronal survival and its depletion results in FTLD-related deficits. Moreover, PPIA-/- mouse represents a useful animal model to understand the molecular mechanism behind TDP-43 pathology and its involvement in FTLD-TDP.

Keywords: Animal model, Cognitive, Degeneration

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ND39 | The role of Group I metabotropic glutamate autoreceptors in ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by death of motorneurons. One major cause of the pathology is glutamate(Glu)-mediated excitotoxicity. Our previous studies demonstrated that Group-I metabotropic Glu-receptors (mGluR1, mGluR5) at glutamatergic terminals enormously enhanced drug-receptor affinity and produced abnormal Glu release in spinal cord of 120 days-old SOD1G93A mice at the late (120 days) stage of disease. Here we studied the modulation of Glu release by mGluR1/5 activation in SOD1G93A spinal cord at pre-symptomatic (30 and 60 days) and early symptomatic (90 days) ALS stages. 0,3 μM 3,5-DHPG, a mGluR1/5 agonist, induced abnormal Glu release in 90 days-old early-symptomatic SOD1G93A mice through both mGluR1 and mGluR5.3,5-DHPG induced also excessive increase of cytoplasmic Ca2+concentration, due to Ca2+release through IP3-sensitive channels. Confocal microscopy and Western Blot, demonstrated that mGluR1/5expression was increased in SOD1G93A mice. No abnormal 3,5-DHPG-mediated release were observed in 30 or 60 days-old pre-symptomatic SOD1G93A mice. The present and previous data suggest that mGluR1and mGluR5are hyperactive in SOD1G93A mice at early-and late-symptomatic phases of ALS, not at pre-symptomatic stages, suggesting that their hyperactivity is linked to ALS progression. Selective antagonists could be useful for new therapeutic approaches in ALS.

Keywords: Animal model, Degeneration

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ND40 | A bone disease in the retina; how Autosomal Dominant Osteopetrosis Type-II (ADO2) affects inner retinal circuitry

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Autosomal Dominant Osteopetrosis Type-II (ADO2) is a rare, under-investigated disease with a prevalence rate of 1:20000. A heterozygous missense mutation in the lysosomal chloride channel transporter 7 (CLC-7) is responsible for the resulting increase in bone density. Considered as a bone disease alone, the penetrance is 66%. The CLC family consists of chloride channels and electrogenic chloride/hydrogen exchangers, the latter describing the CLC-7 channel. It is known that, due to the importance of phagocytosis carried out by lysosomes in photoreceptors, a homozygous mutation in CLC-7 compromises retinal functioning. Photoreceptors must recycle photopigments at a high metabolic rate, resulting in a high demand for phagocytosis. Without functioning lysosomes, this process will be negatively impacted. Our aim is to investigate the retinal function and morphology of CD1 Clcn7 heterozygous mice. The experimental protocol consisted of electrophysiology in vivo through flash-electroretinogram, the collection of retinal tissue and immunohistochemistry for Clcn7 expression and stress markers in the retina. Preliminary data from ERG show a subtle difference in the latency of the b-wave, but no observable differences in the amplitude. Immunohistochemistry does not show clear changes in oxidative stress markers, although, a locational difference in Clcn7 expression is observed in the retinal pigmented epithelium.

Keywords: Electrophysiology, Biomarkers, Degeneration

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ND41 | The thalamocortical coherence in acute and chronic dopamine depletion

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Parkinson's disease (PD) is characterized by abnormal basal ganglia (BG) dysfunctions. Despite the crucial role of thalamus in BG, little is known concerning the thalamo-cortical interplay. We investigated the thalamocortical coherence in low (13-25 Hz) and high beta (25-40 Hz) frequencies intervals, in motor thalamus (MTh) and in its modulator, the nucleus reticularis thalami (NRT). In order to disentangle possible compensatory mechanisms that intervene before the chronic dopamine (DA) loss, we performed study in acute and chronic DA depletion states, obtained by means of tetrodotoxin or 6-hydroxydopamine infusion into the medial forebrain bundle, respectively. We observed that the MTh-cortical coherence increased in both acute and chronic DA depletion states in high beta band, whilst NRT-cortical coherence decreased only in acute state in both low and high beta band. These results underline the presence of compensatory mechanisms in acute state determining the dissociation between NRT and cortex, whilst the tardive mechanisms induce pathological cortical association with MTh. Since the NRT is the main thalamic modulator and it is involved in acute depletion states, it could have a crucial role in PD.

Keywords: Animal model, Electrophysiology, Degeneration

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ND42 | Neuroprotective effects of saffron and its components in animal model of retinal neurodegeneration

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PURPOSE. Saffron "Repron" is a high interest spice in the medical field, whose effects in various diseases are proven. In the last decade, saffron has shown neuroprotective effects in Age-related Macular Degeneration (AMD) in animal models and clinical trials. The project aim is to identify the relevant components in the neuroprotective actions. – METHODS. Using light exposed Sprague Dawley rats as an AMD model, we divided animals into 4 groups of treatment; vehicle, saffron, crocins components and safranal + crocetin components. The treatment was administrated for 7 days before, during and for 7 days after the light damage. We then performed flash-Electroretinogram recordings, looking at flicker responses as well as, a-wave, b-wave and Oscillatory amplitude responses, to evaluate the preservation of retinal function in vivo. Animals were then sacrificed and eyes collected for further immunohistochemistry analysis. We evaluated outer nuclear layer thickness through nuclear labeling and assessed retinal inflammation by immunofluorescence analysis of microglia and quantification of stress-markers such as bFGF-2 and GFAP. – RESULTS. Our results show that crocins and saffron show similar neuroprotective effects, confirming that crocins are the active molecules in neuroprotection. Finally, total saffron is more convenient for both therapeutic and commercial reasons.

Keywords: Animal model, Electrophysiology, Degeneration

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ND43 | Motor neuron degeneration and disease progression in amyotrophic lateral sclerosis are accelerated by the disruption of the astrocytic TNFR1-GDNF axis

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Neurodegeneration in Amyotrophic Lateral Sclerosis (ALS) can be conditioned by a deleterious interaction between motor neurons and the astrocytes. Astrocytes perform several activities that are essential to preserve CNS homeostasis, secreting a variety of mediators by which they can influence their cellular neighbours. Among these factors, astrocytes secrete GDNF, a potent neuroprotective agent. The modulation of the production of endogenous GDNF by astroglia may therefore have therapeutic implications in ALS. We identified TNF receptor 1 (TNFR1) signalling as a major promoter of GDNF synthesis and release from human and mouse spinal cord astrocytes in vitro and in vivo. To determine whether endogenously produced TNFalpha can also prompt the production of GDNF in the CNS, we focused on SOD-1G93A ALS transgenic mice, whose affected tissues spontaneously exhibit consistent levels of TNFalpha and TNFR1 at the onset and later stages of the disease. In SOD1G93A spinal cords, we verified a strict correlation in the expression of TNFalpha, TNFR1 and GDNF at different stages of disease progression. The ablation of TNFR1 completely abolished GDNF rises in both SOD1G93A astrocytes and spinal cords, accelerating motor neuron degeneration and disease progression. Therefore, the astrocytic TNFR1-GDNF axis could represent a novel target for therapeutic intervention in ALS.

Keywords: Degeneration, Neuron-glia communication

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ND44 | The protein kinase LRRK2 is involved in glutamate release by interacting and phosphorylating synapsin I in nerve terminals

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Parkinson's disease (PD) is the second most common neurodegenerative disease. Although the majority of cases are sporadic, about 10% of cases demonstrate a classic Mendelian familial inheritance; in particular, mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene are linked to late-onset autosomal dominant PD. Despite its predominance in PD, the physiological function of LRRK2 isn't fully understood. LRRK2 is composed of several protein-to-protein interaction domains and an enzymatic core with GTPase and kinase activities suggesting a role in intracellular signaling. At this regard, we demonstrated that LRRK2 interacts with synapsin I (Syn I), an important phosphoprotein involved in neuronal development and regulation of neurotransmitter release and, by phosphorylating, affects Syn I binding with synaptic vesicles and actin. Functionally, through superfused synaptosomes and patch-clamp recordings, we provided additional evidence that LRRK2 influences the glutamate release depending on its kinase activity in a Syn I-dependent manner, describing a mechanism that could be altered in case of LRRK2 pathogenic mutations. Given that only few LRRK2 substrates have been described and their relevance in disease is still unclear, it is tempting to speculate that Syn I represents a checkpoint that links LRRK2 activity to the regulation of excitatory neurotransmission in health and disease.

Keywords: Plasticity

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ND45 | mTORC1 in primary neurons: analysis of the role of oxygen and glucose in its kinase activity

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Ischemic stroke is a sudden vascular accident involving the reduction or disruption of brain blood flow. This situation generates a complex cascade of signals, called ischemic cascade that may causes the loss of several brain function because of neuronal damage. This perturbation of blood supply trigger a deprivation of nutrients, such as growth factors, oxygen and glucose to some brain regions. Several studies trying to find potential therapeutic targets to reduce ischemic damage in brain. Among then, mTORC1 complex appears to play an essential role controlling the dichotomy of catabolism/anabolism in neurons. Our aim is to elucidate the impact of deprivation of growth factors, oxygen and/or glucose (OGD), on mTORC1 activity through PI3K/Akt pathway, analysing the phosphorylation degree of two mTORC1 substrates: P70S6K and 4EBP-1 as reporters. We used an in vitro model of OGD in primary culture neurons from mouse cerebral cortex. Our results shown that OGD caused variations in the mTORC1 activity, as well as changes in neuronal morphology and viability, as indicated the activation of caspase 3. Therefore, maintenance of Akt/mTOR activity may be a crucial mechanism to promote neuronal survival in the presence of an ischemic event.

Keywords: Molecular biology, Degeneration, Regeneration

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ND46 | Evaluation of functional asymmetry in hemiparkinsonian rat using the Tail Suspension Test

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The postural instability and motor asymmetry are two widely recognized cardinal motor features of Parkinson's Disease (PD). The mechanisms underlying such lateralization are poorly known. Unilaterally injected 6-hydroxydopamine (6-OHDA) rats provide a useful model of hemiparkinson and apomorphine-induced rotation counting in the open field test (OFT) is a reliable method for the quantification of motor asymmetries. In order to characterise the time-course of postural and motor asymmetries, we performed a detailed analysis of the relationships between the tail suspension swing test (TST) and the behaviours recorded in OFT, in spontaneous and apomorphine-induced condition and striatal dopamine (DA) immunostaining, in 6-OHDA-lesioned rats. We performed a 10-trials/session sampling of swing behaviour. A swing was counted whenever the animal moved its head out of the vertical axis to either side. The data showed a strong correlation between the apomorphine-induced swings in the TST and apomorphine-induced motor asymmetry in the OFT (r=0.95). The TST showed a strong differentiation between spontaneous and apomorphine-induced behaviour. Furthermore, we observed a high correlation (r=0.94) among the striatal degeneration and TST-measured postural bias. Since this TST is fast, simple and inexpensive it could be used for more accurate behavioural assessment of toxin-induced neurodegenerative progression.

Keywords: Animal model, Biomarkers, Degeneration

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ND47 | Targeting the mitochondria-tyrosine kinase axis to prevent age-associated neuronal decline

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Aging is the progressive and physiological changing of biological functions responsible for increased risk of diseases developement. Nervous system is particularly sensitive to consequences of aging, such as altered metabolism and organelles dysfunction. Among these, mitochondrial impairment rapresents the driving force of aging process, especially through the uncontrolled overproduction of reactive oxygen species. In recent years it has been suggested that modulation of mitochondrial functionality and removal of damaged mitochondria can be regulated by tyrosine kinases, whose signaling is frequently altered in neurodegenerative diseases. In particular, cAbl and Src tyrosine kinases activity are frequently affected in neurodegenerative disease. Here we focused on Alzheimer's disease cellular models to verify a possible role of cAbl and Src kinases on mitochondrial homeostasis. We investigated if nutraceuticals compounds (i.e. polyphenols and carotenoides) modulate mitochondrial functionality or removal through a different activation or localization of cAbl and Src. Given the correlation between oxidative stress and aberrant tyrosine kinase signaling, we also focused on a possible nutraceuticals-induced modulation of the master regulator of oxidative stress Nrf2 through tyrosine kinases signaling. Taken together our findings aim to shed light on a new approach based on nutritional implementation to ameliorate age-associated neuronal decline.

Keywords: Molecular biology, Ageing, Degeneration

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NI15 | Protective effect of the histamine H4 receptor antagonist, JNJ7777120, in a rat model of cerebral ischemia

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Cerebral ischemia is a multifactorial pathology characterized by different events evolving in time. Extracellular levels of histamine, increase in the ischemic areas after focal cerebral ischemia induced by occlusion of the middle cerebral artery (MCAo) in the rat. The histamine H4 receptor (H4R) is predominantly expressed in several cell types of immune system and in numerous areas of the Central Nervous System (CNS) where is involved in regulating inflammation. Our aim was to assess the putative neuroprotective effects of the potent and selective H4R antagonist, JNJ7777120, chronically administered (1 mg/kg, i.p., twice/day for 7 days) on damage parameters in a model of focal ischemia induced in the rat by the transient (1 hour) MCAo. Chronic treatment with JNJ7777120, significantly protected from the neurological deficit and from body weight loss after tMCAo. Seven days after the ischemic insult, JNJ7777120 reduced the volume of the ischemic cortical and striatal damage, the number of activated microglia and astrocyte in the ischemic cortex and striatum, and decreased the plasma levels of IL-1beta and TNF-alfa, while increased the levels of IL-10. Results indicate that the selective antagonist of H4R, JNJ7777120, systemically and chronically administered after ischemia exerts a protective effect against brain transient ischemia.

Keywords: Animal model, Brain injury, Stroke

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NI16 | Exploring the cross-talk between microglia and oligodendrocyte progenitors in cerebral ischemia

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Oligodendrocytes are severely affected by ischemia contributing to stroke-associated deficits. Recent data showed that the subpopulation of oligodendrocyte precursor cells (OPCs) expressing the P2Y-like receptor GPR17 actively responds to the ischemic injury by increasing both their proliferation rate and migratory ability but, at later stages, only a few percentage of these cells undergoes maturation. This limited post-stroke repair is likely due to the local unfavorable inflammatory milieu mediated by infiltrating macrophages and resident microglia. Here, we aimed at understanding the time-dependent role of microglia/macrophages following cerebral ischemia and how these cells contribute to OPC responses after permanent middle cerebral artery occlusion (MCAo) in conditional GPR17-iCreERT2xCAG-eGFP transgenic mice. Results showed a correlation between the number of GPR17-expressing OPCs (GFP+-cells) and microglia/macrophages close to the lesion starting from 72h after MCAo. Moreover, partial depletion of microglia/macrophages at different time points after ischemia alters OPC reaction. Finally, both in vitro and in vivo studies pointed out that extracellular vesicles (EVs) produced by regenerative microglia positively affect OPC behavior, unveiling EVs as important players in microglia-OPCs cross-talk. Elucidating how microglial EVs produce these effects will be relevant for developing new strategies to improve recovery after stroke. Supported by Fondazione Cariplo grant 2015-0910 to MF.

Keywords: Inflammation, Immune system, Stroke

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NI17 | White matter microstructure alterations correlate with terminally differentiated CD8+ effector T cell depletion in the peripheral blood in mania: combined DTI and immunological investigation in the different phases of bipolar disorder

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BACKGROUND. White matter (WM) microstructural abnormalities and signs of immunological activation were consistently demonstrated in bipolar disorder (BD). However, their relationship remains unclear. –METHOD. In 60 BD patients (each different phase of illness) we investigated: (i) diffusion tensor imaging (DTI)-derived parameters using a tract-based spatial statistics (TBSS) approach; (ii) composition of circulating T cell subpopulations and cytokine production by flow cytometry; (iii) potential relationships between WM and immunological data. – RESULTS. We found: (i) a significant widespread WM alteration mainly in mania, with involvement of the body of corpus callosum (BCC) and superior corona radiata (SCR); (ii) a significant increase in CD4+ T cells and decrease in CD8+ T cells, as well as their subpopulations effector memory (CD8+CD28-CD45RA-), terminal effector memory (CD8+CD28-CD45RA+) and CD8+IFNγ+ in mania; (iii) a significant relationship between WM and immunological alterations in the cohort, and a significant correlation of WM abnormalities in BCC and SCR with reduced frequencies of CD8+CD28-CD45RA+ and CD8+IFNγ+ T cells in mania. – CONCLUSIONS. Our data show that WM abnormalities in mania are correlated with reduction in circulating terminally differentiated CD8+ T effector cells which are prone to tissue migration, suggesting that these T cells could play a role in WM alteration in BD.

Keywords: Neuroimaging, Brain injury, Inflammation, Degeneration, Immune system

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NI18 | Analysis of the Immunomodulatory Potential of Mesenchymal Stem Cell-derived Extracellular Vesicles in a Model of Alzheimer's Disease

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AIMS. Evaluation of the role of preconditioned human Bone Marrow Mesenchymal Stem Cells-derived Extracellular Vesicles (hBM-MSC-EVs) as a therapeutic strategy to modulate inflammatory response in in vitro and in vivo AD model. – METHOD. We induced an immunosuppressive hMSC phenotype by preconditioning cells with pro-inflammatory cytokines. Preconditioned hMSC-EVs were then isolated from cell culture medium by ultracentrifugation. In vitro experiments were performed to test EV immunomodulatory effects: primary murine microglia were treated with EVs 2h after administration of an inflammatory challenge and cytokine release was assessed after 48h. 7-month-old 3xTg mice were intranasally injected with 15x109 EVs and sacrificed after 3 weeks for the analysis of cortical and hippocampal microglial phenotype. Golgi Cox was used for the analysis of neuronal dendritic branching. – RESULTS. hMSC-EVs seem to foster, in vitro, microglia M2 phenotype as evidenced by the negative modulation on the pro-inflammatory cytokine IL-6 and the increased release of the anti-inflammatory IL-10. In vivo EV treatment is able to reduce microglial activation and increase dendritic spine density in hippocampal CA1 region of injected 3xTg mice compared to the controls. – CONCLUSION. Preconditioned-hMSC-EVs may represent an exploiting tool to dampen neuroinflammation as well as to restore synaptic integrity in AD.

Keywords: Animal model, Inflammation, Degeneration, Immune system, Stem cells

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NI19 | Coenzyme A metabolism controls pathogenic features in myelin-specific T cells by linking metabolic reprogramming to alteration of intracellular signaling pathways

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The role of Coenzyme A (CoA) metabolism in the development of autoimmune diseases has never been directly investigated. In our study, we evaluated the role of CoA metabolism in the control of autoreactive T-cell pathogenicity by using murine experimental autoimmune encephalomyelitis (EAE) as a model of autoimmune disease. We demonstrated that CoA fueling with pantethine, a CoA precursor, affected, in vitro, essential immune-related processes of autoreactive T-cells such as cell proliferation, cytokine production, and cell motility. Accordingly, our in vivo experiments showed that preventive treatment with pantethine inhibited EAE development and significantly ameliorated the disease course when administered after the disease onset. Notably, by a bioinformatics analysis, we obtained results suggesting a direct role of CoA synthase (CoASY), involved in the CoA synthesis pathway, in the regulation of immune response through effects on MAPK, RAC1 and mTOR signaling pathways. Interestingly, CoASY silencing decreased the inhibitory effect of pantethine on the proliferation rate of autoreactive T cells and enhanced proliferation rate of autoreactive T-cells. In conclusion, we demonstrated a new role of CoA synthesis pathway and CoASY in the regulation of autoreactive T-cell pathogenic features, suggesting CoA fueling may represent a novel therapeutic approach for the treatment of autoimmune diseases.

Keywords: Animal model, Inflammation, Immune system

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NI20 | Role of mitochondria in the activation of neuroinflammation in A53T mice a model of Parkinson's disease

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Recent findings propose mitochondrial dysfunction as a condition that can lead the neuro-inflammatory process associated with Parkinson's disease (PD). Our preliminary results in the animal model of PD, represented by 12 months old A53T transgenic mice, demonstrate that the accumulation of α-synuclein in the striatum and midbrain is accompanied by the degeneration of dopaminergic neurons (TH- positive), by the increase of markers of neuroinflammation (GFAP e IBA-1) and by the changes in the levels of expression of NCX1 and NCX3 in the striatum and midbrain, respectively. These data lead to suppose a mechanism in which the reduced expression of NCX3 in the mesencepahlic neurons, probably associated with the expression of α-synuclein, leads to a mitochondrial dysfunction that in turn causes dopaminergic neuronal loss. This effect might then stimulate the release of pro-inflammatory factors leading to microglia activation in the striatum through a NCX1-dependent mechanism. The present study was addressed to explore mitochondrial function in neurons obtained from mice expressing the human A53T variant of a-synuclein. Experiments were performed in mesencephalic and striatal neurons prepared from 14 days old embryos (E14). Mitochondrial function was monitored with confocal microscopy in order to measure m[Ca2+], mitochondrial membrane potential and mitochondrial free radical production.

Keywords: Inflammation, Degeneration, Neuron-glia communication

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NI21 | Carnosine prevents amyloid-beta-induced inflammation in microglial cells

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Carnosine is a natural dipeptide widely distributed in mammalian tissues and exists at particularly high concentrations in skeletal and cardiac muscles and brain. A growing body of evidence shows that carnosine is involved in many cellular defense mechanisms against oxidative stress, including inhibition of amyloid-beta aggregation, modulation of nitric oxide (NO) metabolism, and scavenging both reactive nitrogen and oxygen species. Microglia exert a dual role in the pathogenesis of Alzheimer's disease; on one hand promoting the clearance of amyloid-beta via phagocytosis, on the other hand increasing neuroinflammation through the secretion of inflammatory mediators and free radicals. The results of our study, by using a well-validated model of amyloid-beta-induced neuroinflammation, showed that carnosine prevents cell death in microglia challenged with amyloid-beta oligomers through a multimodal mechanism of action. Specifically, carnosine lowered oxidative stress decreasing the expression of amyloid-beta-induced enzymes, iNOS and Nox, and the concentrations of both NO and superoxide. In our experimental model, carnosine decreased the secretion of pro-inflammatory cytokines such as IL-1beta and IFN-gamma simultaneously increasing the release of IL-10 and TGF-beta1. These data suggest a novel multimodal mechanism of action of carnosine underlying its protective effects and a therapeutic potential of carnosine against neuroinflammatory processes in Alzheimer's disease.

Keywords: Molecular biology, Inflammation, Degeneration, Immune system, Protein aggregation

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NI22 | Role of CXCL13 in the patophysiology of ALS: study in transgenic SOD1G93A mice

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Growing evidence suggests a prominent role of the immune system in the pathoprogression of Amyotrophic Lateral Sclerosis (ALS), the most common and adult-onset motor neuron (MN) disease. We recently observed that the pro-inflammatory CXCL13 chemokine, a chemoattractant of Th17 cells, was increased in the CNS of ALS SOD1G93A mouse models. This the effect was more pronounced in fast (129Sv-SOD1G93A) than in slow (C57-SOD1G93A) ALS progressing mice, even before symptoms onset. We investigated the effect of intra-cerebro-ventricular administration of a neutralizing monoclonal antibody (mAb) against CXL13 in 129Sv-SOD1G93A mice. Unexpectedly, CXCL13 neutralization accelerated the disease progression in 129Sv-SOD1G93A mice and increased MN death, astrocytosis and muscle denervation compared to control group. Notably, mAb-treated mice exhibited higher CXCL13 protein levels in the CSF, while showing a reduction of chemokine transcript in the spinal cord. These data suggest that mAb-CXCL13 complexes have increased the chemokine half-life without neutralizing, but rather boosting, its pathological activity in the CNS of 129Sv-SOD1G93A mice. Strategies aimed to inhibit CXCL13 production are underway to clarify its role in ALS. However, its differential expression in fast and slow progressing ALS mice suggests that CXCL13 could be a potential biomarker of disease severity useful for ALS patients stratification.

Keywords: Animal model, Inflammation, Biomarkers, Immune system

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NI23 | Differential local tissue permissiveness influences the final fate of GPR17-expressing oligodendrocyte precursors in two distinct models of demyelination

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Multiple sclerosis (MS) is a chronic immune-mediated disease in which inflammation and myelin disruption lead to impaired electrical conduction. Promoting remyelination is recognized as a novel strategy to foster repair in MS. The membrane receptor GPR17 is expressed by oligodendrocyte precursors cells (OPCs), regulates their maturation and, after reaching its highest levels in immature oligodendrocytes, it has to be down-regulated to allow terminal maturation. Here, by means of an inducible reporter mouse line, we followed the fate of GPR17-expressing cells in two distinct models of MS: the experimental autoimmune encephalomyelitis (EAE), characterized by marked inflammation, and the cuprizone-induced demyelination, where myelin dysfunction is achieved by a toxic insult. In both models, demyelination led to a strong local increase of GFP-positive cells. However, whereas in EAE mice, GFP-expressing OPCs were blocked at immature stages, in the cuprizone model they differentiated to mature oligodendrocyte contributing to remyelination. Our data suggest that these distinct fates are caused by different permissiveness of the local CNS environment, such as the presence of proinflammatory factors. For this reason, new potential remyelinating strategies targeting GPR17 should also be combined with anti-inflammatory drugs. – Sponsored by FISM n. 2013/R/1 to MPA and Fondazione Cariplo n. 2014-1207 to DL.

Keywords: Animal model, Inflammation, Remyelination

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NP16 | Pharmacological characterization of NMDA autoreceptors regulating glutamate release in the hippocampus with anti-GluN antibodies: relevance to anti-NMDA receptor autoimmune diseases

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We used selective anti-GluN antibodies recognizing the outer sequence of the receptor proteins to identify the pharmacological profile of the NMDA autoreceptors regulating glutamate release in the hippocampus. We also evaluated whether antibodies can alter the surface expression of NMDA autoreceptors and whether the presence of anti-GluN antibody-antigen complexes in synaptosomal plasmamembranes can enhance complement-induced glutamate release. Mouse hippocampal nerve terminals were pre-incubated with the antibodies and labeled with [3H]D-aspartate ([3H]D-Asp). We compared [3H]D-Asp release evoked by NMDA/glycine or mouse complement in control and antibody-treated synaptosomes. Concomitantly, we investigated the expression of GluN subunit proteins with confocal microscopy and biotinylation/immunoblotting analysis. NMDA-evoked release of [3H]D-Asp is significantly reduced by anti-GluN1 and anti-GluN3A antibodies and almost abolished by anti-GluN2A and anti-GluN2B antibodies. The impaired function of NMDA receptors is linked to an antibody-induced internalization of these receptors. Interestingly, complement-evoked glutamate release from synaptosomes incubated with anti-GluN1 and anti-GluN2B antibody is significantly reduced, when compared to control synaptosomes, and not increased, as hypothesized. Our approach unveils a complex assembly of receptor subunits participating to the composition of native hippocampal NMDA autoreceptors. Our results can add new insights on the molecular events that may occur in patients suffering from anti-GluN autoimmune diseases.

Keywords: Molecular biology

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NP17 | Electrophysiological and biochemical characterization in transgenic mouse model with lack of serotonergic synthesis

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Intact striatal function depends on the dynamic interaction between the dopaminergic and serotonergic signaling systems. 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 L-3,4-dihydroxyphenylalanine (L-Dopa) into dopamine but they cannot manage its reuptake and degradation, bringing to abnormal dopaminergic receptors stimulation and altered glutamatergic transmission. We used a transgenic mouse model displaying lack of 5-HT synthesis (Tph2 -/-), while retaining 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, through whole-cell patch clamp and sharp recordings. To evaluate if the lack of 5-HT impacts on glutamatergic transmission, we examined spontaneous excitatory activity in SPNs. The results showed a lack of LTP and a lower frequency of glutamatergic spontaneous activity in KO mice compared to the other groups, while LTD was normally expressed in all three genotypes. These data suggest that the serotonergic system is necessary for intact striatal function, confirming its role in corticostriatal plasticity.

Keywords: Electrophysiology, Degeneration, Plasticity

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NP18 | Treatment with a beta 2-adrenergic agonist restores dendritic pathology in a mouse model of Down syndrome

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The intellectual disability (ID) that characterizes Down syndrome (DS), a genetic condition due to triplication of chromosome 21, is attributable to impairment of neurogenesis and dendritic development. The goal of this study was to establish whether it is possible to pharmacologically improve brain development in DS by exploiting the Ts65Dn mouse model of DS. We recently screened two libraries of FDA-approved drugs in neural progenitor cells derived from Ts65Dn mice for a drug-repurposing project. We found that the beta 2-adrenergic agonist clenbuterol favored neurogenesis and neuron maturation. Based on this evidence, we daily treated Ts65Dn pups with clenbuterol (10 µg/kg, 0.5 mg/kg, 1 mg/kg or 2 mg/kg) from postnatal day 3 (P3) to P15. We examined the effect of treatment on the hippocampal dentate gyrus, a region fundamental for declarative memory. We found that the lowest dose of clenbuterol was sufficient to fully restore dendritic spine density. The highest dose only, however, was able to moderately improve neurogenesis. This study provides novel evidence that clenbuterol is able to fully restore spinogenesis in the hippocampus of a DS model. Our study suggests that clenbuterol, an antiasthmatic drug used in children, may represent a suitable therapy to correct dendritic pathology in DS.

Keywords: Animal model, Cognitive, Plasticity

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NP19 | HCN1 novel mutations in familiar generalized epilepsy

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels control neuronal excitability and their dysfunction has been linked to epileptogenesis but, since 2014, only five individuals with epileptic encephalopathy bearing de novo HCN1 variants have been reported so far. We describe here clinical, genetic and functional data of novel patients with a mild phenotype of genetic epilepsy with febrile seizures plus spectrum carring de novo variants in HCN1 gene that lead to missense substitutions altering highly conserved amino acids: p.Cys329Ser localized at the channel pore, and p.Val414Met in the C-linker region. Both the probands exhibited infantile- or late infantile-onset febrile tonic-clonic seizures. To determine the functional impact on the biophysical properties of the channel, we performed whole-cell patch-clamp recordings in CHO cells transiently transfected with the homotetrameric form of the channels and found that the two mutations behaved differently. In particular, p.Cys329Ser showed a mild loss of function due to a reduction in the current density while the p.Val414Met a gain of function due to a positive shift in the activation curve. In conclusion, our results suggest that pathogenic variations in HCN1 are not exceptional and illustrate how HCN1 has a pivotal function in the control of neuronal excitability.

Keywords: Electrophysiology

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NP20 | Effects of Bmal1 gene deletion in GLAST positive cells on retinal morphology and physiology

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Mammalian physiological functions are modulated with a circadian rhythm both at cellular and tissue level. Light is the main driver of this rhythmicity modulating a wide range of endogenous clock genes that are present in almost all cell populations. Recent studies by Barca-Mayo et al. have shown that the selective deletion of the clock gene Bmal1 in GLAST positive cells alters locomotor activity and cognition in mice. We wonder whether such a selective deletion in GLAST positive cells of the retina might impinge on retinal function. Interestingly, Storch et al. found that adult mice lacking Bmal1 in all retinal cells modify their retinal information processing. Here, we studied whether conditional deletion in adult life of the gene Bmal1 in astrocytes and Muller cells interferes with retinal function and morphology. We recorded retinal light responses in Bmal1cKO and control subjects both in vivo (flash-Electroretinogram) and ex-vivo (high-density CMOS multi electrode array). In addition, we collected retinal tissue for morphological and immunohistochemical analyses. In our experimental condition preliminary results show no major differences between control and Bmal1cKO, suggesting that Bmal1 deletion in GLAST positive cells does not impact on retinal physiology and morphology.

Keywords: Electrophysiology, Visual perception, Neuron-glia communication

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NP21 | Intergenerational influences of parental Approaching/ Avoiding phenotypes on offspring behaviors in C57BL/6J mice

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Recent studies underline the transmission of phenotypic traits from parents to subsequent generations. Aberrant traits of approach or avoidance (A/A) can constitute a risk to several psychopathologies, however the impact of such parental phenotypes on offspring's development is not been widely investigated yet. The present project aims at investigating the intergenerational influences of maternal and paternal Approaching (AP) / Avoiding (AV) phenotypes on offspring's A/A, novelty response, exploratory behaviors and anxiety levels. Furthermore, the role of (bi)parental care and oxytocinergic modulation upon dopamine-expressing neurons in ventral tegmental area (VTA) is investigating. Using the A/A Y-Maze we categorized AP and AV male and female mice. Selected animals were tested in the Open Field (OF) and the Elevated Plus Maze (EPM) and mated with control mice. Undisturbed observation of (bi)parental care and retrieval test were performed to verify the influence of parental phenotype on parental care. The intergenerational transmission of phenotype was evaluated in F1 by the A/A Y-Maze categorization and in the OF and EPM tests. Finally, the oxytocinergic modulation upon dopamine-expressing VTA neurons in parents and offspring was investigated by immunofluorescence. Results indicate an intergenerational and sex-biased effects of maternal and paternal AP/AV phenotype on offspring.

Keywords: Animal model, Plasticity

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NP22 | Specification of the Drosophila Orcokinin A neurons

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One of the major challenges in the field of Developmental Neurobiology is to understand the basic mechanisms of cell specification. Therefore, the main objective of this project is to advance in our understanding about generation and maintenance of neuronal diversity through the study of the Orcokinin A neuropeptidergic neurons in Drosophila melanogaster. Orcokinin A is mainly expressed in the Central Nervous System (CNS) of both larvae and adults. However, our research focuses on the larval Ventral Nerve Cord (VNC), where one Orcokinin A neuron is born in each abdominal hemineuromere from 1 to 5 segment. First, we have found that these cells are generated at late stage of lineage progression in the Neuroblast 5-3 (NB 5-3) during the castor-grainyhead temporal window. Second, we have found that their correct specification depends on Hox genes input. In particular, Ubx and abd-A appear to be involved in the establishment of the Orcokinin A terminal fate, while Antp and Abd-B seems to trigger a different fate in the segments they govern. Finally, we have found that although the Dpp pathway is active in these neurons, it does not seem to determine the Orcokinin A fate. Additionally, the Notch pathway is inactive in these neurons.

Keywords: Animal model, Stem cells

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NP23 | Chronic enhancement of neuronal activity promotes morphological and functional in vitro maturation of Mecp2 null developing neurons

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Given the timing of Rett Syndrome (RTT) onset, most of the studies so far investigated the role of Mecp2 during adulthood. Recent data, however, demonstrate that early signs of the pathology can be observed well before the onset of typical symptoms. We have reported that the lack of Mecp2 affects different features of early neuronal maturation, including activity. Since activity has an important role in driving neuronal differentiation, we have tested whether the enhancement of neuronal excitability can rescue Mecp2 null neurons maturation. We use differentiated neuroprogenitor cell cultures, obtained from E15 cortices, to follow each phase of neuronal development. We show that Mecp2 null differentiating neurons recapitulate many phenotypes already observed in RTT models such as the transcriptional down-regulation of genes involved in neuronal excitability, morphological impairment, reduced responsiveness to external stimuli and, consequently, defective integration in a developing neuronal network. Interestingly, by slightly inducing neuronal depolarization during differentiation we can rescue parts of these defective phenotypes. Thus, we demonstrate that early functional impairments occurring in Mecp2 null neurons during in vitro differentiation likely concur to the poor neuronal network maturity, a typical feature of RTT patient brain.

Keywords: Plasticity, Stem cells

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NO6 | Modeling immunoediting in glioma progression

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The occurrence of a mutual reshape of tumor cell and immune system during tumor progression is a widely accepted notion in different cancers including gliomas. The importance of this phenomenon in shaping glioma progression and the mechanisms governing it, however, are not fully elucidated. We used a well-characterized glioma model, based on somatic gene transfer of PDGF-B and novel custom image-analysis tools to define the in vivo immune cell composition at different stages of progression. Complementing this, genome-wide transcriptomics on purified tumor cells coherently pointed to the progression-related reorganization of glioma-immune system interactions. We show that the inability of low-grade glioma cells to propagate upon grafting in the brain of syngeneic immunocompetent mice, positively correlates with the abundance of infiltrating CD8+ lymphocytes in donor tumors and correlates with a highly immunostimulatory transcriptional profile. Importantly, during tumor progression glioma cells downregulate these genes and the composition of their immune infiltrate accordingly shifts towards a pro-tumorigenic phenotype. Challenging low-grade, immune-stimulatory, gliomas with grafting into immunodeficient hosts revealed the crucial role of the adaptive immune system in constraining glioma progression. Finally, we observed that although progression still takes place in immunodeficient mice, it is apparently slower, likely due to a far milder selection.

Keywords: Animal model, Immune system, Cancer

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NO7 | Nucleolin expression in the Neurovascular Unit as a potential regulator in glioblastoma neovascularization

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Treatment of glioblastoma is currently one of the most challenging problems in neuro-oncology. Since malignant gliomas are among the most vascularized tumors, one of the most important aspect for the development of malignancy is the interaction of tumor cells with the cells of the neurovascular unit (NVU). We investigated the role of Nucleolin (NCL), a multifunctional phosphoprotein ubiquitously distributed in cells of different tissues and with multiple roles in normal cell growth and metabolism. NCL has been demonstrated to be overexpressed in highly proliferative cells as well as in tumor cells. The analysis of NCL expression in high grade gliomas, compared to normal developing brain, reveals differential subcellular localization. During normal development, NCL shows a nucleoplasm and nucleolar localization in the NVU cells, whereas in tumor cells and in the tumor NVU cells, NCL presents a nucleoplasm expression as well as a specific plasma membrane and cytoplasm localization. NCL could be considered the hallmark of different cell populations that may play different roles in glioblastoma growth and neovascularization, and thus can represent a potential new molecular target for both anti-proliferative and anti-angiogenic therapy.

Keywords: Biomarkers, Cancer

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NO8 | New xenogeneic engraftment assay in immune-competent mouse embryos for Glioblastoma multiforme

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INTRODUCTION. Glioblastoma Multiforme (GBM), a WHO Grade IV glioma arising from glial cells, is a very heterogeneous and infiltrative tumor of the CNS. Therefore, their complete surgical resection is difficult and leads to recurrence and therapy resistance. Limitations of currently used xenograft animal models are absence of immune response, slow growth and lack of invasion. Here, we aim to implement a new xenogeneic GBM engraftment assay in immune-competent mouse embryos. – METHODS. Injection of a single-cell suspension of dsRED-fluorescently labeled human U87MG cells in the lateral ventricle of wildtype mouse embryos. – RESULTS. Injected tumor cells successfully engraft, resulting in extensive tumor growth and integration without negatively influencing the viability of embryos. Number of tumors, their size, location and tumor cell fate choice was assessed up to six days after tumor cell injection. Remarkably, tumors show infiltrative growth pattern, neovascularization and interaction with the host tissue and immune system. – CONCLUSIONS. This model allows to study tumor growth, infiltration and interactions with the host tissue, in vivo. This approach has the potential to rapidly assess patient-specific tumor properties and can be used for gene delivery, drug testing and thus opens new avenues for the screening of personalized therapies to improve GBM treatment.

Keywords: Animal model, Neuroimaging, Cancer

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NO9 | Exploitation of the synergic action of a microRNA pool as differentiation therapy of Glioblastoma Multiforme

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Glioblastoma-multiforme (GBM) is the most fatal type of Gliomas. 70% of GBM patients experience recurrence, which has been related to drug-resistant glioma stem-like cells (GSCs). Therapies that target GSCs might be effective to overcome drug resistance and reduce recurrence. Exploiting mechanism that control differentiation of normal neural stem cells (NSCs) could be an effective “differentiation therapy” to eradicate GSCs and overcome tumour recurrence. MicroRNAs (miRNAs) are small noncoding RNAs regulating gene expression at the post-transcriptional level, that are dysregulated in several pathological conditions, including cancer. We recently identified a “pool” of 11 miRNAs that is necessary and sufficient to drive neuronal differentiation of murine hippocampal adult NSCs, through synergic repressive action on target mRNAs in parallel pathways (Pons-Espinal et al., Stem Cell Reports 2017). The 11 miRNAs of the “pool” and their targets are conserved in human and often dysregulated in GBM. To exploit the potential of the miRNA pool as differentiation therapy of GBM, we administered the 11 miRNAs to human glioblastoma cells (U87mg, grade IV GBM model). Interestingly, the pool inhibits growth of U87mg in 3D spheroid cultures. Identification of mechanisms of action of the “pool” in U87mg cells is in progress.

Keywords: Molecular biology, Stem cells, Cancer

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NO10 | Faraway, so close! Modelling brain cancer in Drosophila

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Despite the obvious differences between humans and flies, the analogies of their nervous systems make Drosophila melanogaster an excellent organism in which to model human brain cancers. Glioblastoma multiforme (GBM) is the most common and incurable brain cancer of the adult. It is characterised by a subset of undifferentiated and highly tumourigenic cells, called GBM stem cells (GSC), responsible for malignancy and relapses. Inactivation of the tumour suppressor gene (TSG) PTEN is frequent in primary GBM, resulting in the inhibition of a second TSG, the polarity determinant lgl, due to an ectopic activity of aPKC. Deregulation of this molecular axis is sufficient to reprogramme human neural progenitors into GSC. First, we have proved that the PTEN/aPKC/Lgl axis is conserved also in Drosophila. Second, we have disrupted this conserved axis in type II neuroblasts, a cell population with a lineage similar to that of the mammalian neural stem cells.We obtained a model of invasive tumour that persists and keeps growing in the adult, leading the animals to premature death. This neurogenic model recapitulates many traits typical of human brain cancers, and may help obtain more information on GBM origin, which has long being discussed.

Keywords: Animal model, Stem cells, Cancer

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ND48 | Axo-glial interplay in oligodendrocyte specification and myelination: role of JNK1

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The C-Jun N-terminal kinase 1 (JNK1) participates in several mechanisms during brain development. Moreover, 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. The somatosensory cortex was reacted with anti-PDGFRalpha antibodies to label oligodendrocyte precursor cells (OPCs) and with anti-myelin binding protein (MBP) antibodies to label the myelinating ones. Immunohistochemical analyses revealed a significant increase in the density of OPCs in KO mice, and a lower MBP expression, indicative of reduced myelination. MBP expression was also altered in the corpus callosum where we found that the nodes of Ranvier labelled by contactin-associated protein 1 displayed a higher density and an increased length in KO mice. With the aim to dissect JNK1-dependent cell autonomous components, we performed in vitro cultures of rat OPCs treated with DJNKi (JNK inhibitor). Immunohistochemical analyses of the Neural/Glial antigen-2 and Ki67 revealed a higher proliferative rate of the OPCs treated with DJNKi and an altered cell morphology. Our findings suggest that JNK1 takes part in oligodendocyte development and in the axo-glial interplay. Further experiments will disentangle the relative contribution of JNK1 in oligodendrocytes or neurons to the observed phenotype.

Keywords: Neuron-glia communication

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ND49 | Ubiquitin-proteasome system is early involved in dying-back presynaptic degeneration induced by NGF-withdrawal in septo-hippocampal neurons

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Dysfunction of Basal Forebrain Cholinergic Neurons (BFCNs), depending on Nerve Growth Factor (NGF) supply for survival/differentiation and innervating the cortical and hippocampal regions involved in memory/learning processes, correlates with cognitive decline in Alzheimer's Disease (AD). Aberration in ubiquitin-proteasome system (UPS) is a pivotal AD hallmark but whether it plays a causative or secondary role in early synaptic failure remains unclear. By taking advantage of newly-developed protocol of cholinergic-enriched septo-hippocampal primary cultures, we have previously reported that impairment of NGF/TrkA signaling triggers an early activation of "dying-back" degenerative processes accompanied by deficit in presynaptic excitatory neurotransmission due to loss of selected vesicles trafficking proteins. Here, we show that the UPS is early stimulated in -6h NGF-deprived BFCNs and that its in vitro pharmacological suppression is able to attenuate the reduction of synapsin I, SNAP-25 and a-synuclein and, then, to rescue the decrease of miniature excitatory post-synaptic currents (mEPSCs) frequency without any change in other pre- and post- synaptic markers. NGF replacement therapy can be beneficial to contrast the early cognitive and synaptic dysfunction of BFCNs occurring in vivo in incipient and mild AD by targeting the UPS-regulated degradation of distinct vesicles trafficking proteins subserving the neurotransmitter release at presynaptic terminals.

Keywords: Molecular biology, Electrophysiology, Degeneration

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ND50 | Characterization of the mitochondrial aerobic metabolism at the pre- and perisynaptic districts of the SOD1G93A mouse model of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease due to the progressive degeneration of cortical, brainstem and spinal motor neurons. Several mechanisms are involved in ALS neurodegeneration, including metabolic dysfunction. Since we found profound alteration of glutamatergic synaptic activity and glutamate release, we investigated here the aerobic metabolism in two specific compartments actively involved in neurotransmission: synaptosomes (model of the presynaptic district) and gliosomes (model of the perisynaptic astrocyte processes), isolated from spinal cord of SOD1G93A mice at different disease stages. Results show that ATP/AMP ratio was lower in synaptosomes from SOD1G93A vs. control mice. The energy impairment was linked to altered oxidative phosphorylation (OxPhos) and increment of lipid peroxidation. These metabolic dysfunctions started at the very pre-symptomatic stage and did not depend on reduced number of mitochondria or different expression of OxPhos proteins. Conversely, gliosomes showed a reduction of the ATP/AMP ratio only at the late stages of the disease and an increment of oxidative stress also in absence of a significant OxPhos activity decrement. These results support the idea that these two districts may differently contribute to the synaptic damage in ALS and that the presynaptic neuronal moiety plays a pivotal role in synaptic energy metabolism dysfunctions.

Keywords: Spinal cord injury, Degeneration

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ND51 | Physiopathology of light exposure in the eye

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INTRODUCTION. Neurodegenerative process shares some common features like their progression in time and space. Age-related macular degeneration (AMD), is a multifactorial disease leading to blindness condition. Degeneration starts from the fovea, the central part of the retina, to expand, in later stages, to periphery. A consolidated model to mimic this pathology in rats is the light damage model, obtained by exposing albino rats, raised at 5 lux, for 24 hours to 1000 lux. In this model degeneration starts from the dorsal side of the retina expanding to the ventral side. Interestingly, following light damage, we observed not only retinal damage, but also corneal damage. – AIM. In this work we follow the progression of corneal damage and photoreceptor degeneration by mean of functional and morphological studies. – RESULTS AND CONCLUSION. We performed morphometric analysis and immunohistochemical techniques in retina and cornea monitoring neuroinflammatory markers, microglia and neuroprotective factors. We functionally followed the progression of neurodegeneration by mean of flash-electroretinograms. Moreover, by mean of patch-clamp recordings from retinal ganglion cells, we highlight the impact that photoreceptor degeneration has on retinal circuitry and therefore on visual output.

Keywords: Electrophysiology, Inflammation, Degeneration, Visual perception

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ND52 | Progress in C9-ALS therapy: patient specific iPSC-derived lines as in vitro model to test Morpholino oligomers efficacy

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Amyotrophic lateral sclerosis (ALS) is a fatal disorder characterized by progressive degeneration of motor neurons (MNs). GGGGCC repeat expansions in C9ORF72 gene are the most common identified genetic cause, even if their pathogenic mechanisms are still unknown. Patient specific induced pluripotent stem cell (iPSC) can provide fundamental insights to understand C9-ALS pathogenesis and to develop an effective therapy. In this study, we reprogrammed iPSCs from C9-ALS patients and differentiated them into affected MNs. First, we characterized the pathological phenotype of the C9-ALS lines compared to controls, evaluating cells survival, RAN products expression, TDP43 inclusion presence, dysregulation of putative RBPs interacting with RNA foci and R-loops formation. Antisense oligonucleotides (ASOs) designed to bind complementary mRNA and interferer with specific biological processes, are now being tested for the human disease treatment. We exploited this strategy also for C9-ALS designing two different ASOs with Morpholino chemistry: against the expansion motif or against the whole gene. We transfected ALS-MNs with Morpholinos and evaluated any modification of the pathological markers previously identified. Results obtained suggest that our in vitro model is a valuable tool to deepen the knowledge of C9ORF72 pathogenesis and to validate promising therapeutic strategies such as Morpholino-mediated approach.

Keywords: Molecular biology, Degeneration, Stem cells

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ND53 | A2a-D2 heterodimers on striatal astrocytes: biochemical and biophysical evidence

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The adenosine A2A and dopamine D2 receptor-receptor interaction (RRI) at the striatal neurons has opened up new perspectives on the molecular mechanisms connected to neuropsychiatric disorders development (e.g. Parkinson's disease, schizophrenia). Considering the glia involvement in neuropsychiatric disease vulnerability, we investigated this RRI on the astrocyte processes prepared from adult rat striatum. Studying glutamate release, we observed the effects of A2A and D2 activation/blockade, the A2A-D2 RRI and interference by the synthetic peptide VLRRRRKRVN (corresponding to the D2 region involved in electrostatic interaction underlying A2A-D2 heterodimerization) or intracytoplasmic homocysteine (inhibiting D2-mediated effect on gliotransmitter release, without interfering with the A2A-mediated antagonism of the D2 effect). We report biochemical and biophysical evidence for A2A-D2 RRI. The co-immunoprecipitation of A2A and D2 receptors in astrocyte processes demonstrated their co-expression on the plasma membrane, while the proximity ligation assay (PLA) detected their strict co-localization on astrocytes. In conclusion, our findings indicate the physical interaction of A2A-D2 receptors and their crucial integrative role at the striatal astrocyte processes. As striatal astrocytes role in the pathophysiology of Parkinson's disease is increasingly recognized, these findings may shed light on the pathogenic mechanisms of this disease and contribute to the development of new drugs for its treatment.

Keywords: Neuron-glia communication

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ND54 | Monitoring of glucose, lactate and motion in brain of freely moving rats by simultaneous telemetry

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In brain, maintaining the membrane potential of neurons, recycling neurotransmitter and axonal-dendritic transport are responsible of most of energy consumption. Glucose is the major source of energy for the mammalian brain under physiological conditions, but addictionally lactate can be used as an alternative bioenergetic substrate. We describe a new approach to simultaneously detect, in real time, extracellular glucose and lactate in brain by use of Enzymatic microelectrode biosensors and a biotelemetric device fixed to the animal's head. Biotelemetry is a technique for real-time transmission and recording of physiological parameters. Our device consists of a dual-channel, single-supply miniature potentiostat-I/V converter, a microcontroller unit, a signal transmitter, and a miniaturized microvibration sensor. The biotelemetry device has been used for accurate transduction of the anodic oxidation currents generated on the surface of implanted glucose and lactate biosensors. Biotelemetric device and Biosensors implanted in the striatum were fixed to the animal's head. Physiological and pharmacological stimulations were given in order to induce neural activation in striatum and to modify the motor behavior in awake, freely moving animals and untethered animals.

Keywords: Animal model, Electrophysiology, Degeneration, Stroke, Neuron-glia communication

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ND55 | Development of new cranial motor neuron differentiation protocol from human iPS cells carrying ALS mutations

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Human induced pluripotent stem cells (iPSCs) are widely used for in vitro disease modeling, offering the possibility to generate disease-relevant cell types, thanks to their pluripotent character. Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease of the motor system, caused by a progressive degeneration of motor neurons (MNs). Not all MN subtypes are equally vulnerable to ALS disease although pathogenic proteins are typically expressed in all subpopulations of MNs. ALS prognosis depends on the site of onset of the first symptoms and the bulbar ALS is the form with the worst prognosis. This form affects primarily cranial motor neurons of the branchiomotor and visceral motor subtype. We have developed a fast and efficient method to convert human iPSCs into cranial motor neurons. Our method is based on stable integration of an inducible vector that allows controlling the activation of Ngn2, Isl1 and Phox2a (NIP), obtaining electrophysiologically mature cells at day 12 of differentiation. Importantly, we have extended our method to iPSCs carrying ALS mutations, thus providing a useful tool to analyse the cellular and molecular bases of motor neuron vulnerability in pathological conditions.

Keywords: Molecular biology, Degeneration, Stem cells

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ND56 | Long non-coding RNAs in Motorneurons differentiation and degeneration

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease leading to motoneuron (MN) death. The identification of mutations in RNA binding proteins (FUS, TDP-43) points to an essential role of abnormal RNA metabolism in ALS neurodegeneration. However, the role of non-coding RNAs in MN degeneration and differentiation is still poorly characterized. My PhD project aims to highlight the biological contribution of the non-coding transcriptome to MN differentiation and in FUS-ALS etiology, specifically focusing on long non-coding RNAs (lncRNAs). We analysed the transcriptome of in vitro differentiated MNs from HB9::GFP mouse embryonic stem cells (mESCs) carrying an aggressive ALS-FUS mutation. RNA-seq data revealed coding and non-coding species altered upon FUS mutation. We identified 19 deregulated lncRNAs in FUS mutant MNs. These candidates were selected for their increased expression along MN differentiation. Among those species, lncMN-2 is one of the most up-regulated in MNs, suggesting a relevant function in these cells. To explore its activity in MN differentiation, we have recently knocked-out lncMN-2 by a CRiSPR-Cas9 strategy. Furthermore, our data show a deregulation of lncMN-2 in FUS mutant MNs, stimulating the investigation of the possible interaction between lncMN-2 and FUS (both WT and mutant) in order to elucidate lncMN-2 role in FUS-ALS context.

Keywords: Molecular biology, Degeneration, Stem cells

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ND57 | Different pro-nerve growth factor protein variants elicit different biological outcome in PC12 cells

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The nerve growth factor (NGF) is a neurotrophin produced as a precursor proNGF. Both proNGF and mature NGF (mNGF) are biologically active. mNGF, challenging the tropomyosin receptor A (TrkA) or to the p75 neurotrophin receptor (p75NTR)-TrkA complex, exerts neurotrophic effects. ProNGF can bind p75NRT/TrkA and/or the p75NRT-Sortilin complex, respectively eliciting neurotrophic and neurotoxic effects. We aimed to characterize the role of the two main murine proNGF variants, proNGF-A and proNGF-B, studying their biological effects on PC12 cells. To this aim, we first characterized the receptors phenotype of the PC12 cells, maintained in different culture conditions and then we studied the effect of proNGF variants on cell viability and differentiation. The presence of all of the possible mNGF/proNGF receptors on cell surface correlated with cell cycle phase and was promoted by serum starvation and/or by pretreatment with mNGF (priming). Both mNGF and proNGF-A promoted cell survival and differentiation in primed PC12 cells, while proNGF-B displayed neurotoxic properties. The neurotrophic effects of mNGF and proNGF-A were TrkA-dependent, while the blockade of p75NRT counteracted or even reverted the effects of proNGF-B. Our results suggest that proNGF-B and mNGF/proNGF-A preferentially challenge different receptor complexes, eliciting specific and opposite biological effects in PC12 cells.

Keywords: Biomechanics

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ND58 | MPTP-Induced changes in behavioral test scores and extracellular levels in the striatum of freely moving mice

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Parkinson disease (PD) is a neurodegenerative disorder characterized by the death of dopaminergic neurons of the substantia nigra pars compacta. The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is often used to induce an animal model of PD. MTPT was administered to a group of freely moving mice with the aim to underline if the behavioral changes are related with the MPTP-induced variations in dialysate dopamine (DA) concentration. The behaviour of mice was detected with open field, ethogran, grid and swimming tests. Mice underwent to stereotaxic surgery with the implantation of a microdialysis probe in the striatum: the DA concentrations were estimated by HPLC. The first dose of MPTP induced an increase in DA concentrations. Dose after dose we observed a reduction in DA baseline in comparison with control mice with a trend to recovery after the end of the treatment. During the MPTP administration, the behavioral tests showed a worsening, while 7 days after the end of the treatment open field and ethogran tests showed a completely recovery while grid and swimming tests demonstrated a variable recovery. Behavioral tests are reliable tools in for vivo evaluation of MPTP-induced neurochimical damage mainly because of the correlation between their scores and DA concentrations in dialysates.

Keywords: Animal model, Electrophysiology, Brain injury, Degeneration, Neuron-glia communication

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ND59 | Neuroprotective effects of stray-dried rosmarinus officinalis powder extract in OGD-injured human neuronal-like cells

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Rosmarinus officinalis, known as Rosemary, is an aromatic evergreen shrub, originated from the Mediterranean region, whose extract (RE) is rich of several biologically active compounds showing positive effects including general antioxidant properties due to phenolic phytochemicals and beneficial synergistic cooperation of all molecules content. Neuroprotective effects have also been attributed to RE, even if the molecular mechanism is still unknown. Onset and progression of homeostatic imbalances observed in the development of various neurodegenerative diseases, has been associated with a GJ-dependent increased membrane permeability and a number of Connexin (Cx), including Cx43, alterations are differently involved. Here we studied in vitro RE effects on cell survival on SH-SY5Y and A-172 cell lines in an oxygen glucose deprivation injury model. The RE influence on Cx43 levels was also investigated and compared to ioxynil octanoato, a selective Cx43-GJs inhibitor. Our results showed that RE exerts a protective action increasing cell viability and metabolic turnover. Moreover, it reduced the Cx43-based cell coupling of injured cells suggesting that this effect may be the molecular basis for its beneficial effects to be exploited for preventive treatment against the risk of some neurodegenerative disorders.

Keywords: Biomarkers, Degeneration, Neuron-glia communication

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ND60 | Protective effect of Genistein-loaded transferosomes against H2O2-induced oxidative stress in PC12 cells

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Oxidative stress is a condition determined by an excessive amount of reactive oxygen species (ROS). Overproduction of ROS has been regarded as an important factor characterizing different neurodegenerative disorders including Parkinson's disease. The experiments were performed in PC12 cells, a neuronal model, used to evaluate the potential antioxidant properties of Genistein (GEN), the predominant component in soy products. Four formulations of Genistein-loaded transferosomes (GEN-TFs) were used as drug delivery systems in PC12 cells exposed to oxidative stress induced by hydrogen peroxide (H2O2) for 24h. The preliminary results obtained by MTT and LDH assay showed that the cytotoxic effect of H2O2 was significantly attenuated by GEN-TF2 administration, underlining its possible protective activity against oxidative damage. In addition, the fluorescent GENT-TF2 uptake was evaluated and confirmed by flow cytometry in PC12 cells. Subsequently it has been shown, by Dichlorofluorescein-diacetate assay, GEN-TF2 was able to decrease the ROS amount in presence of oxidative damage. Moreover, the apoptotic cell percentage was significantly reduced in the samples treated with GEN-TF2, confirming its protective effect. In conclusion, these obtained data assigned to GEN-TF2, drug delivery system, a potential antioxidant activity and its possible application as adjuvant therapy in oxidative stress-related neurodegenerative diseases, such as Parkinson.

Keywords: Brain injury, Ageing, Degeneration

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ND61 | Innovative 3D cellular model for the study of neurodegenerative diseases

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Neurodegeneration is one of the main causes of disability and death afflicting over 50 million people worldwide. The importance of biological models is still fundamental for research on neurodegenerative diseases, but they do not completely recapitulate what happens in the real tissue. With this work we want to create a complex 3D cell culture to obtain tissue-like conditions. We selected two biomaterials to perform 3D in vitro model, alginate and gelatin, both natural materials. Alginate is suitable for maintain 3D structure after ionic crosslink with calcium chloride, and gelatin is a good material for cells' viability sustainment. We tested the biocompatibility of hydrogels with many concentrations of alginate and gelatin with HeLa and SH-SY5Y cell lines. We observed also the increase in viability with increasing concentration of alginate and gelatin. Afterwards, the bioinks were tested with a 3D bioplotter, Cellink INKREDIBLE+, that allows to deposit the materials in a defined 3D space and does not affect cells viability. Our results show the possibility to obtain a 3D tissue in an in vitro model, maintaining many of the characteristics of the real tissue, in order to obtain an innovative and realistic cell culture for neurodegeneration modelling.

Keywords: Molecular biology, Biomarkers, Degeneration

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ND62 | Mitophagy impairment in a peripheral model of ALS

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Mitochondria impairment is widely considered an important issue in Amyotrophic Lateral Sclerosis (ALS) and morphological alterations of these organelles can be readily found in tissues from patients and animal models, while no data are reported in Peripheral Blood Mononuclear Cells (PBMCs), a good peripheral model of the disease. Thus, aim of this work is to study mitochondrial dynamism, caspase-dependent apoptosis and mitophagy in PBMCs of sporadic ALS (sALS) patients. In our model, TEM analysis evidenced the presence of atypical mitochondria, confirmed using MitoTracker staining. Surprisingly, we did not find any significant change in proteins involved in mitochondrial dynamism and in caspase-dependent apoptosis, either in RT-qPCR and WB analysis. Interestingly, we observed a significant increase in LC3-II/LC3-I ratio and in PINK1 levels in PBMCs of sALS patients compared to CTRL. These data suggested us an accumulation of damaged mitochondria, confirmed by flow cytometry analysis and by co-localization of PINK1 and LC3 by immunofluorescence microscopy. Our data may indicate that in patients' PBMCs the impairment of mitophagy pathway lead to an inefficient turnover, resulting in the accumulation of altered and unfunctional mitochondria. As suggested by other works, the presence of damaged mitochondria could contribute to cells' degeneration in ALS patients.

Keywords: Molecular biology, Degeneration

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ND63 | Transcriptome and proteome analysis of FXS patient-derived iPSC lines to investigate FMRP role and its interactors during neural development

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Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability, caused by the silencing of the fragile X mental retardation 1 (FMR1) gene. This mutation leads to the loss of fragile X mental retardation protein (FMRP), which plays a crucial role in neuronal development controlling specific mRNAs translation. Patient-specific induced Pluripotent Stem Cells (iPSCs) can be converted in neural precursor cells (NPCs) and neurons, offering the chance to study cell populations affected by neurological disorders. IPSC carrying FMR1 mutation can be differentiated towards a neural fate and used as an in vitro model system to study FXS. Our project aims at better understanding the FMRP network, using different FXS patient iPSC lines and mutant FMRP lines derived by CRISPR/Cas9 gene editing and their isogenic controls. We will perform RNA-seq on control and mutant iPSC-derived neurons and, in parallel, FMRP CLIP-seq analysis in control iPSC-derived neurons, in order to identify those mRNAs that are directly targeted. This analysis will allow to understand how FMRP loss of expression in iPSC-derived neurons have an impact on targets involved in FXS phenotype. Our final goal is to identify candidate mRNAs deregulated by FMRP and to elucidate their functional role in FXS.

Keywords: Molecular biology, Degeneration, Stem cells

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ND64 | Lysosomal function and dysfunction in astroglia

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Astrocytes engulf and degrade dead cells and protein aggregates via the lysosomal pathway and dysregulation of astrocyte functions is involved in neurodegenerative diseases (NDs). To uncover novel physiological mechanisms in astrocytes and understand how lysosomal dysfunction is linked to NDs, we investigated the role of Leucine rich repeat kinase 2 (LRRK2) in astrocytes. LRRK2 is a kinase that impinges on the lysosomal pathway in different tissues and alteration of LRRK2 kinase activity is associated with Parkinson's disease, a common ND. LRRK2 is expressed in mouse striatal astrocytes and Lrrk2 deficiency affects the morphology of these cells at the ultrastructural level in brain slices. Interestingly, morphological changes in the lysosomal pathway reflect on a reduced ability of lysosome to refill Ca2+ in Lrrk2-/- primary astrocytes. Since impairment in the lysosomal Ca2+ impacts general Ca2+ homeostasis, we assessed whether LRRK2 plays a role in the regulation of Ca2+ handling in astrocytes in brain slices. Our results shows that astrocyte Ca2+ response is significantly altered in Lrrk2-/- mice. We also observed exaggerated lysosomal Ca2+ signals in primary astrocytes carrying the pathogenic mutation. Overall, our findings support future research aimed at understanding the mechanisms behind LRRK2-linked astrocyte function and dysfunction.

Keywords: Animal model, Ageing, Degeneration, Protein aggregation, Neuron-glia communication

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ND65 | Autophagy enhancement rescues neurons from toxicity induced by amyloidogenic peptides

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SNC amyloidosis, including Alzheimer's, Parkinson's and Huntington's diseases, share with prion disease protein misfolding process, that starts amyloidogenic path and generates soluble oligomers to which are ascribed neurotoxic and proinflammatory properties. Among the neurotoxicity mechanism, alteration of proteostasis represents an intriguing hypotesis and a promising target for innovative unifying therapy. This work arises from the observation that protease-resistant fragment of prion fragment (PrP90-231) produces neuronal death through internalization as insoluble, protease-resistant aggregates which localized into acidophilyc structures with features of lysosomes and autophagolysosomes. Given the importance of the autophagy in proteostasis manteinance we sought to understand if PrP90-231 detection into autophagolysosomes reveals a neurotoxic mechanism or a protective reactive strategy of neurons. We observed that PrP90-231 causes mesencephalic neurons death throught lysosomal impairment, cytosolic diffusion of hydrolitic enzymes and mitochondrial damage. Analysis by immunoblotting and TEM of autophagic flux activity showed that PrP90-231 produces enhancement of lysosomes and autopagholysosomes being the latter filled with undigested material. Increasing autophagy by pharmacological blockade of mTOR reduced neuronal sensitivity to PrP90-231 and the amount of peptide within autophagolysosomes. We conclude that restoring proteostatic ability of neurons may be effective in inhibiting neurotoxicity related to amyloid oligomers.

Keywords: Degeneration, Imaging, Protein aggregation

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ND66 | Neurotoxic impacts of neonicotinoid insecticides in a mouse model

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Neonicotinoids are nicotine-derived insecticides used worldwide for pest management. Recent in vitro studies showed neonicotinoids ability to bind mammalian nicotinic acetylcholine receptors (nAChRs). Considering the large exposure to these insecticides and the key role of nAChRs in the nervous system development, more studies are needed to disclose possible neurotoxic effects. This project was aimed at investigating the neonicotinoids effects in primary neuronal cultures and brains of prenatal exposed-mice. To provide relevance for human exposure, the determination of the experimental doses was based on the estimated human exposure or the acceptable daily intake set by regulatory agencies. Our findings show that the exposure to low levels of neonicotinoids might induce impairments during critical phases of neuro/immune development. In detail, neonicotinoids affected the neuron viability in vitro down by nanomolar concentration and impaired both synaptic markers (synaptophysin) and dendritic arborization. Imidacloprid (at micromolar concentration) was also able to reduce LPS-induced production of TNFalpha in cultured microglia, exhibiting immunosuppressive effects. The results of ex vivo investigations on brains from newborn mice in utero-exposed to imidacloprid confirmed the in vitro data. These data can pave the way for a proper risk assessment and for a best regulation of neonicotinoids use preventing potentially pollution-related diseases.

Keywords: Molecular biology, Inflammation, Degeneration

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ND67 | Oleuropein aglycone stabilizes the monomeric α-synuclein and favours the growth of non-toxic aggregates

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α-synuclein plays a key role in the pathogenesis of Parkinson's disease (PD); its deposits are found as amyloid fibrils in Lewy bodies and Lewy neurites, the histopathological hallmarks of PD. Amyloid fibrillation is a progressive mechanism proceeding through the transient formation of oligomeric intermediates widely considered as the most toxic species. Consequently, a promising approach of intervention against PD might be preventing α-synuclein build-up and aggregation. A possible strategy involves the use of small molecules able to slow down the aggregation processor to alter oligomers conformation favouring a non-pathogenic one. Here, we show that oleuropein aglycone (OleA), the main Extra Virgin Olive Oil (EVOO) polyphenol, exhibits anti-amyloidogenic power in vitro by interacting with α-synuclein monomers thus hindering the growth of on-pathway oligomers. We investigated the molecular basis of such interference by biophysical techniques together with limited proteolysis assay; aggregate morphology was monitored by electron microscopy. We also found that OleA reduces the cytotoxicity of α-synuclein aggregates by hindering their binding to cell membrane components and preventing the resulting in oxidative cellular damages. Finally, we found that OleA stabilizes monomeric α-synuclein and favours the growth of stable non-toxic aggregates with no tendency to evolve into other amyloids.

Keywords: Molecular biology, Degeneration, Protein aggregation

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ND68 | Development and characterization of a new electrochemical sensor for in vitro study of dopamine auto-oxidation

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Oxidative stress, caused by reactive oxygen species, (ROS) is responsible of the dopaminergic neuronal death at nigro-striatal level, as it occurs in Parkinson's Disease (PD). ROS are responsible of the non-enzymatic oxidation of Dopamine (auto-oxidation). In this study it was developed a novel electrochemical device able to monitor the auto-oxidation of DA, induced by hydrogen peroxide (HP). The system consists of an electrochemical cell containing a buffer solution at ph 7,4 able to reproducing the cerebral extracellular environment, an Ag/AgCl pseudoreference electrode, an auxiliary electrode, two working electrodes, one made with epoxy/carbon and the other one with platinum/iridium. Both sensors were coated with a polimer (poly-dopamine). The developed system allowed the oxidation of DA and HP at the platinum surface while the only DA was oxidized at carbon surface. The polymerization was able to shield the interfering molecule such as ascorbic acid (AA). This relative selectivity of the electrochemical transducers allowed the discrimination between DA and HP and recording their changes in the homogeneous phase by constant potential amperometry. The device has been used for studying the time curse of DA auto-oxidation in the extracellular compartment of PC12 cells exposed to HP and the protective properties of several antioxidants.

Keywords: Animal model, Electrophysiology, Degeneration, Neuron-glia communication

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ND69 | Novel models of stretch-induced injury in mouse oligodendrocytes and organotypic culture of cerebellar slices: study of pathophysiological mechanisms

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Mechanical strain applied to the brain tissue occurs during development and following traumatic brain injury (TBI). Understanding how the mechanical forces lead to tissue damage and particularly to myelin breakdown and reactive oxygen species (ROS) production remains a considerable challenge. We hypothesized that stretch-induced injury can initiate oligodendroglial damage and demyelination and we aimed to decipher the cellular and molecular responses of oligodendrocytes when subjected to mild (20% strain) and moderate (30% strain) injury. In oligodendrocyte-enriched primary culture, mild injury reduced cell surface area and caused cell loss in both mature and immature oligodendrocytes. The observed damage was more pronounced after moderate injury. In 158N oligodendroglial cell culture, moderate injury markedly reduced the amount of myelin protein PLP. Both mild and moderate injury resulted in an increased production of reactive oxygen species accompanied by protein oxidation and strain-dependant alteration of anti-oxidant defence. Cerebellar slices were subjected only to a “moderate” stretch, showing an alteration in the expression of myelin genes and proteins. In conclusion, this study suggests that mechanical stretch causes loss of oligodendrocytes and demyelination after injury.

Keywords: Molecular biology, Brain injury, Biomechanics

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ND70 | Evaluation of immune system status in a SMA murine model

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Spinal muscular atrophy (SMA) represents the most common genetic cause of infant death: it is a neurodegenerative disease characterized by motor neuron degeneration and muscle atrophy. SMA is due to the deletion or mutation of the telomeric survival motor neuron 1 gene (SMN1): its homologous, SMN2 gene, encodes a truncated protein which can modulate SMA severity. Beside the classical “neuron-centric” view, it is now clear that SMA pathogenesis is more complex than previously retained. Interestingly recent works also reported immune organ defects in SMA murine models, that probably play a role in the disease onset/progression. The purpose of our work is to analyze the immune system status of SMA delta 7 pups (a SMA murine model) through histological and functional/molecular analysis. According to the literature, our preliminary data, confirm that SMA spleen size is significantly reduced compared to WT at 0, 1, 5 and 12 postnatal days. Furthermore spleen architecture appears altered. We also counted the white blood cells (WBC) at 0, 1, 5, 7, 8 and 12 postnatal days: at all the time points the WBC number is significantly reduced in SMA. These results suggest that immune system alterations could contribute to SMA pathogenesis.

Keywords: Brain injury, Degeneration, Immune system

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ND71 | II3NeuAc-Gg4: a new neurotrophic player

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GM1 ganglioside (II3Neu5Ac-Gg4Cer) has been considered a master regulator of the nervous system due to its neurotrophic and neuroprotective function in vitro and in vivo. GM1 is essential in maintaining a healthy nervous system as observed in the consequence of its genetic deletion, and its therapeutic potential appears consonant with its neuroprotective role, as shown by running preclinical-clinical studies. Unlucky, the molecular mechanisms behind these processes are largely unknown. To clarify this point we decide to investigate the importance of its oligosaccharide portion (II3Neu5Ac-Gg4). Here, we pointed out a specific role of II3Neu5Ac-Gg4 for important neurotrophic function of GM1. In murine neuroblastoma cell line, we found that II3Neu5Ac-Gg4 interact directly with TrkA-NGF complexes, leading to MAPK cascade activation. This is followed by neurodifferentiation and upregulation of several biochemical mechanisms with neuroprotective potential. Using mouse primary neurons, we highlighted that II3Neu5Ac-Gg4 is able to influence the differentiation processes by accelerating the neuronal differentiation from both morphological and biochemical point by activating TrkA-MAPK signaling. We surmise that the neurotrophic effect of GM1 is due to a direct interaction between II3Neu5Ac-Gg4 and TrkA at the PM level. Trying to define the molecular mechanism of GM1, we have potentially found a new neurotrophic player.

Keywords: Brain injury, Spinal cord injury, Degeneration, Regeneration, Plasticity

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ND72 | Early deficits of cerebellar plasticity in a mouse model of Alzheimer's disease

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In this study was examined the cerebellar involvement in a mice model of Alzheimer's disease (AD) at the pre-plaque stage. To this aim, behavioral, electrophysiological, and molecular investigations were carried out in TgCRND8 mice at 2-months of age, before plaque formation. Foot-printing analysis and balance beam test revealed that TgCRND8 mice had a significant motor balance and coordination impairments respect to their littermates. The electrophysiological recordings, using the patch-clamp technique on cerebellar slices, showed an alteration of long-term depression at the parallel fiber – Purkinje cell (PF-PC) synapse, considered the critical cellular mechanism for motor learning. We also showed alteration of the noradrenergic modulation of the PF-PC synapse in Tg mice compared to wild-type mice. Finally, western blot analysis on cerebellar lysates of 2-months old TgCRND8 mice showed an enhanced activation of NADPH oxidase, that leads to the increase of reactive oxygen species. Taken together these results suggest that the cerebellum is affected in the early stage of AD.

Keywords: Molecular biology, Electrophysiology, Ageing

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ND73 | Amyloid and prion-copper complexes: redox reactivity in membrane-like environment

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The dyshomeostasis of metals and misfolded proteins contribute to neurodegeneration. Transition metals and radicals are not only factors contributing to protein aggregation but also the interaction between membrane and amyloidogenic protein may play an important role. The presence of membranes can affect the protein structure and the redox activity of the metal-peptide complexes, by influencing the availability of copper binding sites. It is known that alpha-synuclein, beta-amyloid and prions strongly interact with neurolipids. Therefore, we have studied the catechol oxidation in SDS micelles catalysed by copper and the main fragments of beta-amyloid/prion implicated in metal binding. Prions and beta-amyloid exhibit very different behavior when added to model membrane systems: in presence of SDS the reactivity of [copper-prion] towards catechols is significantly quenched, as observed with alpha-synuclein. Conversely, the interaction between SDS and [copper-beta-amyloid] slows only the reaction rate, not resulting in the shutdown of substrate oxidation. Moreover, neuronal proteins can be subject to oxidative modifications, improving the misfolding and aggregation. We have investigated how metals and catechols can induce peptide oxidation and how the membrane can influence the pattern of modifications.

Keywords: Ageing, Degeneration, Protein aggregation

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ND74 | Oleuropein Aglycone and its metabolite Hydroxytyrosol interfere differently with toxic Aβ1-42 aggregation

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Several data highlight the role played by phenolic components of extra virgin olive oil, particularly oleuropein aglycone (OleA) and its main metabolite hydroxytyrosol (HT), against amyloid aggregation. In this sense, particular emphasis has been given to Aβ1-42 aggregation path involved in the onset and progression of Alzheimer's disease (AD). Drug discovery efforts are focused at preventing the formation of toxic aggregates and/or at favouring their disaggregation. Recent data have shown that OleA interferes with some proteins aggregation path (b2-microglobulin, tau and transthyretin). However, there are limited studies exploring the molecular level of their interaction to Aβ1-42 preventing fibril formation. So, we sought to elucidate the molecular and cellular determinants of OleA and HT protection against protein aggregation and/or aggregates cytotoxicity by a set of in vitro experiments carried out using biophysical analysis and cell biology techniques. Our results besides confirming previous data on OleA/Aβ1-42 aggregation, highlight a modulation of the molecular mechanism of HT/Aβ1-42 aggregation; in particular, HT was found to accelerate the protein aggregation thus skipping the appearance of toxic oligomers. Our data offer the possibility to validate and optimize the use of OleA and/or HT to rationally design novel drugs for possible use in AD prevention and therapy.

Keywords: Molecular biology, Imaging, Protein aggregation, Biophysics

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ND75 | Neuronal proteins and 3-hydroxykynurenine: implications in neurodegenerative diseases

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Studies have shown that dysregulation of kynurenine pathway, the main catabolic pathway for tryptophan, is strongly associated with neurodegenerative processes in Parkinson's and Alzheimer's diseases. The aminophenol 3-hydroxykynurenine (3OHKyn) is a neurotoxic metabolite of kynurenine that is found throughout the tissues of the human body and is able to cross the blood-brain barrier thus penetrating the brain. Little is currently known on the dangerous effects of oxidative stress (also related to metal ions excess) and the simultaneous presence of kynurenine metabolites in neural environment, but the documented reactivity of 3OHKyn towards nucleophilic amino acid residues in proteins may result in the alteration of physical and chemical properties of the proteins (eg through cross-linking, fragmentation, oxidation, peroxide formation, unfolding and altered solubility). Therefore, considering the likely involvement of kynurenine metabolites in neurodegenerative disorders and the lacking in literature of data reporting their interaction with neuronal proteins, we have studied the reactivity of 3OHKyn towards alpha-synuclein and amyloid-beta peptide fragments. In order to identify the modification sites and analyze the impact of derivatization by kynurenine metabolites on protein physical and chemical properties, these adducts have been characterized through different spectrometric (LC-MS/MS) and spectroscopic (UV-Vis, fluorescence, CD, NMR) techniques.

Keywords: Degeneration

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ND76 | Coordination of non-heme iron to a fragment of alfa-synuclein C-terminus and implication in oxidative stress

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Alpha-synuclein is one of the most abundant brain protein. It is implied in the pre-synaptic vesicles recycle, in cellular membrane binding and in dopamine metabolism. Its native sequence (140 amino acids) can be divided in three different regions. Residues 1 to 60 configure the N terminus of the protein, which contains KTKEGV repetitions. Amino acids 61 to 95 represent the non–amyloid-beta component (NAC), while residues from 96 to 140 configure the C-terminus. This last region has an high concentration of negative charged residues, which makes it possibly implicated in iron(II)/iron(III) coordination. To have further insights regarding the implication of iron-synuclein complexes in catecholamine metabolism, a representative sequence of C-terminus has been synthesized (Ac-119DPDNEAYEMPSEEG132-NH2). It contains the Tyr125 and Ser129 residues, whose phosphorylation seems to have a crucial role in iron coordination. Preliminary studies on iron binding and catalytic oxidation of catecholamines have been performed. Future perspectives are directed toward the synthesis of the phosphorylated sequences, to evaluate the effect of increased affinity on catalysis.

Keywords: Degeneration

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ND77 | Modulation of intracellular Ca2+ concentration in brain microvascular endothelial cells actively induced by brain targeted liposomes

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AIMS. The aim of our study is to evaluate the interaction at the neurovascular unit of liposomes (mApoE-PA-LIP) functionalized with ApoE-derived peptide (mApoE) and phosphatidic acid (PA). In light of our previous results (Re et al.,2010), we assess mApoE-PA-LIP activities on human cerebral microvascular cells (hCMEC/D3) as an in vitro human BBB model. – METHODS. The intracellular Ca2+ concentration was measured by digital imaging microscopy in hCMEC/D3 maintained in a low-profile chamber in presence of PSS solution (NaCl 150 mM; KCl 6 mM; MgCl2 1mM; CaCl2 1.5mM; HEPES 10mM; Glucose 10mM). We pre-incubated hCMEC/D3 cells with 4µm acetoxy-methyl-ester Fura-2 AM for 30 minutes at 37°C. Afterwards, we perfused the cells with mApoE-LIP or mApoE-PA-LIP (in PSS) to evaluate the ATP (50µM) evoked calcium waves. – RESULTS. The interaction of mApoE-PA-LIP with the hCMEC/D3 actively induced a modulation in the duration of the ATP induced calcium waves. We found an increase (mean ± se, 136 ± 3.75 sec, n=52, p-value <0.05) of the duration of the ATP evoked calcium waves in presence of mApoE-PA-LIP in comparison to mApoE-LIP perfusion (mean ± se, 125 ± 1.95 sec, n=52). mApoE-LIP and "PSS alone" do not prolong ATP evoked calcium waves. – CONCLUSIONS. Our data confirm that the specific liposome functionalization with phosphatidic acid may be linked to the enhanced calcium waves evoked in hCMEC/D3 by ATP. This finding suggests an intriguing issue involving PA intracellular pathways and its possible implications in the modulation of calcium waves duration in hCMEC/D3 cells.

Keywords: Nanomaterials/nanoparticles, Degeneration, Imaging

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ND78 | Electrophysiological, molecular and behavioral effects of Transcranial Magnetic Stimulation (TMS) in the early model of Parkinson

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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, though the long-term use of this dopamine precursor is complicated by highly disabling fluctuations and dyskinesias. In this context, Repetitive Transcranial Magnetic Stimulation (rTMS), a non-invasive neuromodulatory technique, can be used as a tool to explore cerebral reorganization and promote functional recovery in disease conditions characterized by aberrant forms of synaptic plasticity. In order to evaluate the effects of TMS in the treatment of PD symptoms at an early stage, we used partially 6-OHDA-lesioned rats. Intracellular recordings from cortical striatal slices obtained from these animals show that a single session of TMS is able to induce a recovery of plasticity, while the molecular analysis of postsynaptic density exhibits a significant reduction of GluN2B subunit-containing NMDA receptor. The behavioral data associated with these results include an improvement of the akinesia's contralateral limb and the amelioration of gait. In conclusion, the modifications induced by an acute treatment could represent a useful paradigm to exploit the great therapeutic potential of TMS.

Keywords: Electrophysiology, Degeneration, Plasticity

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ND79 | Metabotropic glutamate receptor type 5 effects on ALS progression

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by motor neuron (MN) death, whose aetiologyis not clear, although glutamate(Glu)-mediated excitotoxicity represents one major cause. 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 knocking-down mGluR1 in SOD1G93A mice significantly prolongs survival and ameliorates disease progression. To study the function of mGluR5 in ALS, we investigated the effects of the genetic down-regulation of mGluR5 (SOD1G93AmGluR5+/-) or its ablation (SOD-1G93AmGluR5-/-) in SOD1G93A mice. SOD1G93AmGluR5+/-mice showed delayed disease onset and prolonged survival probability, accompanied by spinal motoneuron preservation, decreased astrocyte and microglia activation, and normalization of the excessive cytosolic [Ca2+]I and Glu release. Unexpectedly, motor skills were improved in male SOD1G93AmGluR5+/- mice only. SOD1G93AmGluR5-/- mice presented a more evident amelioration of all disease features, including motor skills, both in males and females. These results represent a proof of concept supporting the idea that mGluR5 represents a useful target for promising pharmacological treatment in ALS.

Keywords: Animal model, Degeneration

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ND80 | TDP-43 and R loops relation in Amyotrophic Lateral Sclerosis

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R loops are three-stranded nucleic acid structures composed by a RNA-DNA hybrid and displaced single-stranded DNA which accumulation can induce genomic instability in several neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). The prevention or repair of R loop associated DNA damage is mediated by TDP-43, that once mutated is involved in ALS through formation of cytoplasmic aggregates containing inert RNA and associated proteins, called stress granules. R loops presence was measured in lymphoblastoid cell lines (LCLs) derived from a mutated TDP-43 ALS patient (mutTDP43), a sporadic ALS (sALS) patient and a healthy control (Ctrl) by flow cytometry, revealing their significant accumulation in mutTDP43 LCLs. Co-localization of R loops with TDP-43 and stress granules was investigated by immunofluorescence, showing a strong segregation of R loops with TDP43 and stress granules in the perinuclear area of mutTDP43 LCLs. Co-immunoprecipitation (Co-IP) confirmed the obtained data, demonstrating relevant TDP-43 and R loops interaction in whole lysate of mutTDP43 LCLs in comparison with chromatin fraction. We can hypothesize from the obtained data that translocation of mutated TDP-43 in cytoplasmic cellular compartment can lead to co-localization with R-loops and segregation in cytoplasmic stress granules.

Keywords: Molecular biology, Degeneration, Protein aggregation

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ND81 | Caenorhabditis elegans as simplified animal model to elucidate the molecular mechanisms underlying the propagation of tau pathology in traumatic brain injury

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Traumatic brain injury (TBI), a risk factor for Alzheimer's disease and chronic traumatic encephalopathy, induces a tau pathology that spreads in the brain in a prion-like manner, causing functional and histophatological abnormalities. Using a well established pre-clinical TBI mouse model, we have demonstrated that tau in its phosphorylated form, propagates from the site of injury to remote brain regions and that can be transmitted to naïve mice by intracerebral inoculation. To dissect the molecular mechanisms underlying in vivo the spreading and toxicity of abnormal tau protein produced after brain injury, we employed the nematode *Caenorhabditis elegans.* This worm has a short lifespan and many overlapping proteins to humans. As such, it has the potential to serve as a research model from which large volumes of data can be produced in a short space of time. We observed that homogenates prepared from brains of TBI-injured mice induced functional deficits in *C. elegans* accompanied by specific neuromuscular synaptic damage. Similar results were obtained in nematodes exposed to brain homogenates from transgenic mice overexpressing mutant human tau P301L, arguing a direct role of misfolded/aggregated tau in driving the toxic effects. These findings indicated that *C. elegans* can be used as tractable model to establish a causal relationship between misfolded/aggregated tau and neuronal dysfunction. This nematode may also represents a novel platform to screen candidate agents able to modify the prion-like properties of phosphorylated-tau and impair tau progression.

Keywords: C. elegans, phosphorylated tau, traumatic brain injury, prion

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NI24 | 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 (IR), type 2 diabetes (T2D), obesity and Alzheimer's Disease (AD). Recent studies have shown that diet-induced changes in insulin sensitivity contribute to alterations in brain insulin signaling and cognitive functions. In fact, IR could be the common pathogenetic mechanism underlying AD and T2D, affecting glucose metabolism and utilization in different organs including the brain. Multiple factors, determined endogenously as well as environmentally contribute to the risks of AD and T2D. It is important to identify the underlying molecular mechanisms for designing novel therapeutic strategies. The aim of this study is to understand, by using in vivo and in vitro models, the molecular mechanisms leading to cognitive impairment associated with metabolic disorders. PET and optical in vivo imaging data, analysing glucose metabolism and inflammation, will be presented on an animal model on high fat diet, which shows glucose and insulin intolerance and increased anxiety. In addition, ex vivo brain tissues analyses investigating synaptic alterations and stress pathways will be shown. Furthermore, in vitro data on neural cell models treated with palmitate will be presented to recapitulate the in vivo condition of elevated free fatty acids typically accompanying IR and T2D.

Keywords: Molecular biology, Animal model, Cognitive, Inflammation, Biomarkers

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NI25 | Characterization of a novel CAPS Knock-in mouse model to exploit novel approaches for the modulation of the NLRP3 inflammasome

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Cryopirin associated periodic syndromes (CAPS) are autoinflammatory diseases associated to NLRP3 gene mutations, leading to inflammasome hyperactivity and IL-1β hypersecretion. Three phenotypes (FCAS, MWS and CINCA) represent a continuum of the same disease characterized by different severity degrees. CINCA patients, affected by the most severe form, presented recurrent fever, systemic inflammation and central nervous system disabilities. We engineered N475K mutation in mouse NLRP3 gene and generated a knock-in (KI) model that presents two different phenotypes: heterozygous and homozygous form. Although both KI mimic CAPS human disease, homozygous (homo)-KI mice presented a much more severe phenotype and exhibited typical features of CINCA patients. Homo-KI mice showed severe skin rush and growth delay respect to Wild Type (WT) controls. Survival and body weight were severely decreased. Interestingly, IL-1β secretion was strongly increased respect to WT and heterozygous littermate mice. Homo-KI mice presented severe cerebral complication that prevent walking. Hystological analysis showed a diffuse inflammatory phenotype characterized by delay in cerebellum development and altered lamination of cerebral cortex. In conclusion, homo-KI mice recapitulate clinical and immunological features of CINCA patients. In the future this mouse model could be used to gain insights into the mechanisms associated to neurological defects and their consequent treatment.

Keywords: Animal model, Brain injury, Inflammation

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NI26 | Activation of the hydroxycarboxylic acid receptor-2 by monomethyl fumarate triggers different pathways in different cell types

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We demonstrated that monomethyl-fumarate (MMF), the bioactive metabolite of the drug dimethyl-fumarate, modulates microglia activation through a novel 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 dimethyl-fumarate 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-pathway. Our preliminary 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. Unexpectedly, in activated-IEC, both MMF and butyrate exerted an anti-inflammatory effect, albeit HCAR2-dependent only for MMF. Altogether these data suggest a cell- and environment-biased activation of different pathways in HCAR2 signaling.

Keywords: Inflammation, Immune system

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NI27 | Early, norepinephrine-dependent, activation of the hematopoietic niche upon induction of experimental autoimmune encephalomyelitis

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In the bone marrow (BM), mesenchymal stem cells (MSC) contribute to the homeostasis of the hematopoietic niche through the production of factors which promote a quiescent hematopoietic stem cell (HSC) state (1). The sympathetic nervous system negatively controls the expression of these factors through the neurotransmitter norepinephrine (NE), whose interaction with beta 3-adrenergic receptors (B3AR) expressed by MSC leads to the mobilization of HSC (2). In the model for multiple sclerosis (3), experimental autoimmune encephalomyelitis (EAE), immunization with myelin antigens results in the activation of peripheral lymphoid organs where pathogenic T cells are generated. PURPOSE: Our goal is to define the role of the NE-dependent activation of the hematopoietic niche in the development of EAE. – METHODS. Femora and thymuses were harvested at different days post EAE induction (dpi) with Myelin Oligodendrocyte Glycoprotein (MOG35-55) and analyzed by FACS, real-time PCR and ELISA. – RESULTS. From 3 dpi, we observed a significant increase in NE level in BM and thymus, associated with a reduced expression of MSC-specific genes controlled by NE-mediated signaling. Analysis of common lymphoid and myeloid progenitors showed a lymphoid bias of hematopoiesis in BM. Parallel investigation indicated an early activation of the thymus in EAE, with a burst in maturation of inherent precursors from 3 dpi resulting in an elevated number of CD4+ T cells. BM-derived precursors increased in thymus from 7 dpi, which would be expected to sustain T cell development. Blockade of B3AR through chemical inhibition impaired the generation of lymphoid progenitors in BM upon EAE induction and prevented the mobilization of HSC. This was associated with a reduced number of BM precursors in the thymus, likely accounting for the consequent impairment of T-cell generation. – CONCLUSIONS. These data indicate that EAE clinical onset, which occurs around 11 dpi, is preceded by an early generation of lymphoid progenitors in BM, followed by a later increase of T-cell precursors and by a boost of T-cell maturation in the thymus. These events are controlled by NE through the activation of B3-AR-expressing MSC, which were inhibited, at least in part, upon blockade of B3-AR. – BIBLIOGRAPHY. 1. Mendez-Ferrer *et al*., Nature 466: 829 (2010); 2. Katayama *et al*., Cell 124: 407 (2006); 3. Mendel *et al.*, Eur. J. Immunol. 25: 1951 (1995)

Keywords: Inflammation, Immune system, Stem cells

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NI28 | Adenosine A2B receptors and sphingosine kinase/ sphingosine-1-phosphate signalling axis control maturation of oligodendrocyte precursor cells in vitro

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Oligodendrocyte-formed myelin sheaths play important roles in the neuronal functions in the central nervous system. In demyelinating diseases, such as Multiple Sclerosis (MS), the remyelinating process is somehow hindered. Restoration of the myelin sheaths requires the differentiation of the oligodendrocyte precursor cells (OPCs) into mature oligodendrocytes (OLs). Adenosine and sphingosine kinase/ sphingosine1-phosphate signaling axis (SphK/S1P) play important roles in remyelination processes. Remarkably, Fingolimod (FTY720), approved as orally active drug for relapsing MS, also modulates S1P receptors. The role of adenosine A2B receptor (ADORA2B) and SphK/S1P signaling on oligodendrogenesis in rat cultured OPCs was investigated by patch clamp experiments coupled to Real Time PCR and Western Blot. BAY60-6583 (0.1-30 microM), a selective ADORA2B agonist, reduced the amplitude of outward currents. This effect was blocked by MRS1706 (10 microM), a selective ADORA2B antagonist. FTY720phosphate (FTY720P) mimicked and partially occluded the effect of BAY60-6583. When applied at nanomolar concentrations, FTY720P produced an increase in outward currents. SphK1 phosphorylation was enhanced after acute treatment with BAY60-6583, demonstrating an interaction between SphK/S1P pathway and A2B activation. Chronic ADORA2B stimulation reduced the expression of mature OL markers. Our data shows that novel pathways activated by ADORA2B and SphK/S1P are involved in the maturation of OPCs.

Keywords: Electrophysiology, Brain injury, Inflammation, Remyelination

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NI29 | Occurrence of neurodevelopmental disorders in children of women with multiple sclerosis treated with natalizumab during pregnancy

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BACKGROUND. According to recent epidemiological evidences (Portaccio, 2018) foetal exposure to natalizumab increases miscarriage and malformation risks, with clues for neurodevelopmental disorders, including autism spectrum disorder (ASD). – METHODS. We collected clinical and paraclinical data of two dizygotic twins with ASD and malformations, who were exposed to natalizumab for nearly 8 weeks of gestation. We also describe our findings on the immunogenetic association between maternal immune activation during pregnancy and fetal risk of ASD. – RESULTS. Very low-weight associated with congenital anomalies were reported at the pre-term twins birth. Other clinical and instrumental data, as well as other ASD-related risk factors were unremarkable. Our immunogenetic results on different datasets (Guerini, 2018a and 2018b) indicate NK-activating genetic axis KIR2DS1-HLAC2+/HLA-G*14bp+ and HLA-G*01:05N to be associated with both ASD and recurrent miscarriage. – CONCLUSIONS. In line with ours and other studies (Fu, 2017; Estes, 2016; Gandoglia, 2017) we propose a pathogenic link in which the natalizumab-driven VLA-4 blockade on uterine NK-cell surface may alter key NK cell functions like migration, tolerogenicity and cytotoxicity, possibly leading to ASD-predisposing uterine immune activation.

Keywords: Molecular biology, Brain injury, Inflammation

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NI30 | Blocking of CSFR1 impairs oligodendrocyte differentiation

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Glial cells are the most abundant cell type in the Central Nervous System (CNS) and they play very important roles during development and disease. As such, crosstalk amongst the different glial cell types, i.e. astrocytes, oligodendrocytes and microglia, as well as with neurons, is essential for the maintenance of a healthy nervous system. In the CNS, oligodendrocytes' cytoplasmic membranes ensheath axons forming myelin sheets, enabling fast saltatory nerve conduction and keeping axon integrity among other functions. Microglial cells play a very important role in the maintenance of oligodendrocyte progenitor cells (OPCs) by providing trophic support, and they can also modulate oligodendrocyte differentiation. It is therefore important to understand the mechanisms that underlay their communication. Indeed, unravelling how microglia influences oligodendrocyte differentiation and myelination is critical to uncover novel strategies for myelin repair. The growth factor Colony Stimulation Factor 1 (CSF-1) regulates microglia proliferation and survival through the activation of the tyrosine kinase receptor CSF-1R, expressed in microglia during development and in adults. We used Plx5622, a highly specific CSF-1R inhibitor, to analyse the role of microglia in oligodendrocyte differentiation and survival. We have shown that depletion of microglia in early embryogenesis impairs OPCs maintenance and differentiation in the neural tube of wt mice, as shown by the numbers of Pdgfra and OLIG2 positive cells. Furthermore, mice lacking microglia show a decrease in the number of differentiated myelinating oligodendrocytes, as assessed by the myelin protein Plp expression. As microglia seems to be crucial for oligodendrocyte survival and differentiation during development, we decided to explore whether microglia exert the same functions in a model of multiple sclerosis. We used cuprizone to induce acute or chronic demyelinating lesions followed by Plx5622 treatment. After a short demyelination time and 4 weeks of Plx5622 administration, around 30% of the microglia population remained and the number of OPCs was increased. Overall, microglia relationship with oligodendrocytes seems to be context-dependent. Thus microglia are necessary for oligodendrocyte specification and differentiation during development whereas a modulation of microglia population might have a beneficial effect in the production of new oligodendrocyte progenitors after a demyelination process.

Keywords: Animal model, Neuroimaging, Brain injury, Inflammation, Remyelination, Plasticity, Imaging

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NI31 | Exploring the role of the immune system in the susceptibility to cocaine use disorder following early-life stress

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Childhood maltreatment is a risk factor for developing substance use disorder (SUD), but the mechanisms underlying this relationship have not been determined. Adverse childhood experiences affect the immune system and the immune system mediates the effects of psychostimulants. However, whether alterations in this system are causal for SUD in individuals who have experienced early life stress is unknown. We performed ex vivo and in vivo experiments in mice and humans to explore the function of the immune system in the early-life stress-induced susceptibility to the neurobehavioral effects of cocaine. In mice, exposure to social stress (S-S) at an early age permanently sensitized the peripheral (splenocytes) and brain (microglia) immune responses to cocaine. In the brain, microglial activation in the ventral tegmental area (VTA) of S-S mice was associated with functional alterations in dopaminergic neurotransmission. Preventing immune activation during the S-S exposure reverted the effects of dopamine in the VTA and the cocaine-induced behavior to control levels. In humans, cocaine modulated Toll-like receptor 4-mediated immunity, an effect that was enhanced in cocaine addicts who had experienced a difficult childhood. Collectively, our findings demonstrate that sensitization to cocaine in early-life-stressed individuals involves brain and peripheral immune responses in both mice and humans.

Keywords: Molecular biology, Animal model, Neuron-glia communication

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NI32 | MiRNAs content in the exosomes derived from immunomodulatory MSC as regulators of neuroinflammation in ALS

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Activated glial cells-mediated neuroinflammation characterizes Amyotrophic Lateral Sclerosis (ALS). Mesenchymal stem cells (MSC)-treated ALS mouse model (mSOD1) showed reduced neuroinflammation levels. We postulated that MSC modulate microglial phenotype in part through exosome-mediated transfer of specific microRNAs. We identified nine microRNAs significantly upregulated in IFNgamma-stimulated MSC. All nine microRNAs are within the IFNgamma-primed MSC-derived exosomes in particular miR-466q and miR-467f are significantly overexpressed. To ascertain whether these microRNAs could modulate microglial activation, we transfected lipopolysaccharide-activated murine microglial N9 line cells with microRNA mimics and analyzed the expression of genes associated with proinflammatory (M1-like) and anti-inflammatory (M2-like) phenotypes. Some specific microRNA, significantly induced the upregulation of M2-like genes, while miR-466q and miR-467f downregulate proinflammatory genes in activated microglia. To analyze the miRNAs mode of action, we have used MirWalk, KEGG Pathway and PANTHER databases to predict possible target genes of the two microRNAs and the pathways involved, including MAPK and inflammasome pathways, which we are presently validating. We verified in vitro that exosomes, containing these modulatory microRNAs, induce microglia switch from M1-like to M2-like phenotype. We obtained similar results in isolated-mSOD1microglia. These data are prerequisite to obtain an in-vivo proof-of-concept that exosomes administration to mSOD1 can recapitulate the MSC beneficial effect.

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NI33 | Alemtuzumab differentially affects effector and regulatory immune cell subsets

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BACKGROUND. Alemtuzumab is a human monoclonal antibody targeting CD52 causing a transient decrease in immune cell subsets followed by repopulation. Differential targeting of immune cells may be associated to its efficacy and/or side effects, including a 30% risk in secondary autoimmune diseases. - METHODS. Numbers of B, T and NK cells over the first year of treatment were evaluated by conventional flow cytometry (N=14 patients). In order to obtain proportion of effector and regulatory T, B and NK cell subsets, a highly standardized method for flow cytometry, using tubes pre-filled with lyophilized fluorescent antibodies(Lyotubes), was employed (N= 7 patients). – RESULTS. Immediate post-treatment (PT) decrease in absolute numbers (AN) of all immune cell subsets was observed compared to baseline. At subsequent time-points, AN of CD3+ T was persistently decreased; AN of CD19 + B cells were increased at 12 months PT; AN of NK cells were unchanged. Among B lymphocytes, proportions of Bregulatory and Bmemory cells increased 3 months PT; among T lymphocytes, there was a trend to an increase in Th1 cells at 3 months PT; total T regulatory cells (Treg) were persistently decreased PT. CD-56brightNK cells increased in half subjects PT. – DISCUSSION. Alemtuzumab differentially affects immune effector and regulatory subsets.

Keywords: Biomarkers, Immune system

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NI34 | MRI-diagnosed white matter lesions in the brain of VLBW babies: risk factor analysis

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In the last decades, new type of white matter injury connected with the prematurity started attracting attention of neonatologists: punctate white matter lesions (PWML) frequently seen on MRI at term equivalent age. We have conducted a retrospective study in order to analyze prevalence of PWML on term-equivalent age MRI, and investigate related clinical risk factors. Study included 321 very low birth weight infants consecutively scanned at term equivalent age. MRI scans were performed at 1,5 T system and included T1, T2, diffusion and susceptibility weighted (SWI) sequences. Images were reviewed in order to evaluate prevalence, and type of PWML (hemorrhagic or non-hemorrhagic according to SWI appearance). Clinical data were collected retrospectively. Univariate and multivariate analysis of risk factors was performed. Sixty-one infant (19%) presented PWML, in 26 cases 6 or more PWML were present, while in 15 cases PWML were seen on SWI indicating haemorrhagic nature of the lesions. Incomplete antenatal steroid treatment (OR 2.71) and intubation (OR=10.1) resulted significant risk factors for PWML ≥6. Risk factor associated with SWI+ PWML was the presence of GMH-IVH (OR=8.67). Respiratory distress emerged as an important risk factor in the development of PWML. Further studies could help to corroborate our findings.

Keywords: Neuroimaging, Brain injury, Inflammation

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NI35 | Placement inflammation and MRI-detected brain lesions in very premature infants

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INTRODUCTION. The role of placental inflammation in the development of brain lesions is still debated. – METHODS. 286 very premature infants with available placental histology and MRI at term equivalent age were selected. Perinatal data were studied with univariate and multivariate analyses to identify significant risk factors for germinal matrix-intraventricular haemorrhage (GMH-IVH), cerebellar haemorrhage (CBH), cystic periventricular leukomalacia (c-PVL) and punctate white matter lesions (PWML). – RESULTS. Apgar score at 5 min ≤ 5 (OR=6,62), incomplete or no antenatal steroids (OR=2,45), mechanical ventilation in the first 72 hours of life (OR =2,14) and surgical ligation of ductus arteriosus (OR=3,45) were significant for GMH-IVH (prevalence of 23,8%) together with umbilical vein vasculitis (most significant factor for placental inflammation, OR=3,80) and villous infarction (sign of fetal vascular impairment, OR =5,94). In our study, placental characteristics, including chorioamnionitis, were not identified as independent risk factors for white matter lesions, including both c-PVL (prevalence: 2,4%) and PWML (prevalence: 19,9%). – CONCLUSIONS. Placental inflammation represents a risk factors for the development of GMH-IVH, a disease occurring in the first days of life. Chorioamnionitis is not associated with white matter lesions, in contrast with previous studies based mainly on ultrasound findings.

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NI36 | Monomethyl fumarate prevents inflammation-driven synaptopathy by counteracting miR-142-3p action in experimental MS

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Excitotoxic synaptopathy is emerging as an early pathophysiological hallmark of multiple sclerosis (MS) and of its mouse model, experimental autoimmune encephalomyelitis (EAE). It includes increased glutamategic transmission induced by inflammation that, in the long-term, can cause disabling neuronal damages. Since EAE/MS synaptopathy is precocious and potentially reversible, it represents an attractive therapeutic target. Thus, we asked whether EAE synaptopathy could be directly targeted by disease-modifying treatments, like the oral drug dimethyl fumarate (DMF), which metabolizes to active metabolite monomethyl fumarate (MMF). Ex vivo electrophysiological experiments in EAE cerebellar slices showed that acute incubation of MMF was able to correct glutamategic current abnormalities. Mechanistically, we observed that MMF reduces the expression of a crucial effector of the Il-1b excitotoxic signal, as miR-142-3p, with consequent increase of its target the glial glutamate transporter GLAST/EAAT1. Consistently, therapeutic intracerebroventricular administration of MMF in EAE mice, was able to mitigate cerebellar synaptic dysfunctions, mimicking the milder EAE phenotype of heterozygous mice with disrupted miR-142 gene. Overall, our findings highlighted a novel direct neuroprotective mechanism through which MMF is able to restore glutamate homeostasis in EAE cerebellum and, thus, to prevent glutamate-dependent excitotoxic damages, by perturbing the detrimental Il-1b-miR-142-3p-GLAST/EAAT1 regulatory axis typical of the EAE cerebellum.

Keywords: Molecular biology, Electrophysiology, Inflammation

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NI37 | Punctate white matter lesions (PWML) and adenosine blood levels in premature infants

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PWML are MRI diagnosed minor forms of white matter damage with high prevalence (20%) in premature infants. Pathophysiology remains unclear (i.e. intrinsic vulnerability, inflammation, oxidative stress, etc) but early biomarkers are needed. We investigated whether blood adenosine levels could predict the vulnerability to the develop PWML. Dried blood spots were prospectively collected for newborn screening program for congenital disease and Adenosine concentration was measured by Tandem Mass Spectrometry. Fifty-six very low birth weight newborns with gestational age at birth below 31 wks were included, 32 babies repeated the exam twice (Day 3 and Day 15), 27 babies were sampled three times (Day 3, Day15 and Day 30) and 23 babies up to four times (Day 3, 15, 30 and 40). PWML were diagnosed using MRI at term equivalent age. Blood adenosine concentrations increased over time from a median of 0.75 µM at Day 3 to 1.46 µM at Day 40. Adenosine blood concentration >1.58 µM at day 15 was significantly associated with PWML at MRI (OR [95%CI] of 50.0 [3.6-688.3], p-value < 0.001). These findings suggest a potential role for blood adenosine concentration as a biomarker of the frequent PWML diagnosed at term corrected age in premature babies.

Keywords: Neuroimaging, Brain injury, Biomarkers

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NI38 | Blood adenosine levels in very low birth weight infants and neurological follow-up at 12 and 24 months

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INTRODUCTION. Inflammation and oxidative stress seems to alter white matter development in very low birth weight (VLBW) infants, leading to white matter injury. We investigated the role of adenosine as a neuroinflammatory biomarker, assessing how its blood levels can predict the neurological outcome at 12 and 24 months of corrected age in this population. – METHODS. 32 VLBW neonates were included. Adenosine level was assessed by Mass Spectrometry using dried blood spots collected for the metabolic screening at 15±2 days of life. Griffiths Mental Developmental Scale (GMDS) was performed at 12 and 24 months of corrected age. – RESULTS. Out of 32 enrolled patients, 27 completed GMDS at 12, and 25 at 24 months. Pearson's correlation coefficient for adenosine/GMDS was of -0.52 at 12 months, and -0,5 at 24 months. Only one out of 13 infants with adenosine levels below 1 µM showed an abnormal GMDS score (< 85) at 12 of corrected age. – CONCLUSIONS. A medium strength linear association between adenosine levels at 15 days of life and a lower Griffith score at 12 and 24 months of corrected age was found. Our results suggest that adenosine could be a promising early biomarker for neurological outcome in VLBW infants.

Keywords: Cognitive, Inflammation, Biomarkers

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NI39 | Personalizing health care in Multiple Sclerosis using systems medicine tools: presentation of the cytomics data

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Development of personalized health care for multiple sclerosis (MS) is hindered by a poor understanding of the biological processes underlying the disease, their interactions, and the heterogeneity between patients. By combining integrative omics, imaging and clinical data, we aim at developing algorithms that can be used in clinical practice to define the prognosis and select the best therapeutic approach. Here, we present cytomics data obtained in four European centers by flow cytometry of immune cell subsets in PBMC samples from 246 MS patients (age 42.5 ± 10 years; sex: 67.5% female; disease duration: 10.6 ± 8 years; subtype: 73.5% RRMS; 20% PMS; 6.5% CIS; mean EDSS: 2.4 ± 1.7) and 77 healthy controls (HC). Assays were strictly standardized using specifically prepared antibody-cocktail lyotubes. Comparison with HC indicated that: MS patients have significantly lower percentages of naïve-Treg and Th1/17 cells and higher percentage of total Treg and Breg cells; patients receiving high-efficacy drugs have less naive-Treg and Th1/17 cells and more total Treg and Breg than patients on other treatments. Such cytomics markers will be used in the development of clinical decision support systems for improving disease management.

Keywords: Inflammation, Biomarkers, Immune system

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NI40 | Bridging pro-inflammatory signals, synaptic transmission and protection in spinal explants in vitro

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Multiple sclerosis is characterized by tissue atrophy involving the brain and the spinal cord, where reactive inflammation contributes to the neurodegenerative processes. Recently, the presence of synapse alterations induced by the inflammatory responses was suggested by experimental and clinical observations, in experimental autoimmune encephalomyelitis mouse model and in patients, respectively. Further knowledge on the interplay between pro-inflammatory agents, neuroglia and synaptic dysfunction is crucial to the design of unconventional protective molecules. Here we report the effects, on spinal cord circuits, of a cytokine cocktail that partly mimics the signature of T lymphocytes sub population Th1. In embryonic mouse spinal organ-cultures, containing neuronal cells and neuroglia, cytokines induced inflammatory responses accompanied by a significant increase in spontaneous synaptic activity. We suggest that cytokines specifically altered signal integration in spinal networks by speeding the decay of $GABA_A$ responses. This hypothesis is supported by the finding that synapse protection by a non-peptidic NGF mimetic molecule prevented both the changes in the time course of GABA events and in network activity that were left unchanged by the cytokine production from astrocytes and microglia present in the cultured tissue. In conclusion, we developed an important tool for the study of synaptic alterations induced by inflammation, that takes into account the role of neuronal and not neuronal resident cells.

Keywords: Organotypic spinal slices, Network activity, Cytokines, Neuroinflammation, Neuroprotection, NGF-mimetic

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NI41 | Glia-to neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations

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Recent evidence indicates synaptic dysfunction as an early mechanism affected in neuroinflammatory diseases, such as multiple sclerosis, which are characterized by chronic microglia activation. However, the mode(s) of action of reactive microglia in causing synaptic defects are not fully understood. In this study we show that inflammatory microglia produce extracellular vesicles (EVs) which are enriched in a set of miRNAs that regulate the expression of key synaptic proteins. Among them, miR-146a-5p, a microglia-specific miRNA not present in hippocampal neurons, controls the expression of presynaptic synaptotagmin1 (Syt1) and postsynaptic neuroligin1 (Nlg1), an adhesion protein which play a crucial role in dendritic spine formation and synaptic stability. By the use of a Renilla-based sensor we provide formal proof that inflammatory EVs transfer their miR-146a-5p cargo to neuron. By western blot and immunofluorescence analysis we show that vesicular miR-146a-5p suppresses Syt 1 and Nlg1 expression in receiving neurons. Notably, microglia-to-neuron miR-146a-5p transfer and down-regulation of neuronal Syt1 and Nlg1 do not occur when EV-neuron contact is inhibited by cloaking crucial vesicular phosphatidylserine residues, and when neurons are exposed to EVs either depleted of miR-146a-5p, produced by pro-regenerative microglia, or storing inactive miR-146a-5p, produced by cells transfected with an anti-miR-146a-5p. Moreover, morphological analysis reveals that prolonged exposure to inflammatory EVs leads to significant decrease in dendritic spine density in hippocampal neurons in vivo and in primary culture, which is rescued in vitro by transfection of a miR-146-insensitive Nlg1 form. Finally, dendritic spine loss is accompanied by a decrease in the density and strength of excitatory synapses, as indicated by reduced mEPSC frequency and amplitude. These findings link inflammatory microglia and enhanced EV production to loss of excitatory synapses, uncovering a previously unrecognized role for microglia-enriched miRNAs, released in association to EVs, in silencing of key synaptic genes.

Keywords: Microglia, Extracellular vesicles, miRNAs, Dendritic spine Multiple sclerosis, Cognitive symptoms

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NI42 | Possible involvement of the Repressor Element 1-Silencing Transcription factor in the pathological process of experimental autoimmune encephalomyelitis

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The development of nervous system imply gene expression modulation coordinated by transcriptional factors, including Repressor Element 1-Silencing Transcription factor (REST), which is implicated in neurodegenerative disorders. We analyzed the expression of REST and its target genes in experimental autoimmune encephalomyelitis (EAE). Time-course analysis confirmed REST increased expression during acute EAE together with the downregulation of some target genes in spinal cord and, unexpectedly, with their upregulation in the striatum. REST gene is expressed as a full-length (fREST) or a truncated transcript, REST4, which lacks the repressor domain and competes with fREST to derepress its targets. Analysis of REST transcripts pointed to an upregulation of fREST in spinal cord and no change in REST4 and vice-versa in striatum. We analyzed REST activity in N2a neuronal cells exposed to supernatant of activated T cells, for mimicking EAE microenvironment. We observed an increase in both fREST and REST4 expression, together with upregulation of its targets, in N2a cells exposed to activated T-cell supernatant. Western-blot analyses performed to assess signaling pathway(s) involved in REST activity show that casein kinase-1 signal, responsible for REST degradation was reduced in N2a cells exposed to activated T-cell supernatant. Our results suggest that REST dysregulation in EAE might occur in response to pathological stimuli through alteration of the balance in the expression of fREST and REST4.

Keywords: REST, neuroinflammation, neuronal cells, T cells, multiple sclerosis, neurodegeneration

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NI43 | A possible role for nerve glial antigen 2 in dendritic cell activation

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In central nervous system, nerve/glial-antigen 2 (NG2) is expressed by oligodendrocyte progenitor cells (OPCs) and by activated of pericyte. We found that induction of experimental autoimmune encephalomyelitis (EAE) in NG2 knock-out (NG2KO) mice results in milder EAE than in wild-type (WT) mice with less intense neuropathology. In addition to macrophages, we found that NG2 was also expressed in WT mice by most T cells and 40-50% dendritic cells (DCs). To assess the possibility that NG2 could play a role in the immune response in EAE, we induced EAE in bone-marrow chimeric mice, generated with WT recipients of NG2KO bone-marrow cells and vice versa. Regardless of their original phenotype, mice receiving NG2KO bone marrow developed milder EAE than those receiving WT bone marrow. While no inherent defect could be associated with NG2KO T cells, assessment of recall T-cell responses showed that, while WT and NG2KO T cells proliferated equally, NG2KO T cells were skewed towards a less inflammatory Th2-type response. Analysis of WT and NG2KO lymph node cells for intracellular expression of IL-12 indicated that the proportion of IL-12-expressing DCs was significantly lower in NG2KO mice. Our preliminary experiments to understand if the expression of NG2 in DCs is constitutive or induced support this hypothesis, showing a switch of sorted NG2-negative to NG2-positive DCs upon activation. Our data suggest that NG2 plays a role in DC activation and could be an important target of inflammation.

Keywords: EAE, neuroimmunology, dendritic cells

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NP24 | Sonic hedgehog signalling pathway on neural stem cells during regenerative processes in a mouse model of motoneuronal loss

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Neuronal loss represents the consequence of direct or indirect insults to neurons, as well as one of the major factors mediating persistent disability. Sonic hedgehog (Shh) signalling, which has been indicated as an important pathway in central nervous system development and neural stem cells (NSCs) function, may have a role in prompting the repairing and modulating actions of endogenous and/or exogenous NSCs in neurodegenerative conditions. Here we studied the Shh pathway on NSCs both in vitro and in a mouse model of spinal motorneuronal degeneration. NSCs were expanded, characterized and the effects of Shh signalling were evaluated in vitro. We analysed the effects of Shh signalling pathway modulation on NSCs in vitro, finding a significant increase of the NSCs growth rate (2.98±0.58 vs. 5.26±0.57, $p<0.05$) and neurospheres diameters (109.9±2.4μm vs. 129.6±3.7μm, $p<0.01$). We then analysed the Shh pathway in a mouse model of spinal motoneuronal depletion induced by Cholera toxin-B conjugated to saporin (CTB-Sap), as compared to intact controls. Our results suggest a crucial role of Shh signalling during regenerative processes and suggest the role of NSCs as a potential strategy to support recovery after spinal motoneuronal degeneration.

Keywords: Degeneration, Plasticity, Stem cells

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NP25 | Adult neural stem cell/progenitor fate potential in vivo is controlled by COUP-TFI within the adult hippocampus

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In the adult hippocampal dentate gyrus (DG), adult radial neural stem cells (rNSCs) are multipotent (i.e. they generate both neurons and astrocytes) while progenitors are fate-restricted to the neuronal lineage. Interestingly, factors positively influencing neurogenesis, such as running, also increase DG astrogliogenesis, whereas pathological conditions, such as inflammation, alter the ratio of neuron and astrocyte production in favor of the latter one. Despite the importance of a tight control of neurogenic versus astrogliogenic potential, the underlying transcriptional program is still largely unknown. In the healthy adult DG we found that a large subset of rNSCs/progenitors co-expressed the transcription factor COUP-TFI, whereas neuroinflammation leaded to its downregulation. By inducible knockouts and lineage tracing experiments we demonstrated that COUP-TFI deletion in adult DG rNSCs and committed neurogenic progenitors reduced neurogenesis and increased astrocyte production. Remarkably, this shift also occured upon COUP-TFI deletion by retroviral targeting of mitotic progenitors, indicating that COUP-TFI is required to repress astrogliogenesis all along the neurogenic lineage. Finally, by gain-of-function experiments we showed that COUP-TFI forced expression prevented astrogliogenesis in both normal and inflammatory conditions, indicating that COUP-TFI is a key transcriptional regulator driving adult DG rNSCs/progenitors towards a neurogenic fate by repressing an astrogliogenic one.

Keywords: Inflammation, Plasticity, Stem cells

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NP26 | Functional connectivity adaptation to disease progression in Multiple Sclerosis: an fMRI study

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Severity of disability and disease burden in Multiple Sclerosis (MS) patients may not coincide. Incremented recruitment of crucial cortical areas has been hypothesized to attenuate the negative effects of structural damage accumulation in patients with no-to-moderate disability. Functional connectivity (FC) changes may be associated with relatively preserved neurological functions in the early phases of the disease. Our aim was to explore global brain FC in a large group of patients with MS. A series of 119 patients (28 males, age 39.9±10.1) with MS at different stages and 41 age- and gender-matched healthy subjects underwent 3.0T MRI including single-shot echo-planar resting state functional MRI, high resolution 3D-T1-weighted and dual-echo images. Images were processed through FSL and homemade MATLAB tools. Patients underwent a neurological evaluation (Expanded Disability Status Scale, EDSS). We found that FC changes strongly depend on brain topology, being disability and FC positively correlated in frontal regions and negatively correlated across cerebellar and temporal/frontal regions. The model that better predicts the FC-EDSS relation between frontal regions increases linearly, as example of maladaptive neuroplasticity. Conversely, FC of the cerebellum with temporal and frontal regions shows an initial upraise, as possible compensatory adaptive neuroplasticity, and then a decrement at higher EDSS score.

Keywords: Neuroimaging, Inflammation, Degeneration

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NP27 | Unveiling neuromodulatory functions of NG2-expressing glia

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NG2-expressing glial cells (NG2+c) serve as oligodendrocyte progenitors during development and adulthood. However, there is evidence that suggests additional/alternative functions for these cells: i) NG2+c express a complex array of factors able to mediate neuromodulatory effects; ii) they receive synaptic inputs and neuronal activity, though they do not appear able to transmit electrical signals; iii) in the adult CNS they maintain a high density, but very few are engaged in myelinogenesis. On these bases, we hypothesised that NG2+c may affect the formation and function of neuronal circuitries. To address this issue, we analysed the activity of hippocampal neurons cultured with primary purified NG2+c for 24 hours. Co-culturing with NG2+c was associated with an increase of the synchrony of spontaneous activity of the neuronal network, as recorded by microelectrode arrays, compared to networks without NG2+c. Moreover, patch-clamp recordings revealed that, in the presence of NG2+c, neurons displayed increased amplitude and frequency of GABAergic mIPSCs. These data are consistent with an acceleration of the maturation of the network activity. Analyses are now ongoing to assess whether such effects are associated with changes in the density and size of Glu/GABAergic synaptic puncta.

Keywords: Neuron-glia communication

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NP28 | Fasudil treatment promotes enhanced gliogenesis of neural stem cells in vitro

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The clinically approved ROCK inhibitor Fasudil is used to treat the subarachnoid haemorrhage, and has been reported to have a positive effect on animal models of neurological disorders including Parkinson's disease and stroke. Although tested on neuronal cell lines, its cellular effect on progenitor populations is not clearly understood, and it is unclear how Fasudil may affect primary neural stem cells (NSCs). The effects of Fasudil were tested on NSC cultures derived from postnatal and adult mouse subventricular zone. Dynamic real-time imaging analysis showed that Fasudil promoted neural outgrowth and radial-like inter-kinetic movement in postnatal NSCs, while analysis for lineage markers including GFAP and Sox2 showed enhanced gliogenesis upon treatment. A similar effect was observed in adult NSCs, as Fasudil promoted gliogenesis markers but not neuronal markers. A contrasting trend was observed in the C17.2 line, where Fasudil differentiated cells to Beta3-tubulin neurons and promoted tangential migration with absence of GFAP, suggesting that its gliogenic effect was specific to primary NSCs. These results provide the first evidence that Fasudil treatment causes no significant toxicity in primary NSCs and can promote differentiation towards a gliogenic phenotype, suggesting the Rho/ROCK cascade plays an important role in neural stem cell regulation.

Keywords: Tissue remodelling, Stem cells

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NP29 | A novel mutation in the CHRNA2 gene detected in an Italian NFLE patient

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BACKGROUND. Nocturnal frontal lobe epilepsy (NFLE) is an idiopathic focal epilepsy arising in childhood or early adolescence. A limited number of loci have been associated with the disease: among them, CHRNA2 gene, encoding the α2 subunit of the neuronal nicotinic acetylcholine receptor, has a low mutation rate with only two reported missense variants. – AIMS. To search for disease-causing mutations in Italian probands affected by NFLE and to investigate their functional effects. – METHODS. Genomic DNA was isolated from peripheral blood. All exons and exon-intron boundaries of CHRNA2 gene were sequenced. Functional studies were performed by in vitro mutagenesis, cloning, transient transfections and whole cell patch clamp recordings. – RESULTS. A novel missense mutation in CHRNA2 gene was detected in heterozygosity in a proband. The variant is located in a highly conserved N-terminal domain involved in the acetylcholine binding. The mutation was also found in the mother who had experienced nocturnal arousals in her adolescence, but no clinical evaluations are available. Functional analysis demonstrated that the mutation caused a decrease in response to both nicotine and acetylcholine applications (80% and 35% of reduction, respectively). – CONCLUSIONS. This mutation in CHRNA2 gene associated with NFLE showed the great loss of function effect among those reported.

Keywords: Molecular biology, Electrophysiology

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NP30 | IL-15/IL-15Ralpha signaling modulates hippocampal synaptic transmission

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Cytokines have several physio-pathological roles in the brain regulating the communication between immune and nervous system. Interleukin 15 (IL-15) and its receptor are ubiquitously expressed in the CNS and they are overexpressed in inflammatory conditions. Nevertheless the role of IL15 system in synaptic transmission is poor known. Here we studied the properties of CA1 hippocampal neurons in IL-15Ralpha KO mice which is known to have deficits in hippocampal-dependent memory (He et al, Jneurosci 2010). CA1 pyramidal neurons in IL15RaKO mice showed an increased frequency of the miniature inhibitory synaptic currents (mIPSCs), suggesting a presynaptic modulation of the probability of GABA release. In addition microglial cells isolated from IL15Ra KO mice showed upregulation of IL1b and BDNF genes. Exogenous application of IL15 (10nM) on WT slices resulted in an increase of mIPSCs frequency and a reduction of the miniature excitatory synaptic currents (mEPSCs) amplitude. These data demonstrated that IL-15/IL-15Ralpha signaling exerts a modulatory activity on excitatory and inhibitory synaptic transmission in hippocampal area suggesting an important role as mediator of communication between immune and nervous systems. Further experiments are needed to evaluate the contribution of neuronal, parenchimal and immune cells to the IL15-mediated effects.

Keywords: Animal model, Electrophysiology, Inflammation, Immune system, Plasticity, Neuron-glia communication

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NP31 | Poster session: Whose hand is it? The role of EBA in body ownership

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The extra-striate body area (EBA) is a body-selective focal region located in the lateral occipito-temporal cortex that responds strongly to images of human bodies and body parts in comparison with other class of stimuli. Whether EBA contributes also to body ownership remains in debate. This issue was investigated using double-pulse transcranial magnetic stimulation (TMS). Prior to TMS experiment, all subjects underwent a fMRI localizer task to determine EBA location at the subject level. TMS was then applied at two time-windows (40-50ms and 100-110ms post-stimulus onset) over either right EBA, left EBA and vertex, while participants performed a body parts identification task in which self or others' right or left hands images were presented. TMS at both time-windows over right EBA induced more errors on other's hands than on self-hands and, when applied at 40-50 ms over right EBA, slowed responses for others' hands more than for self-hands. These findings suggest that EBA, in addition to processing descriptive information about body parts, is part of a network underlying self/other discrimination.

Keywords: Neuroimaging, Cognitive, Visual perception

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NP32 | Supramammillary nucleus modulation of hippocampal activity

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The formation of memories has been studied for decades. The role of the hippocampus in memory was revealed by studies performed with patient H.M, whose bilateral loss of hippocampus resulted in the inability to encode new information in long-term memory. Thus, it is currently hypothesized that memory consolidation depends on the hippocampus. Since this discovery, this brain region has been the focus of studies to understand its properties and its role in memory. The area CA2 has not been studied a lot compared to CA1 or CA3. It may be important in the modulation of memory during strong emotional events. The Supramammillary nucleus (SuM), situated in the hypothalamus, is implicated in emotion and motivation. It has been shown to send monosynaptic input to CA2 containing the vesicular glutamate transporter (VGLUT2). Thus, hippocampal activity may be controlled by SuM during emotional context. The aim of this study is to understand which interneuron population are the potential targets of the SuM fibers. For this we will use a VGLUT2-cre mouse line that has been previously injected with virus so that GFP will be expressed in SuM-VGLUT2 expressing neurons. This will allow visualizing GFP-SuM fibers in CA2 and which cells expressed in this area are targeted by the fibers.

Keywords: Plasticity, Imaging

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NP33 | Age related ventriculomegaly in dogs trained to lay in an fMRI

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Dogs (Canis familiaris) are good models for the study of age related cortico-medullary atrophy causing brain ventricles' enlargement. Dogs trained to lay in an fMRI machine allow for non-invasive research comparable to that performed in humans. We investigated ventricular changes in 18 dogs trained to stay motionless in an MR scanner without sedation (movements below 1.00mm for each translation direction and below 0.01degree for each rotation direction). Dogs were trained to enter the MR scanner and lay motionless during the scan. Anatomical images were acquired using a T1-sequence at regular intervals (range 1-6 years, 2-4 scans). Left and right lateral ventricular volumes were calculated (software: FSL and FEI Amira 6.5), 3D-models were generated for comparative visualization. Preliminary results (N=7) indicated a range of ventricular enlargement over a four-year period between 20.4 % and 63% (median = 36.7%) for the left ventricle and between 16.4% and 172% (median = 58%) for the right ventricle. The dogs' ability to perform the trained task and stay stationary in the MR scanner did not vary. The task is demanding in terms of long-term memory and and sustained attention. Dogs' performance thus suggests compensating mechanisms to counteract the effects of brain atrophy.

Keywords: Animal model, Neuroimaging, Ageing, Plasticity, Imaging

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NP34 | Interleukin-6 affects clinical course and brain plasticity in Multiple Sclerosis patients

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The clinical course of multiple sclerosis (MS) is variable and characterized by the occurrence of clinical relapses, followed by remitting phases of neurological deficits. Brain plasticity measured by means of transcranial magnetic stimulation (TMS) has been found to predict clinical course in correlation with the levels of inflammatory mediators in the cerebrospinal-fluid (CSF) of MS patients. Of note, animal studies showed that pro-inflammatory cytokines modulate synaptic plasticity. A retrospective study on MS patients was conducted to evaluate the impact of IL-6 CSF concentrations on disease course. In a subgroup of MS patients, long term potentiation (LTP)-like cortical plasticity was investigated through TMS. In parallel, we performed preclinical studies by recording electrophysiological field potential on mouse hippocampal slices during acute exposure of IL-6. We found that MS patients with high levels of IL-6 in the CSF had increased risk of clinical relapse during the follow-up period. In parallel, we evidenced a negative correlation between IL-6 CSF levels and the LTP-like effect induced by TMS. Moreover, exogenous application of IL-6 on murine hippocampal slices was able to abolish the induction of LTP. Our results highlight the biological relevance of the pro-inflammatory cytokine IL-6 in the pathophysiology of human MS.

Keywords: Electrophysiology, Inflammation, Plasticity

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NP35 | The striatal GTPase Rhes modulates cocaine-mediated behavioural responses in mice

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The small GTP-binding protein Rhes, highly enriched in rodent and human striatal medium spiny neurons, is regulated by dopamine innervation in adult rats. Previously we demonstrated that Rhes knockout (KO) mice display a motor hypersensitivity induced by amphetamine. Based on these findings, we investigated the potential involvement of Rhes in modulating behavioural responses induced by cocaine administration. We found that Rhes KO mice showed hyperlocomotion to the acute cocaine treatment even at the dose of 7.5mg/kg, which did not affect locomotion in WT-treated. Moreover, when mice were chronically injected at 15mg/kg for 10 Days, we observed a progressive enhancement of locomotor response in WT over time, while in KO animals the highest hyperlocomotion was already reached at Day1. Finally, following 21-day withdrawal the same mice were challenged with 7.5mg/kg cocaine in order to induce a behavioral sensitization. Results showed an exacerbated locomotor response in WT mice at the lower dose of 7.5 mg/kg, similar to previously sensitized KO. Accordingly, western blot studies showed a significant increase of PKA signaling in Rhes KO mice treated with cocaine (30mg/kg). These data suggest a crucial role for this small G protein in modulating long-lasting plasticity events underlying striatal dopamine transmission.

Keywords: Animal model, Plasticity

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NP36 | MOR/DOR targeting: an useful strategy in pain management

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Despite opioids represent the first option for pain management their use is associated with a number of side effects. Different studies demonstrated that the simultaneous MOR and DOR targeting leads to an improved analgesia with reduced side effects, that limit opioids use. In this context, we previously identified the multitarget MOR/DOR ligand LP2 that ip administered in mice resulted a significative long-lasting antinociceptive agent (ED50= 0.9 mg/kg). Building upon these evidences, our efforts were focused on demonstrating whether the LP2 multitarget profile could be useful for persistent pain states. To this aim, LP2 was undertaken to in vitro and in vivo assays. The LP2 ability to promote opioid receptor/G protein and opioid receptor/beta-arrestin 2 interaction in the BRET assay was evaluated. The systemic and peripheral antinociceptive effects of LP2 in inflammatory pain, induced by ip injection of formalin in mice, was investigated. As well as, the effect of LP2, ip administered, on mechanical allodynia in a rat model of neuropathic pain, produced by CCI of the left sciatic nerve, was also established. As emerged by our studies, LP2 was able to activate G-protein pathway over β-arrestin 2, behaving as biased agonist at MOR and mainly at DOR. The biased MOR/DOR agonist LP2 produced a significant antinociception in the mice model of inflammatory pain and produced significant antiallodynic effect in the rat model of neuropathic pain. The LP2 multitarget opioid profile and its functional selectivity for G-protein signaling over beta-arrestin 2 recruitment is consistent with its effectiveness in persistent pain states.

Keywords: Animal model, Inflammation

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NP37 | Heterogeneity of cortical layer II immature neurons in mammals

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The doublecortin-positive (DCX+) pre-natally generated neurons discovered in the layer II of the rodent piriform cortex are considered a reservoir of “immature” neurons in the adult brain. In some non-rodent species they extend into neocortex and in sheep also into subcortical regions (Piumatti et al., 2018). Hence, immature neurons might be more important in large-brained, long-living mammals. We assessed the occurrence, distribution and amount (linear density - cells/mm of layer II) of type 1 (small-bipolar) and type 2 (large-ramified) DCX+ cortical neurons at 4 comparable brain levels in 13 mammalian species endowed with different brain anatomy, lifespan, ecological niche. Sections were immunostained for cell proliferation (Ki-67, BrdU) and immaturity/maturity markers (PSA-NCAM, NeuN). All non-rodent species considered hosted DCX+ neurons in neocortex, with highly heterogeneous linear densities, ranging from 1±1 (bat) to 30±21 (cat) cells/mm. By contrast, morphological and phenotypic features were rather constant: type 2 cells represented 10-20% of the total, mostly expressing NeuN, whereas 15-30% of DCX+ cells were also PSA-NCAM+. BrdU and Ki-67 antigen detection confirmed that all DCX+ neurons were non-newly generated/not proliferating. These results show that “immature” cortical neurons do represent a well preserved, yet, highly heterogeneous feature in mammals, especially considering rodent and non-rodent species.

Keywords: Plasticity

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NP38 | Antibodies for the pharmacological characterization of presynaptic, release-regulating mGlu2-preferring and mGlu3-preferring autoreceptors in mouse CNS

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In previous study, the availability of specific ligands able to differentiate between metabotropic glutamate (mGlu) 2 and 3 receptors allowed the characterization of presynaptic release-regulating group II autoreceptors in cortical and spinal cord nerve endings. Our results suggested the existence of pure mGlu3-preferring autoreceptors in spinal cord glutamate terminals and of mGlu3-containing, mGlu2-preferring autoreceptors in cortical nerve endings. To extend and confirm the pharmacological characterization, selective antibodies recognizing the N-terminal of the mGlu2 and mGlu3 proteins were used in functional and biochemical studies as selective ligands to discriminate between the two receptors. Our results confirmed the existence of mGlu2/3 autoreceptor highly sensitive to the mGlu2 PAM LY566332 but slightly, although significantly, susceptible to the mGlu3 NAM LY2389575. Differently, LY566332-insensitive,LY2389575-sensitive autoreceptors exist in spinal cord terminals. Incubation of cortical synaptosomes with anti-mGlu2 antibody prevented the LY379268-induced inhibition of glutamate exocytosis, that was partly reduced by the anti-mGlu3 antibody. Incubation of spinal cord synaptosomes with anti-mGlu3, but not anti-mGlu2 antibody, nulled the LY379268-mediated reduction of glutamate exocytosis from these terminals. Western blot analysis and confocal microscopy confirmed the functional observations. We confirm the existence of mGlu3-preferring autoreceptors in spinal cord terminals and of mGlu2-preferring, mGlu3-sensitive autoreceptors in mouse cortex.

Keywords: Molecular biology

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NP39 | Regional and cellular distribution of H3.3a and H3.3b histone variants in adult mouse brain

Eleonora Daini[1], A. Vilella[1], E. Belotti[2], L. Schaeffer[2], S. Dimitrov[3], M. Zoli[1]

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Among all epigenetic mechanisms, histone variants (e.g. H3.3) have been recently identified as key regulators of chromatin remodelling involved in neuroplasticity. H3.3 proteins, encoded by H3f3a and H3f3b intron-containing genes, can be actively incorporated into chromatin and their turnover and replacement by new variants, during and outside S phase, may subserve specific cellular functions as well as experience- dependent neuroplasticity. To evaluate the region- and cell-specific distribution of H3.3 isoforms, hemagglutinin (HA)-tagged H3.3A (WT/HA-fH3.3A) and HA-tagged H3.3B (WT/HA-fH3.3B) mice were used. In these animals we performed a detailed analysis of the expression of the two isoform in central nervous system white and grey matter regions using semi-quantitative immunohistochemistry. Moreover, we assessed the cell-specific expression of these proteins by double immunofluorescent staining and confocal microscopy analysis by using specific antibodies against microglia, astrocytes and neurons. Using these approaches we demonstrated that H3.3A and H3.3B have a widespread though different region-specific distribution. Indeed, they are mostly, though not exclusively, expressed by neurons. Clarification of the localization and cellular expression of the two isoforms will permit to define whether H3.3A and H3.3B turnover, alone or together, participates to molecular mechanisms that lead to neuroplasticity in specific neuronal circuits and associated functions.

Keywords: Animal model, Plasticity

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NP40 | Expression of the transcription factors SOX2 and COUP-TF1 in the developing human brain

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SOX2 and COUP-TFI (also known as NR2F1), two transcriptional regulators playing a key role in developing brain, are required for cell fate determination and acquisition of neuronal identity. While most of our knowledge is based on mouse models, very little is known about their distribution in humans, where mutations lead to neurodevelopmental disorders. To this aim, we investigated the expression of SOX2 and COUP-TF1 in the developing human cerebral cortex at different gestational ages. Our analysis reveals a high SOX2 immunoreactivity in the ventricular and subventricular zones, as well as in some scattered post-mitotic cells in the subplate (SP) and cortical plate (CP). COUP-TF1 immunoreactivity shows a low anterior to high posterior levels of expression along the telencephalon as described in rodents in addition to a ventricular low to pial high level of expression across the cerebral cortex. SOX2 and COUP-TF1 are co-expressed by numerous cortical progenitor cells, while they co-localized in few postmitotic cells at the level of SP and CP. Additionally, preliminary results show that SOX2 and COUP-TF1 are expressed in glial cells and neurons respectively. This study confirms the conservation of SOX2 and COUP-TF-1distribution and suggests a novel insights into the proliferative domains in human cortex.

Keywords: Plasticity, Stem cells

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NP41 | Family dogs as a model for the study of age-related cognitive decline

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Domestic dogs (Canis familiaris) are good translational models for age-related cognitive decline, due to similarities with the human in cognitive changes, neuro-histopathology and psychoneuroendocrinology. Most of the related research is performed in laboratory dogs, while dogs kept as pets ("family dogs") are exposed to the same environmental risk factors as humans. They are also subjected only to non-invasive research that is more comparable to that performed in humans. Yet there is no objective test to assess cognitive decline in ageing family dogs. We designed one test for differential exploration of familiar and novel objects (Novelty Test), and one test for visuo-spatial short-term memory (STMT). We tested 127 adult family dogs (Mage = 8.33 years, range 3.0-14.5; F = 65, M = 62). Dogs' latency to approach a novel object in the Novel Test was higher as age increased (Cox Regression: N=106, p= 0.023). Dogs' ability to remember the location of food in the STMT declined with age (Generalised Linear Mixed Model: N=124, p<0.001). We identified 2 tasks to objectively detect age-related differences, potentially relying on working and short-term memory in healthy-ageing family dogs. Future research will investigate the relationship between the test results and neuroimaging data (EEG, fMRI) and biomarkers.

Keywords: Animal model, Cognitive, Ageing

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NO11 | Genetic barcoding reveals the clonal dynamics of glioma progression

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During tumor progression, transformed cells accumulate mutations and gain malignancy. Despite this process has been extensively studied, it is still unclear how easily transformed cells can undertake the whole progression and gain full malignancy. To measure the probability of glioma progression we used a well-characterized murine model of gliomagenesis, induced by overexpressing PDGF-B in embryonic brain to mimic the first hit of gliomagenesis. In order to univocally tag each PDGF-transduced cell, we added to PDGF-transducing vector a degenerated barcode sequence. High-complexity libraries of barcoded retroviruses were injected in mouse embryos, and glioma masses were harvested at different stages. Next generation sequencing of barcoded gliomas revealed a strong bottleneck in glioma progression, since from thousands of initiated cells, just hundreds of them are able to undertake the first stages and, more strikingly, just few of them gain full malignancy and reach the high grade stage. To further study the clonal aspects of progression, we collected PDGF-B expressing cells from a low-malignancy tumor and orthotopically transplanted them in multiple mice. Interestingly, we found that the same pool of clones is able to evolve in a non-predetermined manner, indicating progression as a gradual evolution rather than the selection of pre-existing malignant clones.

Keywords: Molecular biology, Animal model, Cancer

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NO12 | Linking Sox2 activity to its downstream effectors in glioma maintenance, towards the definition of therapeutic targets

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We previously demonstrated an essential role of transcription factor Sox2, for the maintenance of neural cancer stem-like cells using a Sox2 conditional deletion mutant in a mouse model of PDGF-induced high-grade glioma (pHGG). Transplanting wild-type pHGG cells into mouse brain generated lethal tumors, but mice transplanted with Sox2-deleted cells remained tumor-free. Cultured Sox2-deleted pHGG cells show decreased growth-rate, activation of glial differentiation, and increased cell death compared to pHGG cells that express Sox2. Microarray analysis identifies early gene expression changes following Sox2 deletion. In this study, we manipulated the expression of various of these Sox2-target genes within non-Sox2- deleted pHGG cells, to ask if we can reproduce the loss of tumorigenicity obtained with Sox2 deletion, towards the definition of therapeutic targets. Overexpression of four downstream Sox2 target genes encoding known oncosuppressors (Zfp423, Ebf1, Hey2 and Cdkn2B), though not of others (Hopx, Wif1, Sdc4, Cryab, Rgs2), reproduces, to varying degrees, the Sox2-deleted pHGG cells phenotype: growth-rate reduction compared to normal pHGG cells and activation of glial differentiation. Moreover preliminary data show that Cdkn2b is essential to mediate the antioncogenic effect of Sox2 delection. Indeed Cdkn2b-deleted pHGG cells do not stop to proliferate after Sox2-loss. These in vitro data suggest that we identified four key effectors in the loss of tumorigenicity of Sox2-deleted pHGG cells. One next important step will be the transplantation of pHGG cells overexpressing the four factors in mouse brain to address the consequences on in vivo tumorigenicity.

Keywords: Molecular biology, Animal model, Stem cells, Cancer

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NO13 | Caspase-8 expression promotes neo-angiogenesis, tumor progression and chemotherapy resistance in human glioblastoma

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Caspase-8 is a key player in extrinsic apoptosis and its activity is often downregulated in cancer. However, human Caspase-8 expression is retained in some tumors, including glioblastoma, suggesting that it may support cancer growth in these contexts. Glioblastoma is the most aggressive primary brain tumor in adult nervous system and it is associated with a poor prognosis. Here we present data on a novel signal pathway through which Caspase-8 sustains neoplastic transformation in human glioblastoma cellular models. We could show that Caspase-8 promotes neo-angiogenesis and tumor growth in vitro and in vivo. Indeed, Caspase-8 promotes the activation of NF-kB transcription factor, which in turn enhances the expression and secretion of a panel of angiogenic cytokines such as VEGF, IL-6, IL-8, IL-1beta and MCP-1, leading to neovascularization and increased resistance of glioblastoma cells to temozolomide, a chemical compound largely used in chemotherapy. Importantly, the bioinformatics analysis of microarray gene expression data, derived from a set of high-grade human gliomas, showed that high Caspase-8 expression levels correlate with a worse prognosis. Caspase-8 might become a target for new anticancer drugs if it is possible to inhibit its cancer-boosting activity without interfering with its ability to promote cell death.

Keywords: Molecular biology, Cancer

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NO14 | Notch signaling inhibition in glioma cells alters the tumor microenvironment and disease progression

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Notch signaling is believed to be oncogenic in glioma, primarily by virtue of its stem cell promoting activity. However, surprisingly, inactivating mutations in Notch pathway components have been identified in human glioma subtypes and Notch inhibition dramatically accelerates tumor progression in mouse models of glioma. In order to investigate the mechanisms underlying the tumor suppressive function of Notch, we combined conditional genetics in mouse models with expression profile analyses of Notch-signaling-depleted tumors. We found that Notch inhibition in tumor cells downregulates expression of genes associated with quiescence of neural stem cells and releases expression of genes involved in stem cell activation and cell cycle progression, thereby promoting an active proliferative state. Unexpectedly, blocking Notch also downregulates genes associated with recruitment and activation of immune cells. This results in an impaired microglia activation that hampers a proper immune response at early stages of tumor formation. 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 regulating both intrinsic and extrinsic properties of tumor cells, and that distinct Notch receptor paralogues are differently engaged in glioma progression.

Keywords: Molecular biology, Immune system, Cancer

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NO15 | Functions of microglia derived extracellular vesicles

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Extracellular vesicles (EVs) are small membrane vesicles involved in different physiological processes. They contain and transfer information (in the form of proteins, lipids and nucleic acids) among cells in different body compartments. The cargo of EVs differs among different cell types [1,2], and the knowledge of EV production and transfer is relevant to better understand cell to cell communication under physiological and pathological conditions [3]. In the central nervous system (CNS), EVs are released by all cell types for cell-to-cell communication. Microglia cells in the CNS represent the innate immune cell population and EVs shed from microglial cells interact with neurons, modulating synaptic activity, and with other parenchymal cells, modulating inflammatory signalling [3]. In pathological conditions such as in glioblastoma multiforme (GBM), factors produced by tumor cells shift microglia phenotype from a pro-inflammatory, protective, to an anti-inflammatory phenotype that favors tumor progression and inhibit innate immune response [4]. We investigated the cross talk among the EVs released from microglia polarized in vitro toward a pro- or anti-inflammatory phenotypes and brain parenchymal cells. In particular, we want to shed light on the effects of EVs on microglial cells, astrocytes, neurons and GBM cells, performing in vitro co-culture experiments. These interactions are also investigated in vivo, studying the effect of EV administration to mice bearing GBM. Our preliminary data suggest that EVs released from microglia are powerful modulators of cell to cell communication in the brain, both in physiological and pathological conditions. – *1. Sica and Mantovani, J Clin Invest. 2012; 2. Li et al., Cli Dev J. 2013; 3. Garzetti et al, J Leukoc Biol. 2014; 4. Robbins and Morelli, Nat Imm. 2014*

Keywords: Molecular biology, Animal model, Brain Cancer

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Poster Session 1 (June 29th, 17:00-18:15)

NEURODEGENERATION | pp. 28-53

ND1 | Giuseppe Arcuria
12 Red Squares App-Coo-Test A valid touch screen application for the quantitative assessment of the upper limb movement disorders in patients with Friedreich's ataxia.

ND2 | Giuseppe Arcuria
App-Coo-Balance-Test A new application to assess static and dynamic balance in patients with cerebellar ataxia.

ND3 | Alice Gualerzi
The biophotonic challenge in neuroscience: development of innovative methods to explore the neurodegeneration mechanisms and discover new biomarkers.

ND4 | Davide Marangon
Inhibition of miR-125a-3p promotes OPC maturation following lysolecithin induced demyelination.

ND5 | Maria Serpente
Non-coding RNAs (ncRNAs) content of Plasma Neural Derived Exosomes (NDEs): new potential biomarkers for Alzheimer's Disease (AD) diagnosis.

ND6 | Maria Piera Cadoni
Understanding the role of VAPB in peripheral blood mononuclear cells of patients affected by sALS.

ND7 | Giulia Santamaria
Mesenchymal Stem Cells Conditioned Secretome: A New Frontier Therapeutic Strategy In The Treatment Of Alzheimer's Disease.

ND8 | Monica Nizzardo
Morpholino conjugated with Cell Penetrating Peptides: a promising therapeutic strategy for Spinal Muscular Atrophy symptomatic cases.

ND9 | Davide Lecca
MicroRNA-125a-3p negatively regulates oligodendrogli al maturation and re-myelination: molecular mechanisms and clinical implications in multiple sclerosis.

ND10 | Carola Torazza
Exosome-shuttled miRNAs derived from mesenchymal stem cells affect the phenotype of spinal cord astrocytes isolated from late disease phase SOD1G93A mice.

ND11 | Davide Aprile
TBC1D24 regulates axonal outgrowth and membrane trafficking at the growth cone in rodent and human neurons.

ND12 | Chloe Hall
Investigating anticonvulsant drugs as potential treatments for Alzheimer's disease.

ND13 | Amel Falco
Direct reprogramming of human fibroblasts to explore neurodegeneration in amyotrophic lateral sclerosis.

ND14 | Virginia Protto
Role of proNGF and effects of electroacupuncture in the septo-hippocampal system of diabetic rats.

ND15 | Eriola Hoxha
Roles of Elovl5 on neuronal function; new insights on spino-cerebellar ataxia type 38.

ND16 | Chris Greene
Cerebrovascular contributions to seizure development and epilepsy.

ND17 | Paola Fabbrizio
P2X7 Receptor Activation Modulates Autophagy in SOD1-G93A Mouse Microglia.

ND18 | Carmen Murano
Modulation of the intrinsic neuronal excitability by multifunctional liposomes tailored for treatment of Alzheimer's disease.

ND19 | Annabel Curle
Neurodegeneration and behavioural and cognitive alterations in mouse model of Autosomal Dominant Osteopetrosis type-2 (ADO2).

ND20 | Nicole Hanley
Tight Junctions of the Blood-brain Barrier as a Therapeutic Target in Epilepsy.

ND21 | Ilaria Lagorio
White matter structural asymmetries and language impairment: MR-DTI and fMRI study.

ND22 | Simona Rossi
Failure of nuclear mRNA export in a cellular model of C9orf72-linked ALS.

ND23 | Carmen Carbone
The Mitopark mouse suggests a causal role for HCN loss of function in the progression of Parkinson's disease.

ND24 | Alice Migazzi
Protein Arginine Methyltransferase 6 is a novel modifier of Huntington's disease pathogenesis.

ND25 | Marco Stazi
A neuronal triggers specific RNAs, local translation of Annexin A2 and cytoskeletal remodeling in Schwann cells.

ND26 | Matteo Pedrazzoli
Glucocorticoid receptor modulation alters spine plasticity, inflammation and behavior performance in 3xTg-AD mice.

NEUROINFLAMMATION | pp. 54-67

NI1 | Valentina Murtaj
Monitoring Neuroinflammation with the TSPO tracer [18F]VC701, after LPS systemic administration in male/female adult and aged mice.

NI2 | Martina Biagioni
Pathogenic role of inflammation in Retinitis Pigmentosa: a target for preventing daylight vision loss.

NI3 | Cristina Mantovani
Role of IL-1 signaling in controlling synaptic function

NI4 | Raffaele Simeoli
Activity assays for evaluation of clinical grade MSC-EV anti-inflammatory properties for use in treatment of drug-resistant epilepsy in children.

NI5 | **Davide Visigalli**
Biomarkers to monitor myelin loss and remodeling.

NI6 | **Natalia Blügental**
LPS-induced inflammation affects biosynthesis of neurosteroids in the chicken pineal gland.

NI7 | **Jessica Garau**
Hydroxychloroquine treatment on lymphoblasts derived from patients with Aicardi-Goutières syndrome.

NI8 | **Alessia Capone**
Cell activation, death and motility of human Th17 cells are finely regulated by specific transcription factor.

NI9 | **Gloria Donninelli**
Anti-inflammatory effect of IL-9 in multiple sclerosis as achieved by modulation of macrophage activation.

NI10 | **Maria Velasco**
Amyloid plaque pathology triggers expression of mechanosensing Piezo1 channels in astrocytes.

NI11 | **Simona Perga**
Moving from systemic to central nervous system inflammation: the role of A20 in the neuropathology of Multiple Sclerosis.

NI12 | **Federica Buffolo**
In vitro and in vivo characterization of REST activity and expression under neuroinflammatory conditions.

NI13 | **Martina Di Nunzio**
Novel approaches to target neuroinflammation: the role of microglia during epileptogenesis.

NI14 | **Marta Lombardi**
Microglia-derived extracellular vesicles regulate the recruitment, proliferation and differentiation of oligodendrocyte precursor cells.

NEURO PLASTICITY | pp. 68-82

NP1 | **Giulia Nato**
Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion.

NP2 | **Bruno Sterlini**
Proline-rich Transmembrane protein 2 (PRRT2) controls neuronal excitability by negatively modulating Na+ channel activity.

NP3 | **Valentina Zamboni**
Hyperactivity of Rac1-GTPase pathway impairs neuritogenesis of cortical neurons by altering actin dynamics.

NP4 | **Loredana Poeta**
Neurodevelopment disorders linked to ARX mutations in different genetic models: how to compensate the damage.

NP5 | **Laure Tabouy**
Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders.

NP6 | **Giuseppe Aceto**
GSK3β in somatosensory Cortex: modulation of timing-dependent long-term depression through direct phosphorylation of Kv4.2 channels.

NP7 | **Gaia Elena Berto**
TTC3: a Down syndrome gene involved in neuronal migration.

NP8 | **Caterina Gasperini**
Identification and functional investigation of mammalian piRNA-pathway in adult hippocampal neurogenesis.

NP9 | **Marco Fogli**
Excitotoxic lesion-induced striatal neuroblasts have a transient life but receive both local and long-range connections.

NP10 | **Francesca Veneri**
Preliminary characterization of a knock-in mouse model for the hyperglycosylating P0D61N Myelin Protein Zero mutation.

NP11 | **Valentina Cerrato**
Multiple origins and modularity in the spatiotemporal emergence of cerebellar astrocyte heterogeneity.

NP12 | **Matteo Di Segni**
Xlr gene as a new candidate for susceptibility to cocaine addiction.

NP13 | **Vanessa Cossu**
Brain Metabolic Response to Metformin: the role of Endoplasmic Reticulum.

NP14 | **Isabella Crisci**
Fate mapping of adult hippocampal neural stem/progenitor cells in a model of neuroinflammation.

NP15 | **Erika Mifsud**
Transgenerational long lasting effects on motivational system of adolescent exposure to nicotine, alcohol and cannabinoids in rats.

NEURO-ONCOLOGY | pp. 83-87

NO1 | **Gianmarco Pallavicini**
Citron kinase inactivation inhibits medulloblastoma progression by inducing apoptosis and cell senescence.

NO2 | **Lorenza Rogna**
Isolation and characterization of putative stem cells from human meningiomas.

NO3 | **Paola Infante**
Itch/βarrestin2-dependent non-proteolytic ubiquitylation of SuFu controls Hedgehog signalling and medulloblastoma tumourigenesis.

NO4 | **Marco Pizzocri**
mApoE-Functionalized Liposomes: a dual-task strategy to Cross the Blood Brain Barrier and to Target Glioblastoma Stem-Cells.

NO5 | **Giuseppe Taurino**
Oligodendroglioma cells are dependent on extracellular glutamine but do not exhibit glutamine anaplerosis.

Poster Session 2 (June 30th, 10:00-11:00)

NEURODEGENERATION | pp. 88-108

ND27 | **Davide Di Censo**
A novel versatile and automated tracking software (TrAQ) for the characterization of behavioural rodent models.

ND28 | **Roberta Schellino**
Pharmacological JNK-pathway inhibition reduces severity of spinal muscular atrophy disease in a mouse model of SMAII.

ND29 | **Francesca Montarolo**
Nuclear receptor related 1 protein (Nurr1) in attention deficit hyperactivity disorder (ADHD): searching for a disease murine model.

ND30 | **Daniela Giovannini**
Atrial Natriuretic Peptides protects dopaminergic neuron-like cells from neurotoxin-induced damage via Up-Regulation of the Wnt/beta-Catenin Pathway.

ND31 | **Francesca Sironi**
Effect of C9orf72 deletion on the pathology progression in a familial amyotrophic lateral sclerosis (ALS) mouse mode.

ND32 | **Cecilia Pandini**
Investigating the involvement of coding and long non coding proto-oncogene c-MYC in ALS.

ND33 | **Ilaria Piano**
Chronic treatment with antioxidant nutraceutical molecules slow down the degenarative process in an animal model of retinitis pigmentosa.

ND34 | **Simona Baldassari**
Modelling autosomal dominant lateral temporal epilepsy (ADLTE) with Human iPSC-derived Neurons.

ND35 | **Carlo Brighi**
Modeling fragile x syndrome with human IPSC-derived neurons.

ND36 | **Conor Delaney**
A role for Colony Stimulating Factor 1 Receptor Signalling in the generation of cerebrovascular and BBB pathology.

ND37 | **Matilde Balbi**
Pharmacological treatment with CTEP, an mGluR5 negative allosteric modulator, in SOD1G93A mice.

ND38 | **Laura Pasetto**
Features of frontotemporal lobar degeneration in the cyclophilin a knock-out mice.

ND39 | **Claudia Rebosio**
The role of Group I metabotropic glutamate autoreceptors in ALS.

ND40 | **Harriet Oxford**
A bone disease in the retina; how Autosomal Dominant Osteopetrosis Type-II (ADO2) affects inner retinal circuitry.

ND41 | **Laura Clara Grandi**
The thalamocortical coherence in acute and chronic dopamine depletion.

ND42 | **Alessio Cavalli**
Neuroprotective effects of saffron and its components in animal model of retinal neurodegeneration.

ND43 | **Giulia Guidotti**
Motor neuron degeneration and disease progression in amyotrophic lateral sclerosis are accelerated by the disruption of the astrocytic TNFR1-GDNF axis.

ND44 | **Antonella Marte**
The protein kinase LRRK2 is involved in glutamate release by interacting and phosphorylating synapsin I in nerve terminals.

ND45 | **Mario Villa González**
mTORC1 in primary neurons: analysis of the role of oxygen and glucose in its kinase activity.

ND46 | **Ilaria Rosa**
Evaluation of functional asymmetry in hemiparkinsonian rat using theTail Suspension Test.

ND47 | **Claudia Cirotti**
Targeting the mitochondria-tyrosine kinase axis to prevent age-associated neuronal decline.

NEUROINFLAMMATION | pp. 109-117

NI15 | **Ilaria Lisa Dettori Gaviano**
Protective effect of the histamine H4 receptor antagonist, JNJ7777120, in a rat model of cerebral ischemia.

NI16 | **Stefano Raffaele**
Exploring the cross-talk between microglia and oligodendrocyte progenitors in cerebral ischemia.

NI17 | **Samuele Tardito**
White matter microstructure alterations correlate with terminally differentiated CD8+ effector T cell depletion in the peripheral blood in mania: combined DTI and immunological investigation in the different phases of bipolar disorder.

NI18 | **Morris Losurdo**
Analysis of the Immunomodulatory Potential of Mesenchymal Stem Cell-derived Extracellular Vesicles in a Model of Alzheimer's Disease.

NI19 | **Tommaso Carlucci**
Coenzyme A metabolism controls pathogenic features in myelin-specific T cells by linking metabolic reprogramming to alteration of intracellular signaling pathways.

NI20 | **Rossana Di Martino**
Role of mitochondria in the activation of neuroinflammation in A53T mice a model of Parkinson's disease.

NI21 | **Giuseppe Caruso**
Carnosine prevents amyloid-beta-induced inflammation in microglial cells.

NI22 | **Maria Chiara Trolese**
Role of CXCL13 in the patophysiology of ALS: study in transgenic SOD1G93A mice.

NI23 | **Camilla Negri**
Differential local tissue permissiveness influences the final fate of GPR17-expressing oligodendrocyte precursors in two distinct models of demyelination.

NEURO PLASTICITY | pp. 118-125

NP16 | **Guendalina Olivero**
Pharmacological characterization of NMDA autoreceptors regulating glutamate release in the hippocampus with anti-GluN antibodies: relevance to anti-NMDA receptor autoimmune diseases.

NP17 | **Valeria Calabrese**
Electrophysiological and biochemical characterization in transgenic mouse model with lack of serotonergic synthesis.

NP18 | **Beatrice Uguagliati**
Treatment with a beta 2-adrenergic agonist restores dendritic pathology in a mouse model of Down syndrome.

NP19 | **Anna Binda**
HCN1 novel mutations in familiar generalized epilepsy.

NP20 | **Serena Riccitelli**
Effects of Bmal1 gene deletion in GLAST positive cells on retinal morphology and physiology.

NP21 | **Erica Berretta**
Intergenerational influences of parental Approaching/Avoiding phenotypes on offspring behaviors in C57BL/6J mice.

NP22 | **Irene Rubio-Ferrera**
Specification of the Drosophila Orcokinin A neurons.

NP23 | **Linda Scaramuzza**
Chronic enhancement of neuronal activity promotes morphological and functional in vitro maturation of Mecp2 null developing neurons.

NEURO-ONCOLOGY | pp. 126-130

NO6 | **Irene Appolloni**
Modeling immunoediting in glioma progression.

NO7 | **Ignazio De Trizio**
Nucleolin expression in the Neurovascular Unit as a potential regulator in glioblastoma neovascularization.

NO8 | **Nadin Hoffman**
New xenogeneic engraftment assay in immune-competent mouse embryos for Glioblastoma multiforme.

NO9 | **Silvia Rancati**
Exploitation of the synergic action of a microRNA pool as differentiation therapy of Glioblastoma Multiforme.

NO10 | **Simona Paglia**
Faraway, so close! Modelling brain cancer in Drosophila.

Poster Session 3 (June 30th, 14:00-15:30)

NEURODEGENERATION | pp. 131-164

ND48 | **Martina Lorenzati**
Axo-glial interplay in oligodendrocyte specification and myelination: role of JNK1.

ND49 | **Valentina Latina**
Ubiquitin-proteasome system is early involved in dying-back presynaptic degeneration induced by NGF-withdrawal in septo-hippocampal neurons.

ND50 | **Silvia Ravera**
Characterization of the mitochondrial aerobic metabolism at the pre- and perisynaptic districts of the SOD1G93A mouse model of amyotrophic lateral sclerosis.

ND51 | **Mattia Di Paolo**
Physiopathology of light exposure in the eye.

ND52 | **Michela Taiana**
Progress in C9-ALS therapy: patient specific iPSC-derived lines as in vitro model to test Morpholino oligomers efficacy.

ND53 | **Simone Pelassa**
A2a-D2 heterodimers on striatal astrocytes: biochemical and biophysical evidence.

ND54 | **Marco Fois**
Monitoring of glucose, lactate and motion in brain of freely moving rats by simultaneous telemetry.

ND55 | **Maria Giovanna Garone**
Development of new cranial motor neuron differentiation protocol from human iPS cells carrying ALS mutations.

ND56 | **Andrea Carvelli**
Long non-coding RNAs in motorneurons differentiation and degeneration.

ND57 | **Martina Albini**
Different pro-nerve growth factor protein variants elicit different biological outcome in PC12 cells.

ND58 | **Andrea Bacciu**
MPTP Induced changes in behavioral test scores and extracellular levels in the striatum of freely moving mice.

ND59 | **Anna Dolcimascolo**
Neuroprotective effects of stray-dried rosmarinus officinalis powder extract in OGD-injured human neuronal-like cells.

ND60 | **Silvia Fancello**
Protective effect of Genistein-loaded transferosomes against H_2O_2-induced oxidative stress in PC12 cells.

ND61 | **Matteo Bordoni**
Innovative 3D cellular model for the study of neurodegenerative diseases.

ND62 | **Valentina Fantini**
Mitophagy impairment in a peripheral model of ALS.

ND63 | **Federico Salaris**
Transcriptome and proteome analysis of FXS patient-derived iPSC lines to investigate FMRP role and its interactors during neural development.

ND64 | **Laura Civiero**
Lysosomal function and dysfunction in astroglia.

ND65 | **Francesco Russello**
Autophagy enhancement rescues neurons from toxicity induced by amyloidogenic peptides.

ND66 | **Alessandro Mariani**
Neurotoxic impacts of neonicotinoid insecticides in a mouse model.

ND67 | **Elena Bruzzone**
Oleuropein aglycone stabilizes the monomeric α-synuclein and favours the growth of non-toxic aggregates.

ND68 | **Paola Arrigo**
Development and characterization of a new electrochemical sensor for in vitro study of dopamine auto-oxidation.

ND69 | **Elena Chierto**
Novel models of stretch-induced injury in mouse oligodendrocytes and organotypic culture of cerebellar slices: study of pathophysiological mechanisms.

ND70 | **Elena Signorino**
Evaluation of immune system status in a SMA murine model.

ND71 | **Elena Chiricozzi**
II3NeuAc-Gg4: a new neurotrophic player.

ND72 | **Pellegrino Lippiello**
Early deficits of cerebellar plasticity in a mouse model of Alzheimer's disease.

ND73 | **Chiara Bacchella**
Amyloid and prion-copper complexes: redox reactivity in membrane-like environment.

ND74 | **Manuela Leri**
Oleuropein Aglycone and its metabolite Hydroxytyrosol interfere differently with toxic Aβ 1-42 aggregation.

ND75 | **Andrea Capucciati**
Neuronal proteins and 3-hydroxykynurenine: implications in neurodegenerative diseases.

ND76 | **Eliana Lo Presti**
Coordination of non-heme iron to a fragment of alfa-synuclein C-terminus and implication in oxidative stress.

ND77 | **Greta Forcaia**
Modulation of intracellular Ca2+ concentration in brain microvascular endothelial cells actively induced by brain targeted liposomes.

ND78 | **Giuseppina Natale**
Electrophysiological, molecular and behavioral effects of Transcranial Magnetic Stimulation (TMS) in the early model of Parkinson.

ND79 | **Tiziana Bonifacino**
Metabotropic glutamate receptor type 5 effects on ALS progression.

ND80 | **Marta Giannini**
TDP-43 and R loops relation in Amyotrophic Lateral Sclerosis.

ND81 | **Margherita Romeo**
Caenorhabditis elegans as simplified animal model to elucidate the molecular mechanisms underlying the propagation of tau pathology in traumatic brain injury.

NEUROINFLAMMATION | pp. 165-184

NI24 | **Silvia Penati**
Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline.

NI25 | **Arinna Bertoni**
Characterization of a novel CAPS Knock-in mouse model to exploit novel approaches for the modulation of the NLRP3 inflammasome.

NI26 | **Benedetta Parodi**
Activation of the hydroxycarboxylic acid receptor-2 by monomethyl fumarate triggers different pathways in different cell types.

NI27 | **Maria Cristina Mariani**
Early, norepinephrine-dependent, activation of the hematopoietic niche upon induction of experimental autoimmune encephalomyelitis.

NI28 | **Federica Irene Cherchi Fusco**
Adenosine A2B receptors and sphingosine kinase/sphingosine-1-phosphate signalling axis control maturation of oligodendrocyte precursor cells in vitro.

NI29 | **Alessandra Carta**
Occurrence of neurodevelopmental disorders in children of women with multiple sclerosis treated with natalizumab during pregnancy.

NI30 | **Alejandra Quiroga**
Blocking of CSFR1 impairs oligodendrocyte differentiation.

NI31 | **Clarissa Catale**
Exploring the role of the immune system in the susceptibility to cocaine use disorder following early-life stress.

NI32 | **Chiara Marini**
MiRNAs content in the exosomes derived from immunomodulatory MSC as regulators of neuroinflammation in ALS.

NI33 | **Serena Palmeri**
Alemtuzumab differentially affects effector and regulatory immune cell subsets.

NI34 | **Mariya Malova**
MRI-diagnosed white matter lesions in the brain of VLBW babies: risk factor analysis.

NI35 | **Laura Costanza De Angelis**
Placental inflammation and mri-detected brain lesions in very premature infants.

NI36 | **Francesca De Vito**
Monomethyl fumarate prevents inflammation-driven synaptopathy by counteracting miR-142-3p action in experimental MS.

NI37 | **Laura Costanza De Angelis**
Punctate white matter lesions (pwml) and adenosine blood levels in premature infants.

NI38 | **Laura Costanza De Angelis**
Blood adenosine levels in very low birth weight infants and neurological follow-up at 12 and 24 months.

NI39 | **Maria Cellerino**
Personalizing health care in Multiple Sclerosis using systems medicine tools: presentation of the cytomics data.

NI40 | **Manuela Medelin**
Bridging pro-inflammatory signals, synaptic transmission and protection in spinal explants in vitro.

NI41 | **Ilaria Prada**
Glia-to neuron transfer of miRNAs via extracellular vesicles: a new mechanism underlying inflammation-induced synaptic alterations.

NI42 | **Valentina Petrosino**
Possible involvement of the Repressor Element 1-Silencing Transcription factor in the pathological process of experimental autoimmune encephalomyelitis.

NI43 | **Giovanni Ferrara**
A possible role for nerve glial antigen 2 in dendritic cell activation.

NEURO PLASTICITY | pp. 185-202

NP24 | **Nunzio Vicario**
Sonic hedgehog signalling pathway on neural stem cells during regenerative processes in a mouse model of motoneuronal loss.

NP25 | **Sara Bonzano**
Adult neural stem cell/progenitor fate potential in vivo is controlled by COUP-TFI within the adult hippocampus.

NP26 | **Costanza Giannì**
Functional connectivity adaptation to disease progression in Multiple Sclerosis: an fMRI study.

NP27 | **Roberta Parolisi**
Unveiling neuromodulatory functions of NG2-expressing glia.

NP28 | **Virginie Sottile**
Fasudil treatment promotes enhanced gliogenesis of neural stem cells in vitro.

NP29 | **Chiara Villa**
A novel mutation in the CHRNA2 gene detected in an Italian NFLE patient.

NP30 | **Laura Carbonari**
IL-15/IL-15Ralpha signaling modulates hippocampal synaptic transmission.

NP31 | **Alizée Pann**
Whose hand is it? The role of EBA in body ownership.

NP32 | **Marielyne Macel**
Supramammillary nucleus modulation of hippocampal activity.

NP33 | **Patrizia Piotti**
Age related ventriculomegaly in dogs trained to lay in an fMRI.

NP34 | **Francesca Romana Rizzo**
Interleukin-6 affects clinical course and brain plasticity in Multiple Sclerosis patients.

NP35 | **Arianna De Rosa**
The striatal GTPase Rhes modulates cocaine-mediated behavioural responses in mice.

NP36 | **Rita Turnaturi**
MOR/DOR targeting: an useful strategy in pain management.

NP37 | **Chiara La Rosa**
Heterogeneity of cortical layer ii immature neurons in mammals.

NP38 | **Francesca Cisani**
Antibodies for the pharmacological characterization of presynaptic, release-regulating mGlu2-preferring and mGlu3-preferring auto-receptors in mouse CNS.

NP39 | **Eleonora Daini**
Regional and cellular distribution of H3.3a and H3.3b histone variants in adult mouse brain.

NP40 | **Benedetta Foglio**
Expression of the transcription factors SOX2 and COUP-TF1 in the developing human brain.

NP41 | **Patrizia Piotti**
Family dogs as a model for the study of age-related cognitive decline.

NEURO-ONCOLOGY | pp. 203-207

NO11 | **Davide Ceresa**
Genetic barcoding reveals the clonal dynamics of glioma progression.

NO12 | **Cristiana Barone**
Linking Sox2 activity to its downstream effectors in glioma maintenance, towards the definition of therapeutic targets.

NO13 | **Giulia Fianco**
Caspase-8 expression promotes neo-angiogenesis, tumor progression and chemotherapy resistance in human glioblastoma.

NO14 | **Elena Parmigiani**
Notch signaling inhibition in glioma cells alters the tumor microenvironment and disease progression.

NO15 | **Carmela Serpe**
Functions of microglia derived extracellular vesicles.

Notes

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