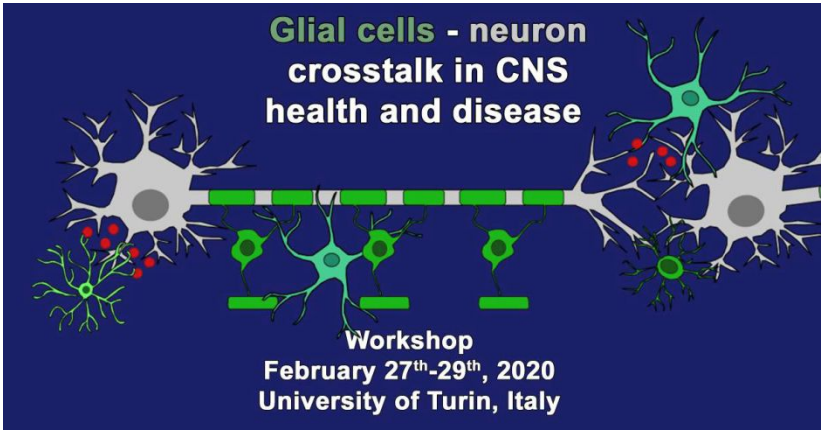

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UNIVERSITÀ DEGLI STUDI DI TORINO
Department of Neurosciences "Rita Levi Montalcini"
Department of excellence

Dottorato di Ricerca in Neuroscienze
Università degli Studi di Torino

NICO
Neuroscience Institute Cavalieri Ottolenghi

SCIENTIFIC PROGRAM

1st day (February 27 2020)

8:30 Registration

9:00 Opening Ceremony Prof. Alessandro Mauro, Director of the Dept of Neuroscience, Univ of Turin; Prof Alessandro Vercelli, Vice-Rector for Biomedical Research of the Univ of Turin and Director of the Neuroscience Institute Cavalieri Ottolenghi, Turin; Prof. Marco Sassoè-Pognetto, Director of the Doctorate Program in Neuroscience of the Univ of Turin.

- SESSION I -
Glia-neuron crosstalk in CNS functions and memory

9:30 Beatrice Vignoli (Univ of Trento, Italy) - *Glial microdomains confine a "molecular memory" enabling long-term information storage for memory consolidation*

10:00 Gertrudis Perea (Cajal Inst, Madrid, Spain) - *GABAergic control of Astrocyte-Neuron signaling in cortical circuits*

10:30-11:00 Coffee break

11:00 Bernadette Basilico (Inst of Science and Technology, Austria) - *Constitutive role of microglia in maintaining synaptic function in mouse hippocampus*

11:30 Christophe Galichet (Francis Crick Inst, London, UK) – *Proliferation and differentiation of Median Eminence Oligodendrocyte Precursor Cells is required for normal function of the hypothalamo-pituitary axis.*

– SESSION II –
Neuroscience Research Methodology

12:00 Marco Perugini (Univ Milano Bicocca, Italy) - *The signal and the noise: Some lessons learned from the replicability crisis in Psychology*

12:30-13:30 Lunch

POSTER SESSION
13:30 - 15:30

Selected oral communications

15:40 Manuela Santo (Sissa, Italy) - *Molecular control of astrogenesis in mouse and human cortex. Role of the transcription factor Foxg1*

16:00 Valeria Bortolotto (Univ of Piemonte Orientale, Italy) - *Novel molecular participants in astrocyte-neural progenitor cells crosstalk in murine hippocampus*

16:20 Corrado Cali (Univ of Turin, Italy and KAUST, Saudi Arabia)- *Investigating Neuroanatomical basis of Brain-Energy Metabolism using 3D models and VR tools*

16:40 Carola Torazza (Univ of Genoa, Italy) - *mGluR5 as a target to modulate the reactive phenotype of astrocytes in the S001G93A mouse model of amyotrophic lateral sclerosis*

17:00-17:20 Coffee break

17:20 Alice Filippini (Unit of Genetics-IRCCS, Brescia, Italy)- *The chaperone Clusterin interferes with the clearance of extra cellular aSynuclein aggregates by astrocytes: implications for aSynuclein spreading and pathology*

17:40 Roberta Parolisi (Univ of Turin, Italy) - *Extracellular Vesicles mediate detrimental and protective action of microglia on myelin lesion*

18:00 Laura Maggi (Sapienza Univ, Italy) - *Interplay between inflammation and neural plasticity: Role of microglia in antidepressant efficacy*

2nd day (February 28 2020)

- SESSION III - Glial cells in CNS aging and pathology

9:00 Chiara Rolando (Univ. of Basel, Switzerland) - *Post-transcriptional regulation of astrocytes: a novel facet to counteract brain aging*

9:30 Rosa Paolicelli (Univ. of Lausanne, Switzerland) - *Synaptic consequences of selective microglial TDP-43 depletion*

10:00 Fabia Filipello (Washington University, St. Louis, USA) - *Microglia immune receptor TREM2 in neurodegenerative diseases*

10:30 Coffee break

11:00 Chiara Zurzolo (Institut Pasteur, Paris, France) - *Roles of Tunneling nanotubes (TNTs) and astrocytes in neurodegenerative diseases*

11:30 Davide De Pietri Tonelli (IIT, Genova, Italy) - *Bmal1-dependent control of astrocyte development and function in brain*

12:00-13:00 Lunch

13:30 Bus transfer to NICO

THEORETICAL-PRACTICAL TRAINING
(bus transfer to the Neuroscience Institute Cavalieri Ottolenghi)
14:30-18:00

- **Dr Christian Feuillet, Miltenyi Biotec/Lavision Biotec** – Light sheet microscopy: Principles and applications in neuroscience

- **Dr Elisa Zuffi, Miltenyi Biotec** - Tools for neural cells research: mouse adult brain dissociation, cell isolation and analysis

Bus transfer to downtown and get together evening

3rd day (February 29 2020)

- SESSION IV -
Glial cells in neurodegeneration

9:00 Eriola Hoxha (Univ of Torino, Italy) - *Disruption of myelin phospholipid composition impairs action potential conduction in a murine model of Spinocerebellar Ataxia type 38*

9:30 Francesca Boscia (Univ of Naples, Italy) - *Enhancing D-aspartate signaling to promote (re)myelination*

10:00 Nunzio Iraci (Univ of Catania, Italy) - *Exosomes as natural messengers of bioactive molecules in the glial-neuronal signaling in Parkinson's disease*

10:30 Coffee break

11:00 Antonella Consiglio (Univ of Barcelona, Spain) - *Novel insights into Parkinson's disease through iPSC-based technology*

- SESSION V -
Neuroscience Research Methodology

11:30 Ludovic Telley (Univ of Lausanne, Switzerland) - *The upcoming role of multi-omics integration in unfolding central nervous development*

12:00-13:30 Lunch and Poster session

- SESSION VI -
Glial cells in CNS development and developmental disorders

14:00 Maria Cecilia Angulo (INSERM Paris, France) - *Developmental cell death regulates lineage-related interneuron-oligodendroglia functional clusters and oligodendrocyte homeostasis*

14:30 Enrica Boda (University of Torino, Italy) - *Inherent heterogeneity of postnatal oligodendrocyte progenitors: lessons from a microcephaly model*

15:00 Valentina Cerrato (University of Torino, Italy) - *The ontogenesis of astrocytes diversity: a remarkably orderly process necessary for the correct cerebellar development and functioning*

15:30 Robert Beattie (Institute of Science and Technology Austria) - *Molecular Mechanisms Regulating Gliogenesis in the Neocortex*

16:00 Closing remarks

LECTURES

Glial microdomains confine a molecular memory enabling long-term information storage for memory consolidation

Beatrice Vignoli

Department of Physics/Nanoscience Laboratory/Department of Cellular, Computational and Integrative Biology (CIBIO) University of Trento, Povo (TN), 38123, Italy

Memory consolidation requires peri-synaptic glia (microdomains) for pro-neurotrophin recycling; but whether this lays a mechanistic foundation for long-term information storage remains enigmatic. Here we demonstrate that persistent synaptic strengthening invited glia microdomains to convert initially internalized (pro)-brain-derived neurotrophic factor (proBDNF) into pro-domain (BDNFpro) and mature BDNF (mBDNF) for synaptic re-use. While BDNF typically phosphorylate TrkB, we uncovered a previously unsuspected role for the cleaved pro-domain, which provide targeting of TrkB/SorCS2 receptor complex at post-synaptic sites. In this way, cooperating BDNFpro and mBDNF reinforce TrkB signaling to sustain long-term potentiation (LTP) and to retain memory in the novel object recognition behavioral test. Thus, the switch from one inactive state to multi-functional one of the proBDNF provides post-synaptic changes that survive the initial activation ("molecular memory"), adding plasticity features to spine functions and global circuits integration for memory consolidation.

GABAergic control of astrocyte-neuron signaling in cortical circuits

Gertrudis Perea

Neuron-Glia Networks Lab, Functional and Systems Neurobiology Department, Cajal Institute, Madrid, Spain

Interneurons are involved in fundamental aspects of brain function playing a key role in the operation of neuronal networks. Thus, fast time course of GABAergic signaling controls the neuronal outputs. The GABAergic signaling to astrocytes has been previously shown, being able to activate intracellular Ca^{2+} signaling. However, the impact of the interneuron-astrocyte signaling into neuronal network operation remains poorly defined. We will discuss new evidence of how astrocytes in the medial prefrontal cortex (mPFC), a critical region involved in goal-directed behaviors, sense and decode interneuron activity; in particular, we will show how astrocytes respond to a particular subpopulation of interneurons, the parvoalbumin positive cells (PV+), which are critical for proper network dynamics. Combining electrophysiological recordings and optogenetics, we will show how astrocytes modulate the inhibition/excitation balance controlling decision making in behaving mice. We have found that specific genetic ablation of $GABA_B$ receptors (GABABRs) in astrocytes profoundly alters gamma oscillations (30-60 Hz) and firing properties of those neurons. Furthermore, optogenetic stimulation of mPFC astrocytes with melanopsin was sufficient to enhance cortical inhibitory synaptic transmission, firing rate of local neurons, gamma oscillations, and improved cognitive performance in wild-type mice.

Our work identifies astrocytes as a hub for controlling inhibition in cortical circuits, providing an independent pathway for the behaviorally relevant midrange time-scale regulation of cortical information processing and consistent goal-directed behaviors.

Constitutive role of microglia in maintaining synaptic function in mouse hippocampus

Bernadette Basilico

Institute of Science and Technology (IST) Austria, Klosterneuburg, Austria.

Microglia are the resident immune cells of CNS, traditionally studied in contexts of trauma, injury and disease. Emerging roles of microglia are related to their ability in continuously scanning the brain environment, maintaining tissue homeostasis and participating in network formation. More and more studies have shed light on the physiological roles of microglia during development and adulthood, highlighting their participation in circuit refinement and maturation.
However, how microglia contribute to neuronal function in the mature CNS is not well understood. Depleting microglia from the brain or altering specific neuron-microglia signaling pathways provided evidence that microglia are critical for proper brain development.
We took advantages of two different models to study how microglia shape neuronal networks in hippocampus. Firstly, we demonstrated that the disruption of neuron-microglia interactions mediated by the CX3CL1/CX3CR1 axis strongly impairs developmental maturation of excitatory hippocampal synapses, leading to abnormal functional features of the presynaptic terminal. More recently, adopting a pharmacological depletion strategy, we highlighted the role for microglia in the maintenance of glutamatergic synapses in hippocampus, contributing constitutively in supporting neuronal functions throughout the life.

Proliferation and differentiation of Median Eminence Oligodendrocyte Precursor Cells is required for normal function of the hypothalamo-pituitary axis.

Christophe Galichet

The Francis Crick Institute, 1 Midland Road, London, NW1 1AT, UK

The hypothalamo-pituitary axis is an essential regulator of metabolism in vertebrates. Upstream of the axis, the hypothalamus centralizes peripheral information and controls accordingly secretion from the six pituitary endocrine cell types. This control is achieved by hypothalamic neurohormones that are secreted either directly into the pituitary, from axonal termini that reach the gland, or into a bed of capillaries located at the base of the third ventricle, at the median eminence (ME), where the hypophyseal portal system collect and deliver them to the gland. Pituitary hormones act in turn on different endocrine glands, tissues and organs. Consequently, pituitary hormones deficiencies, or hypopituitarism, affect important physiological functions and are associated with significant morbidity.

Mutations in the HMG box transcription factor SOX3, both in human and mice, are associated with hypopituitarism. The aetiology of the deficiencies is unclear but we showed that the protein is expressed in the adult murine hypothalamus so its loss is likely to affect this region, and indirectly have an effect on pituitary hormones [1]. We have here further investigated the role of SOX3 and we first demonstrate that pituitary hormone deficiencies appear after weaning. Moreover, conditional deletion of the gene confirms the neural origin of the phenotype. In adult hypothalami, SOX3 is mainly expressed in cells lining the third ventricle, especially in tanycytes, specialised glial cells of the ME, recently shown to have stem cell properties [2] and also in NG2 glia, also known as Oligodendrocyte Precursor Cells (OPCs), in the body of ME [3]. We find that in *Sox3* mutants proliferation of these two types of progenitors is affected, exclusively post-weaning, as the hypopituitarism appears. We then analysed NG2 glia fate by performing lineage-tracing experiments using PDGFRa-CreERT2. In *Sox3*-null mice we observed that these do not differentiate into oligodendrocytes. We therefore propose that this reduction in NG2 glia and oligodendrocytes underline development of hypopituitarism in *Sox3* mutants, where we observe a reduction in pituitary size post-weaning, likely due to defects in endocrine cell maturation. Finally, aspirin has recently been described to induce proliferation and/or differentiation of OPCs [4, 5]. Upon aspirin treatment in *Sox3* mutants, we observe a restoration of OPC proliferation defects and a rescue of pituitary hormonal deficiencies. Our results highlight a previously unrecognised role for SOX3 in ME, and NG2 glia, which are likely to play a central role in formation of a mature hypothalamo-pituitary connection. Furthermore, the effects of aspirin suggest a new potential therapeutic benefit for the treatment of certain types of hypopituitarism.

1. Rizzoti, K., et al., *SOX3 is required during the formation of the hypothalamo-pituitary axis*. Nat Genet, 2004. 36(3): p. 247-55.
2. Robins, S.C., et al., *alpha-Tanycytes of the adult hypothalamic third ventricle include distinct populations of FGF-responsive neural progenitors*. Nat Commun, 2013. 4: p. 2049.
3. Djogo, T., et al., *Adult NG2-Glia Are Required for Median Eminence-Mediated Leptin Sensing and Body Weight Control*. Cell Metab, 2016. 23(5): p. 797-810.
4. Chen, J., et al., *Aspirin promotes oligodendrocyte precursor cell proliferation and differentiation after white matter lesion*. Front Aging Neurosci, 2014. 6: p. 7.
5. Huang, N., et al., *Aspirin Promotes Oligodendroglial Differentiation Through Inhibition of Wnt Signaling Pathway*. Mol Neurobiol, 2015.

The signal and the noise: Some lessons learned from the replicability crisis in Psychology

Marco Perugini

Department of Psychology, University of Milano-Bicocca

The recent replicability crisis in Psychology has attracted much attention and it is starting to have an impact on current research practices. In this talk, after an overview, I will present some suggestions based on the lessons learned from the replicability debate in Psychology. The emphasis will be on methodological issues and research practices increasing the likelihood of designing informative experiments and drawing correct inferences from data, helping therefore researchers to separate the signal from the noise.

Post-transcriptional regulation in adult neurogenesis and brain ageing

Chiara Rolando

Department of Biosciences, University of Milan, Italy

The miRNA Microprocessor, a multimeric complex of the ribonuclease Drosha and the RNA binding protein DGCR8, binds and cleaves double-stranded hairpins in miRNA primary transcripts to generate precursor miRNA that is exported from the nucleus to the cytoplasm for further processing by Dicer. The Microprocessor also has miRNA-independent functions including targeting and cleavage of stem-loop hairpin structures in mRNAs thereby destabilizing the transcripts. We have previously shown that non-canonical target cleavage by Drosha is critical during cortical development and adult neurogenesis. However, the non-canonical role of Drosha in other pathophysiological contexts, like brain ageing is unknown. In order to unravel Drosha-mediated post-transcriptional regulation we performed gene expression profile and proteomic analysis. We found that Drosha and its binding partners have altered expression during brain ageing. In line with this, we could show by conditionally deleting *Drosha* that the knockout astrocytes are reactive and modify their function consistent with age-related changes. We also performed CLIP-seq experiments and identified mRNA targets of Drosha responsible for regulating age-related changes. Taken together our findings reveal a novel miRNA-independent mechanism for Drosha in regulating astrocyte function during brain ageing.

Synaptic consequences of selective microglial TDP-43 depletion

Rosa C. Paolicelli

Department of Physiology, University of Lausanne

Microglia are implicated in a variety of functions in the central nervous system, ranging from shaping neural circuits during early brain development, to surveying the brain parenchyma, and providing trophic support to neurons across the entire lifespan. Microglial phagocytic activity is important for mediating synapse elimination, clearing invading pathogens and removing protein aggregates like amyloid deposits. TDP-43, a DNA-RNA binding protein encoded by the *Tardbp* gene, is a critical regulator of microglial phagocytosis. Mice lacking TDP-43 selectively in microglia (cKO) exhibit drastic synapse loss and enhanced amyloid clearance in a model of Alzheimer's disease (AD). Loss of synapses in *Tardbp* cKO mice, however, is independent of amyloid deposition. Selective loss of TDP-43 in microglia promotes microglial engulfment of synaptic material, and is associated with decreased spine density in the motor/ somatosensory cortex. Here we show that mice lacking TDP-43 in microglia exhibit motor deficit and clasping behavior, and display altered levels of cytokines expression in the cortex and in the spinal cord. Furthermore, aberrant phagocytic microglia induced by TDP-43 depletion during early brain development leads to selective structural defects in the motor cortex, as revealed by MRI analyses.

Overall, our data indicate that dysfunctional microglia significantly affect synapses, thus playing an important role in the pathogenesis of neurodegeneration.

Microglial immune receptor TREM2 in neurodegenerative diseases.

Fabia Filipello

Washington University, St. Louis, USA

Microglial cells are unique brain resident antigen presenting cells that constantly survey central nervous system parenchyma. Microglia express the triggering receptor expressed on myeloid cells 2 (TREM2), an innate immune receptor which plays a critical role in microglial activation, survival, and phagocytosis. The receptor is also cleaved by metalloproteases in a soluble form (sTREM2) which is detectable in the cerebrospinal fluid.

Variants and mutations in the *TREM2* gene have been associated to different types of neurodegenerative disorders such as Nasu-Hakola disease (NHD), Frontotemporal Dementia and to an increased risk to develop Alzheimer' s disease. NHD is a rare autosomal-recessive leukodystrophy characterized by progressive early-onset dementia and by polycystic osseous lesions. So far, the role of TREM2 in human microglia in the context of NHD pathogenesis still remains poorly understood.

My project aims to define the mechanisms by which the homozygous Q33X variant in *TREM2* gene contributes to NHD onset and progression through microglial dysfunction. We have set up a protocol able to differentiate human induced pluripotent stem cells (iPSC) into microglia-like cells (iMGLs). By flow cytometry, immunofluorescence, ELISA and RNA sequencing I characterized the phenotype and features of IPSCs-microglia derived from three individuals belonging to the same family - two NHD patients carrying the Q33X mutation and a healthy sibling-. I showed that NHD iMGLs survived less then healthy iMGLs and present a different inflammatory profile compared to control. As expected, sTREM2 levels detected by ELISA were completely undetectable in NHD iMGLs, since TREM2 protein is not produced by mutant microglia. I am currently investigating whether sTREM2 could be used as a possible therapeutic approach in order to rescue the phenotype observed in NHD iMGLs. In the proposed project, the generation of patient-derived iPSCs could facilitate new opportunities to examine the relationships between genetic risk factors and neurodegenerative diseases such as NHD. Our results will clarify how Q33X mutation impacts on human microglial activity, possibly providing the mechanistic framework needed to develop treatments that restore or enhance TREM2 interactions with its ligands in order to prevent NHD.

Roles of Tunneling nanotubes (TNTs) and astrocytes in neurodegenerative diseases

Chiara Zurzolo

Pasteur Institute, Paris, France

Neurodegenerative diseases (NDs) like Prion disease, Alzheimer's (AD), Parkinson's (PD) and Huntington's (HD) disease are part of a larger group of protein misfolding disorders characterized by the progressive accumulation and spreading of different protein aggregates. Like Prions, misfolded forms of ASYN, tau, Abeta and Htt proteins associated with AD, PD and HD can be transmitted experimentally in cellular and in animal models where act as 'seeds' to recruit the endogenous protein into aggregates. However, the mechanism of intercellular transfer is still debated. We have recently described a novel mechanism of PrP^{Sc} transmission through Tunneling Nanotubes (TNTs)[1]. TNTs are actin-based protrusions connecting cells in culture and represents a novel mechanism of cell-to-cell communication. Furthermore mutant polyQ Htt aggregates appear to highjack TNTs[2] as well as fibrillar and oligomeric ASYN assemblies[3] and Tau fibrils[4]. TNTs appear to form between neurons and between astrocites and neurons. We also studied the role of astrocytes on the intercellular transfer and fate of α-syn fibrils, using *in vitro* and *ex vivo* models. α-syn fibrils can be transferred to neighboring cells, however the transfer efficiency changes depending on the cellular types. We found that α-syn is efficiently transferred from astrocytes-to-astrocytes and from neurons-to-astrocytes, but less efficiently from astrocytes-to-neurons. Overall our in *vitro* and in *ex vivo* culture models indicate astrocytes have a role in degrading asyn fibrils rather then in transfer[6].

We propose that TNTs contribute to the progression of the pathology of NDs by spreading in the brain of misfolded protein assemblies[5]. Thus, understanding the mechanism of TNT formation is important to uncover their physiological function. We demonstrate that despite their similarities, filopodia and TNTs form through distinct molecular mechanisms[7] indicating that they are different structures. Finally, analysis by correlative cryo-EM and tomography[8] show differences in the actin organization and appearance of the two structures revealing their unique identities.

[1] Gousset et al, *NCB* 2009
[2] Costanzo et al,*JCS 2013*
[3] Abounit et al, *EMBO J 2016*
[4]Abounit et al, *Prion* 2016
[5]Victoria and Zurzolo, *JCB* 2017
[6]Loria et al, *Acta Neuropatologica* 2017
[7] Delage et al, *Sci Rep 2016*
[8] Sartori et al, *Nature Comm* 2019

Bmal1-dependent control of astrocyte development and function in brain

Davide De Pietri Tonelli

Neurobiology of miRNA lab., Istituto Italiano di Tecnologia, Genova (Italy).

Physiology and metabolism of the adult mammalian brain are under circadian control, though the role of the molecular clock in brain development are poorly understood. A recent surge of publications overturned the neurocentric view of the circadian system in brain, by demonstrating that astrocytic clock is key contributor of the timekeeping and metabolic systems. Astrocytes and the circadian system develop simultaneously during the perinatal period in rodents, but little is known about the interplay of these processes.

As entry point to address these questions, we deleted the core clock gene *Bmal1* in glial progenitors and astrocytes of the postnatal mouse neocortex. This resulted in impaired proliferation and expansion of astrocytes. To ascertain a possible bidirectional link between neurodevelopmental diseases and Bmal1-dependent postnatal astrocyte expansion, we investigated Bmal1 expression and astrocyte proliferation in postnatal cortices of a mouse model of 22q11.2 deletion syndrome (22q11DS), which confers the most significant genetic risk for autism spectrum disorders and schizophrenia in human. We found a decreased expression of Bmal1 correlating with reduced expansion of astrocytes in postnatal cortices of 22q11DS mice.

Together, these results (1) suggest a possible developmental role for Bmal1 in timing the onset of astrocyte proliferation, which may contribute to neurodevelopmental disorders and associated brain pathologies.

1) Barca-Mayo O and De Pietri Tonelli D., BMAL1 controls astrocyte expansion in postnatal cortex: impact on neurodevelopmental disorders. *Submitted*

Disruption of myelin phospholipid composition impairs action potential conduction in murine model of spinocerebellar ataxia type 38

Eriola Hoxha

Department of Neuroscience Rita Levi-Montalcini, Neuroscience Institute Cavalieri Ottolenghi (NICO), University of Turin, Turin, Italy

SCA38 is due to mutation of the gene ELOngase of Very Long chain fatty acids type 5 (ELOVL5), the rate-limiting enzyme for the production of polyunsaturated fatty acids (PUFA) with more than 18 carbon atoms. Patients with SCA38 present with cerebellar atrophy, ataxia and impairment of both central and peripheral evoked potentials. Mice with a targeted deletion of Elovl5 recapitulate the main SCA38 symptoms, including ataxia and cerebellar hypotrophy.

The aim of this study was to investigate action potential conduction in Elovl5-deficient mice. We found a reduced conduction velocity in both peripheral and central axons. These results indicate that Elovl5 is required for fast action potential conduction in both peripheral and central nerve fibers. Action potential velocity relies on proper myelin sheaths. We analyzed the ultrastructure of myelin in Elovl5-deficient mice, finding an enlarged periodicity with reduced G-ratio across all axonal diameters. Moreover, the myelin MBP and CNPase proteins were less abundant in Elovl5-deficient fibers. Since Elovl5 is crucial to attain normal amounts of PUFA, which are the principal component of myelin, we performed a lipidomic analysis of peripheral and central nerves. Elovl5-deficient mice showed an unbalance between PUFA longer than 18 carbon atoms relative to shorter ones, with an increased ratio of saturated to unsaturated fatty acids. These results suggest that, in SCA38 patients, PUFA alterations might alter myelin structure, causing slower action potential conduction along axons. Cerebellar function is based on precise timing of neural signals. Therefore, action potential conduction deficits caused by myelin alterations might be responsible for cerebellar symptoms.

Enhancing D-Aspartate signaling to promote (re)myelination

Francesca Boscia

Division of Pharmacology, Department of Neuroscience, Reproductive and Dentistry Sciences, School of Medicine, Federico II University of Naples, Naples, Italy

In recent years it has been demonstrated that neuronal activity regulates both myelin plasticity in the adult brain and remyelination after a demyelinating insult. These two processes appear to share similar guiding mechanisms, which may depend on glutamate signaling to OPCs[1]. Indeed, mounting evidence suggests that the activation of Ca^{2+}-dependent pathways through glutamate AMPA and NMDA receptors or the Na^{+}/Ca^{2+} exchanger NCX3 may influence oligodendrocyte maturation, myelin synthesis, and remyelination processes [1,2].

D-Aspartate is a D-amino acid exerting modulatory actions at glutamatergic synapses. Chronic administration of D-Aspartate has been proposed as therapeutic treatment in diseases related to myelin dysfunction and NMDA receptors hypofunction, including schizophrenia and cognitive deficits[3]. By using an *in vivo* remyelination model, we demonstrated that administration of D-Aspartate during remyelination improved motor coordination, accelerated myelin recovery, and significantly increased the number of small-diameter myelinated axons. Chronically administered during demyelination, D-Aspartate also attenuated myelin loss and inflammation. Interestingly, D-Aspartate exposure stimulated OPCs maturation and accelerated developmental myelination in organotypic cerebellar slices. Functional studies demonstrated that D-Aspartate exposure elicited a complex $[Ca^{2+}]_i$ response in oligodendrocyte precursors involving an orchestrated functional crosstalk between glutamate transporters, ionotropic AMPA and NMDA glutamate receptors, and the $Na^{+/}Ca^{2+}$ exchanger NCX3. While blocking NMDA or NCX3 significantly prevented D-Aspartate-induced $[Ca^{2+}]_i$ oscillations, blocking AMPA and glutamate transporters prevented both the initial and oscillatory $[Ca^{2+]}$i response as well as D-Aspartate-induced inward currents in OPCs[4]. Collectively, our findings further confirm the key role of NCX activity for myelin synthesis and indicate that exogenous D-Asp treatment might produce beneficial effects during remyelination processes

References

1.Gautier HO, Evans KA, Volbracht K, James R, Sitnikov S, Lundgaard I, James F, Lao‐Peregrin C, Reynolds R, Franklin RJ, Káradóttir RT (2015) Neuronal activity regulates remyelination via glutamate signalling to oligodendrocyte progenitors. *Nat Commun,* 6:8518.

2.Casamassa A, La Rocca C, Sokolow S, Herchuelz A, Matarese G, Annunziato L, Boscia F (2016) Ncx3 gene ablation impairs oligodendrocyte precursor response and increases susceptibility to experimental autoimmune encephalomyelitis. *Glia,* 64:1124–1137.

3.Errico F, Nistico R, Di Giorgio A, Squillace M, Vitucci D, Galbusera A, Piccinin S, Mango D, Fazio L, Middei S, Trizio S, Mercuri NB, Teule MA, Centonze D, Gozzi A, Blasi G, Bertolino A, Usiello A (2014) Free D-aspartate regulates neuronal dendritic morphology, synaptic plasticity, gray matter volume and brain activity in mammals. *Transl Psychiatry,* 4:e417.

4.de Rosa V, Secondo A, Pannaccione A, Ciccone R, Formisano L, Guida N, Crispino R, Fico A, Polishchuk R, D'Aniello A, Annunziato L, Boscia F. D-Aspartate treatment attenuates myelin damage and stimulates myelin repair (2019). *EMBO Mol Med*, 11 pii:e9278.

Exosomes as natural messengers of bioactive molecules in the glial-neuronal signaling in Parkinson's disease

Nunzio Iraci

Biometec, University of Catania, Italy

Recent experimental evidences suggest that extracellular vesicles (EVs) are important players in the cell-to-cell communication, both in physio- and pathological conditions. EVs are a heterogeneous class of nanoparticles – including exosomes, microvesicles and apoptotic bodies – with different size and biogenesis, and released by virtually all cell types in the microenvironment. They are involved in the exchange of a broad range of bioactive molecules, but much remains to be elucidated with regard to their roles in the brain.

In the context of Parkinson's disease (PD), our previous work demonstrated that astrocytes activated by chemokines, such as Ccl3, exert a robust neuroprotection/regeneration against the PD neurotoxin MPTP, both in vitro and in vivo, but the mechanism(s) underlying the complex cross-talk between astrocytes, neurons and neural stem cells is still unknown.

We hypothesize a potential role for astrocyte-derived EVs in this intercellular signaling.

First, we have characterized astrocytes from both the ventral midbrain (VM) and Striatum (Str) – the two main brain regions involved in PD – and addressed the effect of the Ccl3 on cellular physiology and secretive activity. Then, astrocyte-derived EVs have been isolated by differential ultracentrifugation and characterized for their dimension and concentration by electron microscopy and nanoparticle tracking analysis. We observed that the basal secretion rate is specific for each cerebral area, with VMB releasing more EVs than STR. Moreover, Ccl3 treatment affects both astrocyte morphology and their vesicle secretion, again depending on the specific brain region, in absence of any influence on cellular viability and proliferation. In particular, our ultrastructural analysis showed an enrichment of EVs in the size range of exosomes (~100 nm), with their typical cup-shape. Also, several positive (e.g. CD63, CD9, Tsg101) and negative (e.g. Calnexin, Tom20) exosomal markers have been analysed by western blotting and confirmed the enrichment of exosomes in the astrocyte-derived EVs. Finally, the presence of mRNAs and miRNAs has been evaluated by qPCR, finding both of them associated with astrocyte-derived EVs. These findings support a possible involvement of exosomes in the horizontal transfer of RNAs in the context of glia-neuron communication. This in-depth characterization was preliminary to our next steps of investigation that aim to evaluate, in vitro and in vivo, the impact of astrocyte-derived exosomes on PD target cells.

Novel insights into Parkinson's disease through iPSC-based technology

Antonella Consiglio

Stem Cells and Brain Pathology Unit, Centro de Medicina Regenerativa de Barcelona Hospital Duran i Reynals, Spain

Parkinson´s disease (PD) is an incurable disorder of old age with characteristic impairments of movement, due to the progressive loss of a specific type of dopamine neurons (DAn) in the brain. Despite decades of intense research, the mechanisms underlying PD onset and progression are for the most part still unknown. Understanding how genes and environment come together to increase our propensity for neurodegenerative diseases such as PD is crucial to develop better ways to prevent and treat these disorders. However, studying the human nervous system at the molecular level has always been challenging due to the complexity of the brain, and the difficulty of obtaining live human neurons in the laboratory. The advent of induced pluripotent stem cell (iPSC) technology enables to reprogram human somatic cells to pluripotency, to generate viable human cells affected by the disease. Furthermore, human iPSC-derived neuronal models offer unprecedented access to early stages of the disease, allowing the investigation of the events that initiate the pathologic process in PD. Recently, human iPSC-derived neurons from patients with familial and sporadic PD have been generated in our ad other laboratories and importantly they recapitulate some PD-related cell phenotypes, including abnormal α-synuclein accumulation in vitro, and alterations in the autophagy machinery. During the workshop, I will present the generation and characterization of these PD patient-specific iPSC lines, and I will describe an astrocyte-autonomous process mediating PD-associated degeneration of dopaminergic neurons, that occurs mainly via intracellular accumulation of α-synuclein aggregates in astrocytes and subsequent propagation of such toxic aggregates to surrounding neurons. These new data reveal an important role for glial cells in PD offering potential new targets for therapeutic development.

The upcoming role of multi-omics integration in unfolding central nervous development

Ludovic Telley

Department of Fundamental Neuroscience, University of Lausanne, Switzerland

Ludovic Telley obtained his PhD in Neurosciences at the University of Montpellier (France), where he studied the cerebellar development in the laboratory of Dr Fabrice Ango at the Institut de Génomique Fonctionnelle (IGF). After his graduation, he started a postdoctoral fellowship at the University of Geneva in the laboratory of Prof. Denis Jabaudon. In the following years, he developed a solid dual expertise in single cells transcriptomics and bioinformatics, including state-of-the-art machine learning approaches. He recently started his research group (February 2018) at the University of Lausanne with the support of an ERC Starter Grant. His research is focused on cerebellar development, particularly in understanding the molecular programs that control cerebellar neuron differentiation, interaction and assembly into functional circuits which serve as a basis for the cerebellar various functions.

During his talk he will describe the various single cells multi-omics approaches that have strongly contributed to our current understanding on neuronal development. In particular he will focus on scRNA-seq and provide an overview of the methodology, the bioinformatics pipelines, as well as the perspectives on integrating multiple sources of data to unfold the complexity of neuronal development.

Developmental cell death regulates lineage-related interneuron-oligodendroglia functional clusters and oligodendrocyte homeostasis

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The first wave of oligodendrocyte precursor cells (firstOPCs) and most GABAergic interneurons share common embryonic origins. Cortical firstOPCs are thought to be replaced by other OPC populations shortly after birth, maintaining a consistent OPC density and making postnatal interactions between firstOPCs and ontogenetically-related interneurons unlikely. Challenging these ideas, we show that a cortical firstOPC subpopulation survives and forms functional cell clusters with lineage-related interneurons. Favored by a common embryonic origin, these clusters display unexpected preferential synaptic connectivity and are anatomically maintained after firstOPCs differentiate into myelinating oligodendrocytes. While the concomitant rescue of interneurons and firstOPCs committed to die causes an exacerbated neuronal inhibition, it abolishes interneuron-first OPC high synaptic connectivity. Further, the number of other oligodendroglia populations increases through a non-cell-autonomous mechanism, impacting myelination. These findings demonstrate unprecedented roles of interneuron and firstOPC apoptosis in regulating lineage-related cell interactions and the homeostatic oligodendroglia density.

Inherent heterogeneity of postnatal oligodendrocyte progenitors: lessons from a microcephaly model

Enrica Boda

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In the developing mouse forebrain, temporally distinct waves of oligodendrocyte precursor cells (OPCs) arise from different ventricular germinal zones. These cell subsets eventually populate either dorsal or ventral regions, where they present as transcriptionally and functionally equivalent. Whether developmental heterogeneity influences OPC behavior and molecular features in pathology is largely unknown. Here we show that *in vivo* ablation of Citron-kinase, leading to accumulation of DNA damage, disrupts OPC fate resulting in cell death and senescence in the dorsal and ventral subsets, respectively. This divergence correlates with differential activity of NRF2-mediated anti-oxidant response to DNA lesions in dorsal and ventral OPCs. Depending on their developmental origin, wild-type OPC subsets also show a diverse vulnerability to cisplatin-induced DNA damage. These data indicate that, upon injury, dorsal and ventral OPC subsets unleash molecular and functional diversities that can make them differentially vulnerable to pathological conditions associated with DNA damage.

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

Valentina Cerrato

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

Molecular Mechanisms Regulating Gliogenesis in the Neocortex

Robert Beattie

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The concerted production of the correct number and diversity of neurons and glia is essential for intricate neural circuit assembly. In the developing cerebral cortex, radial glia progenitors (RGPs) are responsible for producing all neocortical neurons and certain glia lineages. We recently performed a quantitative clonal analysis by exploiting the unprecedented resolution of the genetic MADM (Mosaic Analysis with Double Markers) technology and discovered a perhaps unexpected high degree of non-stochasticity and thus deterministic mode of RGP behavior. However, the cellular and molecular mechanisms controlling RGP behavior and proliferation dynamics in neurogenesis and glia generation remain unknown. To this end we identified *Lgl1* as a critical regulatory component. By using a series of quantitative MADM-based experimental paradigms at single RGP resolution we found that *Lgl1* non-cell-autonomously controls embryonic cortical neurogenesis. In contrast, *Lgl1* controls adult neurogenesis in the postnatal subventricular stem cell niche via intrinsic cell-autonomous signaling. We also observed *Lgl1* is cell-autonomously required in early postnatal progenitors for producing the correct number of cortical astrocytes. We traced the developmental origin of increased cortical astrocyte production and observed that astrocyte progenitors (aIPCs) were significantly increased and that a larger fraction of $Lgl1^{-/-}$ mutant aIPCs actively proliferate in late embryonic development, resulting in a larger overall number of mature astrocytes at postnatal stages. We could observe strong genetic interaction between *Lgl1* and *Egfr* suggesting a functional relationship which likely is highly specific for cortical astrocyte generation but not postnatal subventricular neural stem cell behavior. Moreover, we observed $Egfr^{+/+}$ astrocytes in an $Egfr^{+/-}$ background exhibit a competitive dose sensitive advantage when compared to control-MADM (all cells $Egfr^{+/+}$). Collectively, our results obtained from single cell quantitative MADM analysis suggest that NSC-mediated neuron and glia production is tightly regulated through the concerted interplay of sequential *Lgl1*-dependent global and cell intrinsic mechanisms. Furthermore, this work highlights the importance to probe the relative contributions of cell intrinsic gene function and extrinsic tissue-wide mechanisms to the overall phenotype in healthy but also in neurodevelopmental disease conditions.

SELECTED ORAL COMMUNICATIONS

Molecular control of astrogenesis in mouse and human cortex. Role of the transcription factor Foxg1

Carmen Falcone 1,2, Manuela Santo 1, Gabriele Liuzzi 1, Noemi Cannizzaro 1, Clara Grudina 1,3, Erica Valencic 4, Luca Peruzzotti-Jametti 5, Stefano Pluchino 5, Antonello Mallamaci 1

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Astrocytes are essential cells for proper brain functioning, acting in first line during neuronal activity, synapse formation and regulation of neuronal metabolism. The amount of astrocytes available in the mature cortex could strongly affect the functionality of the network, indeed, their production during the first steps of brain development is highly controlled. Astrocytes start to be produced right after the pick of neuronogenesis when a small group of transcription factors promotes astrogenesis onset changing the epigenetic state of astroglial genes. We found that Foxg1, an ancient transcription factor essential for proper brain development, acts as a master gene controlling the temporal activation of astrogenesis. We show that Foxg1 levels within neural stem cells (NSC) are very high during neuronogenesis and they decline just prior the onset of astrogenesis. We demonstrated that the decline in Foxg1 levels is essential to have the activation of astroglial program. We proved, in fact, that Foxg1 can act as a brake toward astrogenesis restricting its activation in the perinatal window. In particular we found that Foxg1 overexpression within NSC, committed these cells to neuronogenesis rather than astrogenesis, in vivo as well as in vitro, both in mouse and human. We showed that Foxg1 inhibition of astrogenesis stem from variegated mechanisms. These include a direct trans-repression of genes that normally push neural stem cells to astroglial fates, as well as an articulated impact on key pathways which modulate astroglial gene transcription. These findings point to an evolutionarily conserved, pivotal role of Foxg1 in fine temporal regulation of astrogenesis. They also suggest that neurological symptoms observed in syndromes with altered Foxg1 allele dosage might be partially caused by an unbalanced astrocyte generation.

Novel molecular participants in astrocyte-neural progenitor cells crosstalk in murine hippocampus

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In adult mammalian subgranular zone of the dentate gyrus new mature neurons are generated starting from a pool of undifferentiated neural progenitor cells (NPC) and are continuously integrated in the preexisting circuitry. This process, named adult hippocampal neurogenesis (AHN) and involved in learning and memory formation, is altered in several neurological diseases and finely regulated by several mechanisms. Recently, our group discovered, among novel molecular regulators of AHN, the transcription factor NF-κB p50 whose absence in vivo results in a significant reduction of AHN correlated with hippocampal-dependent cognitive defects. Surprisingly, NPC derived from wild-type (WT) and p50 knock-out (KO) mice are not different in their neurogenic potential in vitro. These findings prompted us to investigate whether impaired AHN could rely on disrupted signaling from other cells composing the neurogenic niche. We focused our attention on astrocytes, known to provide key soluble pro-neurogenic signals. WT astrocyte conditioned media (ACM) promoted both neuronal and astroglial differentiations, while p50KO ACM only supported astroglial differentiation of WT ahNPC. p50KO NPC showed unresponsiveness to both ACM. LC–MS/MS approach revealed proteins and/or their receptors differentially regulated in p50KO astrocytes. Among those, lipocalin-2 (LCN-2) and chitinase 3-like1 (CHI3L1) are, respectively, upregulated and downregulated in p50KO astrocytes. This initial observation resulted in demonstration that these molecules are novel astroglial-derived pro-neurogenic signals. Furthermore, we discovered that the receptors for astrocyte-derived LCN-2 and Thrombospondin-1 are downregulated in p50KO NPC. Our findings suggest that: i) p50KO mice neurogenic defects may rely on both autonomous and non cell autonomous defects of NPC; ii) in absence of p50, astrocytes may not provide adequate proneurogenic support to NPC but also NPC may be defective in their response to astrocytes. Altogether these results increase our knowledge on the relevance of astrocyte-NPC communication in the modulation of AHN in physiological and, potentially, in pathophysiological conditions.

Investigating Neuroanatomical basis of Brain-Energy Metabolism using 3D models and VR tools.

Corrado Calì 1,2

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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 performed morphometric analysis on segmented 3D Electron Microscopy (EM) imaged volumes, using 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 explicitly expressed in astrocytes and inferred its spatial relationship with synaptic contacts. We compared the neuropil of mice undergoing a novel object recognition (NOR) in the 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 failure to form new synapses, together with a decrease in the levels of glycogen, supposedly the source of lactate. A model of chemical LTP on hippocampal organotypic slices well correlated with the structural effects of learning on spine density and size in vivo. The application of DAB inhibited both LTP and spines turnover. The exogenous application of L-Lactate was able to rescue the behavioral phenotype on mice, and the density increase but not their size. VR analysis highlighted that the presence of glycogen correlated with the presence of synapses. Our results suggest that memory and LTP mobilize glycogen stores, that might accumulate around potentiated synapses.

mGluR5 as a target to modulate the reactive phenotype of astrocytes in the SOD1G93A mouse model of amyotrophic lateral sclerosis.

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Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disorder due to motor neuron (MN) and glial cells degeneration in the spinal cord, brainstem, and motor cortex. One major cause for MN degeneration in ALS is represented by glutamate (Glu)-mediated excitotoxicity. Group I metabotropic glutamate receptors (mGluR1, mGluR5) play a role in ALS, since they are largely over-expressed during disease progression and are involved in the altered neuronal and glial cellular processes. We demonstrated that mGluR1 and mGluR5 produce abnormal Glu release in the spinal cord of the SOD1G93A mouse model of ALS and that halving their expression in SOD1G93AmGluR5+/- mice has a positive impact on in-vivo disease progression. We here investigated the consequences of reduced mGluR5 expression in SOD1G93A mice on the reactive phenotype of spinal cord astrocyte cell cultures from late symptomatic SOD1G93A, age matched SOD1G93AmGluR5+/- and WT mice. [Ca2+]C was increased in SOD1G93A astrocytes under basal and 3,5-DHPG-stimulated conditions. The mGluR5 down-regulation reduced the excessive [Ca2+]C. GFAP, Vimentin and S-100β, three astrogliosis markers, were increased in SOD1G93A astrocytes and over-expression was reduced in SOD1G93AmGluR5+/- astrocytes. mGluR5 down-regulation resulted in a lower cellular presence of misfolded SOD1. The expression and secretion of pro-inflammatory cytokines was strongly reduced in SOD1G93AmGluR5+/- respect to SOD1G93A astrocytes. Mitochondria function was impaired in SOD1G93A astrocytes and this impairment was recovered in SOD1G93AmGluR5+/- astrocytes. Notably, the viability of spinal MNs co-cultured with SOD1G93AmGluR5+/- astrocytes, instead of SOD1G93A astrocytes, was significantly increased. Moreover, after treatment of SOD1G93A astrocytes with CTEP, a negative allosteric modulator of mGluR5, the expression of S-100β and GFAP was reduced respect to controls. Thus, mGluR5 ablation in SOD1G93A mice has a positive impact on astrocytes. This supports the idea that mGluR5 may be a potential therapeutic target aimed at preserving MNs death, possibly by modulating the reactive astroglia phenotype in ALS.

The chaperone Clusterin interferes with the clearance of extracellular αSynuclein aggregates by astrocytes: implications for αSynuclein spreading and pathology

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Clearance of extracellular misfolded/aggregated αSynuclein (αSyn), released by neurons during cell stress or degeneration, is a key process to control the concentration and/or a further aggregation of αSyn in the extracellular space, which could be the basis for the propagation of αSyn and of Parkinson's Disease (PD) pathology. Evidence highlights the presence of molecular chaperones involved in the protein quality control of the extracellular compartment. In this regard, Clusterin has been reported to bind different amyloid proteins, including αSyn, and, interestingly, to influence the uptake and clearance of extracellular beta-amyloid aggregates in astrocytes. In this study we explore whether and how Clusterin affects αSyn pre-formed fibrils (pffs) clearance by astrocytes. Our results show that Clusterin levels are modulated in astrocytes, both in primary and human iPSC-derived astrocytes, upon αSyn pffs priming and in an AAV-based PD mouse model, indicating a functional interaction between Clusterin and αSyn aggregates. Specifically, we observed that astrocytes uptake αSyn pffs through the dynamin-dependent endocytosis and that extracellular Clusterin levels are reduced in the culture media of cells treated with αSyn pffs compared to cells treated with monomer, suggesting that Clu is endocytosed with αSyn pffs. By exploring the role of Clusterin in αSyn aggregates clearance, we found that Clusterin interacts with αSyn pffs in the extracellular compartment and the Clusterin/αSyn pffs complex is internalized by the cells. Moreover, by using Clusterin knock-out (KO) primary astrocytes or the supplement of Clusterin protein in the medium of cells exposed to αSyn pffs we demonstrated that Clusterin acts at level of the endocytic process interfering with αSyn pffs uptake. Collectively, our results indicate that extracellular Clusterin by binding αSyn pffs hampers their uptake/clearance likely masking αSyn pffs receptor-recognition site and, importantly, suggest that targeting Clusterin levels might improve αSyn aggregates clearance and prevent the spreading of αSyn.

Extracellular Vesicles mediate detrimental and protective action of microglia on myelin lesion

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

Interplay between inflammation and neural plasticity: Role of microglia in antidepressant efficacy

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An increasing number of studies show that both inflammation and neural plasticity act as key players in the vulnerability and recovery from psychiatric disorders and neurodegenerative diseases. However, the interplay between these two players has been limitedly explored. With the aim of elucidating Selective Serotonin Reuptake Inhibitors (SSRIs) mechanism of action, as first step, we explored in C57BL/6 adult male mice the effects of an increase in neural plasticity induced by the SSRI fluoxetine on the high- or low inflammatory levels induced, respectively, by 3 weeks of chronic stress or enriched environment. The results show that the increase in neural plasticity affects inflammatory levels and microglial profile in an environment dependent manner, since it counteracts the effect of the environment on inflammation. As second step, we explored whether an immune activation and/or suppression affects neural plasticity. To this aim, we acutely treated mice with lipopolysaccharide or ibuprofen and measured plasticity markers. Interestingly, though having opposite effects on physiological parameters and inflammatory markers, both lipopolysaccharide and ibuprofen significantly reduced long-term potentiation, BDNF expression levels and the phosphorylation of the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor subunit GluR1, compared to control mice. Such effect appears to be dose-dependent since only the higher, but not the lower, dose of both compounds led to a plasticity impairment. Overall, the results obtained show, on the one hand, that an increase in neural plasticity, such as that induced by SSRI, regulates inflammation, and, on the other, that deviations in inflammatory levels in both directions impair neural plasticity. These findings suggest that neural plasticity and inflammation are mutually regulating processes and, to instate the neural plasticity needed for the beneficial effects of SSRI treatment, inflammation should be kept within a strict range

POSTERS

1. Astrocyte contribution to synaptic alterations in Rett syndrome

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Rett syndrome (RTT; OMIM# 312750) is a rare devastating neurodevelopmental disorder that with an incidence of ~ 1:10,000 represents the most common genetic cause of severe intellectual disability in girls. Mutations in the X-linked methyl-CpG-binding protein 2 (MECP2) gene have been reported in over 95% cases of classical forms of RTT. Phenotypic rescue is possible in Mecp2 deficient mice upon reactivation of the endogenous gene, therefore raising the possibility of therapeutic intervention in humans. Although initial studies exclusively supported a neuronal role for MeCP2, recent data suggest that astrocytes lacking Mecp2 are involved in the RTT pathogenesis by exerting a non-cell autonomous effect. Indeed, it was demonstrated that wild-type astrocytes exert a positive effect on null neurons and the exclusive re-expression of Mecp2 in astrocytes significantly improves the phenotype and lifespan of the otherwise null mice. Although a preference for therapeutically acting on neurons, these studies suggest the possibility of targeting astrocytes as complementary strategy for RTT. However, it remains obscure which molecular alterations and key molecules in astrocytes affect neuronal structure and function. Considering that Mecp2 null neurons exhibit severe synaptic defects, we investigated whether the absence of Mecp2 in astrocytes could affect their ability to regulate synaptogenesis. By co-culturing WT neurons with null astrocytes, we demonstrate for the first time that the lack of Mecp2 in cortical astrocytes dramatically influences the synaptogenesis of WT neurons. The observed phenotype does not require contact but is reproducible using the conditioned medium of Mecp2 null astrocytes, suggesting that mutant cells could either secrete neurotoxic factors or do not secrete neurotrophic ones. In this presentation, I will discuss which molecular mechanisms participate to the observed defects in synaptogenesis and whether these defects are attributable only to Mecp2 null cortical astrocytes or broadly to Mecp2 deficient astrocytes.

2. Pro- and anti-inflammatory phenotypes of microglia acutely isolated from SOD1G93A mice during disease progression and effects of the partial deletion of mGluR5

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by death of upper and lower motor neurons (MNs). The etiology of the disease is not completely understood, but one major cause is glutamate(Glu)-mediated excitotoxicity. In addition to MNs there is increasing evidence regarding the contribution of astrocytes and microglia to ALS. Basing on the reported role of Group I metabotropic glutamate receptors (mGluR1 and mGluR5) in ALS, in our previous studies we generated double mutant mice carrying the SOD1G93A mutation and the mGluR5 partial deletion (SOD1G93AmGluR5+/-). These mice showed enhanced survival probability and ameliorated disease progression.

The aim of this study was to investigate the effect of the partial deletion of mGluR5 in microglia cells acutely prepared from mutant mice. We also report our first results on the metabolic alteration of SODG93A mouse-derived microglia. Microglia was purified from motor cortex and spinal cord of WT, SOD1G93A and SOD1G93AmGluR5+/- mice at pre-, early- and late-symptomatic stages of the disease (30, 90, and 120 days, respectively) on Percoll gradient and analyzed by flow cytometry and Western blot. Oxygen consumption and ATP synthesis at the late stage of the disease was determined. The balance between the pro-inflammatory (M1) and the anti-inflammatory (M2) microglia phenotype was studied. The M1/M2 ratio augmented in the spinal cord of SOD1G93A, even though the increase is significant in SOD1G93AmGluR5+/- mice at the late symptomatic phase of the disease only, while did not change in microglia derived from motor cortex. Our results also highlight a bioenergetic impairment in microglia derived from 120 day-old SOD1G93A mice respect to age matched controls. To conclude, the reduction of mGluR5 in SOD1G93AmGluR5+/- mice forces spinal cord microglia toward a more pro-inflammatory phenotype, at least at the late stage of disease progression.

3. ELOVL5 loss impairs myelin composition in a mouse model of Spinocerebellar Ataxia 38 (SCA38)

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

4. A drug-screening platform to identify compounds with neuroprotective potential and assess disease-specific dysfunctions in the context of progressive MS

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Background: The discovery of new agents promoting neuroprotection, blocking further neurodegeneration and supporting myelin differentiation is critically needed for Progressive Multiple Sclerosis. The possibility of generating patient-specific human induced pluripotent stem cells (hiPSC) allow testing and validation of libraries of drugs on disease-specific human cells. The project's aims are: 1) to identify the HIT compounds using in silico approach; 2) to perform drug toxicity test on primary murine cortical neurons; 3) to perform cytokine-, glutamate- and reactive oxygen species-mediated stressor assays on primary murine and hiPSC-derived cortical neurons; 4) to evaluate neuroprotective properties of the HIT compounds on cells exposed to stressors with functional and morphological outcome.
Methods: Repurposed drugs with predictive neuroprotective functions have been pre-selected (n=270) using the bioinformatic tool SPOKE among more than 1500 compounds. To assess their neural toxicity properties E17.5 murine cortical neurons were cultured in 96 well plates and exposed to two doses of the compounds (1 μM and 10 μM) for 24h, followed by cytotoxicity assay. Metabolic (tetrazolium salt), mitochondrial (JC1) and calcium signaling (FLUO4-AM) are functional outcomes. Cerebral organoids were differentiated using STEMCELL proprietary commercial medium.
Results: Neural toxicity of 270 compounds have been evaluated: 146/270 (54%) at 1 μM and 97/270 (36%) at 10 μM promote cell metabolic activity, while 43/270 (16%) compounds were cytotoxic at both concentrations based on tetrazolium assay. NMDA receptor-mediated stressor assay impaired 30-40% of neuronal metabolic activity with N-acetylcysteine served as a positive neuroprotective control. 160/270 non-toxic compounds were tested in neuroprotective assay settings: 32/160 selected HIT compounds will be re-tested for confirmation.
Conclusions: We have selected 160/270 compounds which are non-cytotoxic on murine cortical neurons. These compounds will be further tested for a metabolic and functional neuroprotective role in preventive regimen on both murine and hiPSC-derived neurons. A few selected HITs will be then tested on human cerebral organoids.

5. COUP-TFI/Nr2f1 overexpression in the GLAST-lineage perturbs migration and morphology of an adult-born hippocampal neuron subpopulation

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In the adult mouse hippocampal dentate gyrus (DG), radial glial-like adult neural stem cells (NSCs) generate granule neurons and astrocytes. Adult-born DG neurons play a crucial role in learning and memory; though a small but significant proportion of astrocytes is added to the DG cell network, their function remains completely unknown. Interestingly, running enhances both DG neurogenesis and astrogliogenesis, whereas multiple brain diseases and ageing lead to DG NSC dysfunction favoring astrogliogenesis over neurogenesis; this imbalance could contribute to disease/ageing-related cognitive deficits. This highlights the importance of a tight control of neuronal versus astroglial cell fate decision, most probably linked to intrinsic regulation in the NSC/progenitor pool. We recently identified the transcription factor COUP-TFI/Nr2f1 as a central player in controlling adult DG NSC/progenitor fate choice, where it exerts an anti-astrogliogenic action in both physiological and pathological (i.e. neuroinflammation) conditions enabling proper hippocampal neurogenesis. We are now studying the effect of COUP-TFI/Nr2f1 overexpression in the Glast-lineage (i.e. adult NSC lineage and resident astrocytes). Interestingly, we found that in these mice, where there is decreased DG astrogliogenesis, a subset of newborn granule neurons migrate into atypical DG sub-regions and bear an altered dendritic architecture compared to "normotopic" newborn neurons. Our recent data suggest that these perturbations may occur through non-cell autonomous mechanisms, possibly involving the contribution of newborn and/or pre-existing DG astrocytes in fine-tuning neuronal migration and differentiation within the adult hippocampal neurogenic niche.

6. Dentate gyrus morphogenesis relies on a cortical hem derived astrocytic scaffold

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In the archicortex, granule neuron progenitors are generated in the dentate neuroepithelium (DNE) from where they migrate to reach the presumptive dentate gyrus (DG). Their migration is suggested to rely on a scaffold made of radial glia and/or astrocytes projections, named dentate glial scaffold (DGS). However, its precise role and cellular origin are unclear. We previously showed that SOX9 is required for neuroepithelial progenitors switch from neurogenesis to gliogenesis, because in its absence, embryonic astrocyte specification is severely affected. Here, we performed conditional deletion of Sox9 using different Cre-drivers to perturb astrocytes emergence and showed how DG development is consequently affected.
Conditional deletion of Sox9 using Sox1Cre/+ severely affects DG formation as in adult Sox1Cre/+;Sox9fl/fl mice, DG size is significantly reduced. Embryonically, generation of DG granule neurons and their progenitors is unaffected; however, these fail to reach the developing DG and accumulate close to the DNE, suggesting a migration defect. In agreement, we also observed a dramatic reduction of the DGS in Sox9 mutants. Moreover, earlier in development, we detected a reduction in astrocytic progenitors located in the adjacent cortical hem (CH), which, we hypothesize, are the DGS progenitors. Conversely, Sox9 conditional deletion with Nestin-Cre only partially affects migration of granule neurons and their progenitors. Analyses of Cre activity revealed a delayed recombination of Nestin-Cre in the CH, compared to Sox1Cre, suggesting a preservation of astrocytic specification in this area which allows formation of the DGS in Nestin-Cre;Sox9fl/fl mutants. In agreement, when Sox9 is deleted exclusively in the CH using Wnt3a-Cre, the DGS does not form and granule neuron progenitors accumulate next to the DNE, similarly to Sox1Cre mutants. Our study confirms that SOX9 is required for astrocyte progenitor induction. Moreover, it highlights the astrocytic nature of the DGS and its requirement for embryonic granule neuron migration.

7. Prospective isolation and characterization of mature hippocampal astrocytes from young and aged mice employing cell surface markers

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The proper isolation of astrocytes from different regions of the central nervous system is vital to study astrocyte specific gene expression and to obtain pure serum-free cultures for in vitro approaches. Recently, several astrocytic cell surface proteins including ITGB5 (Foo et al. 2011), GLAST (Jungblut et al. 2012) and ATP1B2 (Kantzer et al. 2017; Batiuk et al. 2017) have emerged as valuable markers to isolate astrocytes from neonatal and adult tissues. In this work, we combined two commercial antibodies, ACSA-1 and ACSA-2 (raised against
GLAST and ATP1B2 surface epitopes, respectively) in order to prospectively isolate and characterize astrocytes from the adult murine hippocampus during aging. We used wild type mice (C57BL6 strain) of 2 and 18 months of age, microdissected the hippocampus and dissociated it with the Neural Tissue Dissociation Kit-Trypsin (Miltenyi). Following myelin removal with a Percoll density gradient, we stained the resulting cell suspension with ACSA-1-PE and ACSA-2-FITC antibodies. Interestingly, we identified three distinct astrocyte subpopulations by flow cytometry: the ACSA-1+, ACSA-2+ and ACSA-1+/ACSA-2+ cells. Our results further showed that the proportion of these subpopulations changes during aging,
reflecting notorious cellular dynamics. ACSA-2+ cells were the minority population at both ages (4,6% and 4,0%, 2 and 18 months respectively). ACSA-1+ cells increased with age (from 46,8% to 76,5%), while ACSA-1+/ACSA-2+ cells decreased with age (from 48,5% to 19,5%). In line with this finding, we found that Atp1b2 (ACSA-2) gene expression diminished in the hippocampus of
a mouse model with accelerated aging. These results show that astrocytes from the adult hippocampus are heterogeneous and display different proportions of cell surface markers depending on age. This should be taken into account when isolating and comparing neonatal, adult and aged astrocytes.

8. The exposure to the viral component Poly I:C leads to an elevation of CX3CL1 level in cortical organotypic cultures

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Prenatal insults (including maternal infections with viral agents) are considered as one of the factors implicated into the development of schizophrenia. They disturb natural neurodevelopmental processes and change physiological neuron – microglia communication. The crucial regulatory systems for neuron – microglia crosstalk are CX3CL1-CX3CR1 and CD200-CD200R.

The aim of the study: The study was designed to examine the impact of the treatment with the viral component – polyinosinic:polycytidylic acid (Poly I:C) on protein levels of CX3CL1, CX3CR1, CD200, CD200R, IL-4, IL-6, IL-10 and TNF-α in cortical organotypic cultures.

Materials and methods: The cortical organotypic cultures were prepared from 6-7-day-old rats. Frontal cortices were sectioned into 350 μm slices and transferred onto inserts. Cultures were maintained for 7 days and then stimulated with Poly I:C (25 μg/ml) for 24 hours. The protein levels of CX3CL1, CX3CR1, CD200 and CD200R were measured in the slices, while the expression of IL-4, IL-6, IL-10 and TNF-α was assessed in the medium by ELISA assays.

Results: The data obtained from ELISA analyses demonstrated that the exposure to Poly I:C significantly increased the level of CX3CL1 in cortical organotypic cultures, leading to disturbances of neuron – microglia interactions.

Conclusions: Our results indicate that the immune activation, caused by Poly I:C, upregulates the protein level of CX3CL1 in cortical organotypic cultures. The observed changes may be interpreted as a compensatory mechanism, leading to the limitation of inflammatory response after Poly I:C immunostimulation. However, our study clearly supports the need for further investigation in the raised matter.

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9. Alteration of microglia function and synaptic activity induced by antibiotic treatment.

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The bidirectional crosstalk between gut microbiota and the central nervous system is becoming increasingly important. Intestinal bacteria are important for the host's health: in fact, changes in their composition and abundance are associated with several diseases which can affect the central nervous system. Various works have highlighted the immunomodulatory role of microbiota: these microorganisms are involved in the maturation and activation of microglia, but nothing is known about their impact on microglia functionality. These cells represent the primary immune system of the brain and play important functions, both during development and in the adult stages. Since the crosstalk between microglia and neurons is essential for the correct building and refinement of the neuronal circuits, we wondered if antibiotic treatment could alter microglia functionality and consequently alter the synaptic activity. Cx3cr1 heterozygous mice were divided in two groups, control group (CTRL) and in a group (ABX) treated with two not-absorbable/broad spectrum antibiotics for two weeks. Several experimental approaches were used in order to investigate hippocampal-microglia features and synaptic activity of hippocampal CA1 pyramidal neurons. Regarding ABX mice, we observed an increasing microglia density, and a reduced re-arrangement ATP response. On the neuron side, we found a decreased synaptic activity (in terms of I/O response and AMPA/NMDA ratio). Despite this alteration in connectivity we didn't see any changes in dendritic spines density, observed by confocal acquisition of Cx3Cr1::CreERT2;Rosa26-CAG::LSL-tomato;Thy1GFP hippocampal brain slices. Considering the alteration of the microglia patrolling, immunofluorescence techniques were used in order to investigate possible neuroinflammatory processes, which involve both microglia and astrocytes. We found an increase in astrocytes density in the ABX group compared to the CTRL group, and also an up-regulation of the C3 (complement factor) by the astrocytes in the ABX group compared to the CTRL. These results suggest that antibiotics are able to alter microglia features and thus alter synaptic activity. Changes in microglia patrolling might induce an inflammatory state that leads to an increase of astrocytes density inside the hippocampus and consequently up-regulation and expression of the C3.

10. Plasticity of microglia: day/night changes in the lateral hypothalamus?

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Microglia constantly scan the microenvironment in order to detect disruptions of brain homeostasis, pathogenic invasions, tissue damage, and they also play an important role in synaptic plasticity mechanisms. Morphological changes of microglial cells in pathological conditions reflect their functional activation and have been extensively described, while plastic changes in normal, unchallenged conditions remain to be explored. We recently observed a daily reorganization of synaptic inputs onto orexin (OX)-containing neurons in the lateral hypothalamus (LH) and focused the present study on the morphology of local microglia during such synaptic rearrangements. The investigation was performed at two different time points in antiphase (nighttime and daytime) in CX3CR1-GFP mice, in which microglia are Green Fluorescent Protein (GFP)-tagged. Either homozygous CX3CR1 (GFP/GFP) mice, in which fractalkine receptor may not be functional, or heterozygous CX3CR1 (+/GFP) mice were used. During the 3 hours preceding sacrifice mice were video-recorded in order to assess their vigilance states. We then used confocal microscopy to collect image stacks of immunolabeled OX neurons and GFP-labeled microglia. 3-D reconstructions obtained with the IMARIS software in an initial, small sample of animals showed microglial cells with more ramified processes at night (when nocturnal rodents are predominantly awake) compared to daytime (the period of sleep predominance). If confirmed by further quantitative observations, a higher degree of ramification during the period of active behavior could imply an active role for microglia not only to ensure homeostatic maintenance in the microenvironment, but also to provide an effective mechanism for the surveillance of the synaptic contacts reached by the highly ramified distal processes. Conversely, less extensive ramifications during resting hours and sleep might suggest a shift towards a scavenging and, possibly, pruning role of microglia, engaged in “cleaning up” the microenvironment at the end of a period dominated by wakefulness and activity.

11. Focus on neuroinflammation and Tamoxifen treatment in the adult hippocampal neurogenic niche.

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

12. Astroglial calcineurin - a novel regulator of CNS proteostasis: between physiology and pathology

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Astroglial cells play important homeostatic functions in the CNS and respond to neuronal activity generating intracellular calcium signals. How the Ca2+ signals regulate homeostatic activities of astrocytes is poorly understood. We have hypothesized that a Ca2+-activated phosphatase calcineurin (CaN) in astrocytes may mediate the translation of Ca2+ signals to homeostatic activities.
To test this hypothesis, we have generated a mouse model with conditional astrocyte-specific CaN KO (ACN-KO mice). We found that, at one month of age, excitability of hippocampal CA1 pyramidal and cerebellar granule neurons is severely impaired due to functional inactivation of Na+/K+ ATPase (NKA) (Tapella et al., 2019).
To further investigate a possible role of CaN as a decoder of the astrocyte-neuron interaction-induced Ca2+ signals, we have used omics approaches to comprehensivly analyze the alterations produced by the deletion of CaN from astrocytes.
Surprisingly, whole transcriptome RNA-sequencing of hippocampal and cerebellar tissues did not reveal significant transcriptional CaN-dependent activity.
Instead, shotgun mass spectrometry proteomics analysis at 1 month of age revealed massive protein expression alteration at the synaptic level. In an in-vitro assay, astroglial protein synthesis was significantly impaired. Bioinformatics analysis revealed overrepresentation of annotations related to neurological diseases including Alzheimer's, Parkinson's, Huntington's diseases, dementia and, in particular, epilepsy and seizures. Moreover, we found that later in life, beginning from 6 mo of age, ACN-KO mice develop elevated risk of epileptic seizures.
Altogether, our data suggest that astroglial CaN regulates proteostasis of the CNS at a posttransciptional level. In conclusion, the deletion of CaN from astroglial cells compromises the CNS homeostasis and predisposes neurons to dysfunction and pathology. This suggests that astroglial CaN may be a key regulator of homeostasis of the nervous tissue.
Reference: Tapella et al., 2019, Glia. doi: 10.1002/glia.23737.

13. D-Aspartate treatment attenuates myelin damage and stimulates myelin repair

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

14. Early microglia activation as a disease-modifying factor in common epilepsies

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The ENIGMA-EPILEPSY consortium (EEC), who is running the largest neuroimaging study in epilepsy, has identified patterns of shared grey matter reduction (cortical thinning) across epilepsy syndromes. Data from healthy human brain gene expression atlas (Allen Atlas) have revealed enrichment for microglial genes in cortical thinning vulnerable regions observed in the epilepsies.
Our aim was to interrogate microglia involvement in cortical thinning and potential functional consequences, using a murine model of acquired epilepsy.
We targeted microglia with a blocker of the Colony Stimulator Factor 1 receptor, PLX3397 that depletes microglia by ~95% after 3 weeks of medicated-diet. After mice are returned to a non-medicated diet, microglia repopulates the brain parenchyma within one week. We depleted microglia in two critical phases of the disease in mice: 1. in a prodromal phase preceding epilepsy onset; 2. in chronic epileptic mice. With both treatment protocols, microglia depletion did not modify the number and duration of spontaneous seizures. Accordingly, depletion in naive mice did not affect synaptic transmission and neuronal excitability as assessed in hippocampal slices and in a Xenopus oocytes model. Depletion of microglia in the prodromal phase mediated significant neuroprotection in the entorhinal cortex as measured by Nissl-staining. Post-mortem MRI showed that neuroprotection was associated with full prevention of cortical thinning in the same region. Importantly, EEC reported entorhinal cortex thinning in human epilepsy syndromes. The prevention of these structural changes was associated with rescue of cognitive deficit in a non-spatial memory test. Differently microglia depletion in chronic epileptic mice did not affect any of these parameters. Our data establish a causal link between the activation of microglia during initial disease development and brain structural changes, which are implicated in cognitive functions that are compromised in human epilepsy. Data highlight microglia as a cellular target for early neuroprotective intervention in human epilepsies.

15. Involvement of glial cells in the resilient or vulnerable response to acute stress: evidence from a preclinical model

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Stress is one of the major risk factors in psychiatric and neurodegenerative diseases (PMID: 11127620). When the stress response is physiologically activated and then inactivated, it can promote adaptive plasticity; when this response is uncontrolled, it can induce maladaptive harmful effects. Changes in the neuroarchitecture of specific brain areas, like the frontal cortex (FC), an area importantly involved in the stress response (PMID: 25482165), have been found in psychiatric patients and rodents exposed to chronic stress (CS) (PMID: 28778394). In this context, the role of glial cells after prolonged stress has been extensively clarified, demonstrating that it directly affects the glutamatergic homeostasis, thus contributing to a maladaptive response (PMID: 25737228). Recent evidence hypothesizes that alterations in cerebral neuroarchitecture are also a consequence of acute stress (AS) (PMID: 26523035), but the role of glia in determining an adaptative/maladaptive response is still poorly understood. Therefore, Sprague-Dawley rats were exposed to footshock stress (PMID: 20052403) and then submitted to the sucrose preference test to identify resilient (RES) and vulnerable (VUL) animals. Considering the homeostatic functions in which glial cells are involved, we investigated, in both RES and VUL rats, structural, cellular and molecular alterations affecting these cells 24 hours after AS in the FC. Results showed that AS exposure determines structural and functional changes of glial cells, differently between RES and VUL subjects. In particular, we detected early glial and inflammatory imbalances in both stressed groups respect to NS rats accompanied by no signs of neuronal damage. This study reveals, for the first time, the involvement of glial cells in response to AS. Results obtained impel to further investigate the functional mechanisms involving these cells in the response to AS and lay the foundations for new potential treatments.

16. Chemokine CX3CL1 attracts microglial processes regulating synaptic function

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Microglia are the resident immune cells of the CNS. Recently, several studies have highlighted the role of microglia in monitoring the brain environment in order to maintain the homeostasis and in regulating network formation. Microglia function is strictly related to their ability to communicate with the neuronal population, through the release of soluble factors or by physically contacting synaptic elements. Indeed, the alteration of microglia-neurons crosstalk leads to the appearance of deficits in synaptic transmission and behaviour. Among the different soluble factors involved in microglia-neuron interaction, the chemokine CX3CL1, produced by neurons, is known to bind his microglial receptor CX3CR1, thus regulating microglial function.
To establish whether CX3CL1 signaling could influence synapses we focused on the interactions between microglia processes and neurons. In Cx3cr1::CreERT2;Rosa26-CAG::LSL-tomato;Thy1::GFP mice we studied the distribution of microglia-neuron contacts in Cx3cl1 KO and WT. In order to assess if CX3CL1 has a central role in the regulation of this interaction, we studied the chemokine distribution in the neuron.
In addition, by fluorescence monitoring in Cx3cr1 heterozygous mice, we observed that CX3CL1 is able to attract microglial processes. These results suggest a relevant role of CX3CL1 in the regulation of microglial-neuron communication and synaptic function. Consistently, in Cx3cl1 KO mice, we observed a decrease in hippocampal connectivity and changes in synaptic properties and plasticity.
We conclude that CX3CL1 represents a pivotal signal in microglia interaction with synapses, attracting microglial processes in order to modulate their function.

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

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In the adult brain, subsets of astrocytes act as neural stem cells in two anatomically defined neurogenic niches: the sub-ventricular zone and hippocampal dentate gyrus. Surprisingly, after excitotoxic lesion striatal astrocytes acquire stem cell properties and generate a large amount of neuroblasts for at least six months. Yet the presence and organization of striatal neurogenic niches and the spatio-temporal dynamics of striatal astrocytes activation and lineage progression remain by large unclear.
Here, through genetic lineage-tracing experiments and 3D reconstructions coupled with mathematical modelling and computer simulations we dissected the transition of striatal astrocytes toward neurogenesis. In the striatum, neurogenic astrocytes are scattered throughout the parenchyma and expand locally, generating clusters of clonally related cells, that we define as striatal niches. These structures are initially composed only of activated astrocytes and transient amplifying progenitors. These latter cells subsequently expand and generate proliferating neuroblasts following a stochastic mode of division and differentiation. Post-mitotic neuroblasts accumulate in the cluster before dispersing as individual cells. Interestingly, striatal astrocytes become activated at a constant rate, resulting in the continuous addition of new striatal niches with time. Nevertheless, the total number of niches does not increase with time indicating that these structures have a transient existence. Thus, continuous striatal neurogenesis occurs through the asynchronous transition of scattered neurogenic astrocytes from quiescence to an active state.
Overall, these data suggest that the neurogenic potential is widespread among striatal astrocytes, and that the striatal parenchyma is largely permissive for de-novo establishment of neurogenic niches.

18. Drug repositioning for remyelination: the approach of multiple-staged phenotypic screening

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One well-acknowledged strategy to prevent multiple sclerosis (MS) progression is the stimulation of endogenous remyelination, which restores impulse conduction preventing axons' degeneration. Currently available therapies for MS are not able to stop or repair CNS damage, so the identification of agents favoring regeneration and neuroprotection is urgent. To speed up the search for remyelinating strategies, researchers' attention has recently focused on drug repositioning. Over the past years, several drug screening for remyelination have been carried out through the phenotypic approach, as it is particularly useful for neurodegenerative diseases, where molecular targets and pathological mechanisms are unknown.

In 2017, our lab team published the results of a multi-tiered phenotypic screening in which a library of 2000 compounds was tested on different oligodendrocyte developmental processes. Among the hit compounds, the neuroprotective drug edaravone appears to be of strong interest since it is already approved for the treatment of acute ischemic stroke and amyotrophic lateral sclerosis. Our studies are in progress to confirm the in vivo regenerative potential of edaravone in a pre-clinical animal model and to identify the molecular targets responsible for its remyelinating activity through a medicinal chemistry approach.

The development of a platform for the sequential screening of drugs that could be repurposed for their remyelinating and neuroprotective potential is the aim of an international project funded by Progressive MS Alliance in which we are involved. The innovative strategy of this study is the use of a bioinformatic platform to select drugs matching with phenotypical and genetics characteristics of the disease and the use of oligodendroglia and neurons derived from human iPSCs of both controls and MS subjects. Multiple-staged phenotypic screenings allow us to evaluate drug activity across the combined data set rather than their status within a single analysis reducing the risks of failure.

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

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

20. Study of blood brain barrier disruption in mct8/dio2 knock-out mice as a model for allan-herndon-dudley syndrome.

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Allan-Herndon-Dudley syndrome (AHDS) is a rare disease in which a highly specific transmembrane transporter of thyroid hormones (T4 and T3), the monocarboxylate transporter 8 (MCT8), is mutated. This syndrome is characterized by peripheral hyperthyroidism together with a cerebral hypothyroidism and severe neurological damage, most likely due to an impaired transport of thyroid hormones across the brain barriers. However, there is still a lack of knowledge of the physiopathology of this complex syndrome. Studies from our group show that brain samples from an AHDS subject present signs of astrogliosis and microgliosis which could be related to blood-brain-barrier (BBB) damage. As Mct8KO mice do not reproduce the neurological syndrome of the patients due to a compensatory mechanism mediated by the enzyme deiodinase 2 (DIO2), the Mct8/Dio2 knockout (KO) mice have been proposed recently as a model for AHDS. Recent results from our group have shown signs of astrogliosis in Mct8/Dio2KO mice. The aim of this study was to investigate if MCT8 deficiency alters the integrity of the BBB. To do this, we evaluated the deposition of Evans Blue (EB) in the brain of Mct8/Dio2KO mice after retro-orbital administration and we studied the infiltration of the immunoglobulin G (IgG), two parameters used for the evaluation of BBB disruption. In order to evaluate the onset and the potential chronification of the damage, we have performed these analyses at different ages. The results showed an increase in the content of EB at all ages studied (P21, P90, and P180) in the brain of Mct8/Dio2KO mice. In agreement with this, immunohistochemical studies revealed a high content of IgG in the brain of Mct8/Dio2KO mice. Overall, our results suggest alterations in the integrity of the BBB as a consequence of MCT8 deficiency that could be related with the astrogliosis described in the Mct8/Dio2KO mice.

21. Protective functions of neuroinflammation in amyotrophic lateral sclerosis.

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Considerable evidence indicates that neurodegeneration in ALS can be conditioned by a deleterious interplay between motor neurons and astrocytes. Astrocytes are the major glial component in the central nervous system, where they fulfill several activities. In physiological and pathological conditions, astrocytes secrete a wide range of mediators, including the trophic factors GDNF and BDNF, two protective agents for motor neurons. Thus, the modulation of the endogenous mechanisms that control the production of astrocytic trophic factors may have therapeutic implications in ALS. We investigated the astrocytic signalling pathways driven by the two pro-inflammatory mediators TNFa and HMGB1 and controlling the astrocytic production of trophic factors. We identified TNFa/TNFR1 signalling as a major promoter of astrocytic GDNF synthesis/release. In SOD1G93A ALS transgenic mice, where the affected tissues spontaneously exhibit high levels of TNFa and TNFR1, we verified a strict correlation in the expression of the TNFa, TNFR1 and GDNF triad at different stages of disease progression. The ablation of TNFR1 completely abolished GDNF rises in both ALS astrocytes and spinal cords, a condition that accelerated motor neuron degeneration and disease progression. In parallel, we examined the role in ALS of HMGB1, a nuclear protein typically released in the extracellular milieu by living cells experiencing physiological stress conditions or by damaged cells. We showed that the interaction of HMGB1 with its receptor RAGE and TLR4 in normal astrocytes promotes neuroprotection via the production of GDNF and BDNF. In ALS mouse spinal cords, we found that HMGB1 is significantly released from motor neurons during disease progression. We postulated that extracellularly released HMGB1 can paracrinally interact with the neighboring astrocytes to counteract the neurodegenerative process. Yet, ALS astrocytes show an impaired capacity to raise trophic factor levels upon HMGB1 stimulation.

22. Anti-inflammatory and neuroprotective effects of Cannabidiol in an animal model of Parkinson's disease

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Several biological mechanisms are involved in the pathogenesis of Parkinson's disease (PD), with an increasingly recognized role for neuroinflammation. Indeed, it has been demonstrated that persistent microglia and astrocyte activation - accompanied by augmented levels of pro-inflammatory mediators in the extracellular space - contribute to exacerbate the degeneration of dopaminergic neurons in the Substantia Nigra pars compacta that drives disease progression. Current PD therapies are purely symptomatic and do not modify disease progression. Cannabidiol (CBD), one of over 100 phytocannabinoids identified in Cannabis sativa, exhibits a large spectrum of therapeutic properties including anti-inflammatory effect, suggesting its potential as disease-modifying agent for PD.

Aim: The aim of this study was to evaluate the effects of chronic treatment with CBD on neurodegenerative and neuroinflammatory processes, and motor deficits using a classic cytotoxic model of PD.
Methods: Sprague–Dawley male rats underwent CBD (10mg/Kg) or vehicle treatment (i.p.) for 4 weeks starting from 6-hydroxydopamine injection in the striatum. The degree of nigrostriatal damage and quantitative and qualitative assessment of neuroinflammatory process was evaluated by immunohistochemistry. Furthermore, the motor performance was assessed by cylinder, rotarod and apomorphine induced rotation test.
Results: Our results showed that CBD treatment significantly dampens neuroinflammatory response and reduces the nigrostriatal damage. Moreover, an improvement of motor performance was reported in the animals treated with CBD compared to controls .
Conclusions: These results further confirm that CBD may have therapeutic utility in PD where neuroinflammatory process is prominent and suggest intriguing symptomatic properties of this drug.

23. Effects of Specific miRNAs Shuttled by Exosomes Derived from Mesenchymal Stem Cells on late symptomatic SOD1G93A Mouse Astrocyte Primary Cultures

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Amyotrophic lateral sclerosis (ALS) is a motor neuron-involving neurodegenerative disease affecting about 4.5 per 100,000 people per year. Despite the significant progress in genetic studies, managed to explain many cases of ALS through mutations in several genes, the cause of a majority of sporadic cases remains unknown, even if the clinical and biomolecular features of genetic and sporadic ALS are very similar. Currently, epigenetics, involving miRNA studies, shows some promising aspects. We previously reported that intravenous administration of mesenchymal stem cells (MSCs) in the SOD1G93A mouse model of ALS produced positive effects on survival and disease progression, also modulating astrocytes and microglia reactive phenotypes. We proposed that MSC effects were paracrine, possibly involving exosome-mediated cell communication. Indeed, unpublished results substantiate the positive impact of MSC-derived exosomes on SOD1G93A mouse-derived astrocytes.Here, we investigated the activity of nine miRNA, which were found up-regulated in IFNɣ-primed MSCs and shuttled by MSC-derived exosomes, on spinal cord astrocyte primary cell cultures from late symptomatic 120 day-old SOD1G93A mice. At this purpose, we transfected SOD1G93A astrocytes with the single synthetic miRNAs and analyzed their effect on the astrocyte phenotype and the involved pathways. Seven out of nine miRNA mimics were able to affect the reactive phenotype of SOD1G93A astrocytes by significantly decreasing the over expression of GFAP, IL1β, and TNFα, detected by confocal microscopy. Four miRNAs (466q, 467f, 466m5p, 466i3p), over expressed in MSCs, were overexpressed also in exosomes. We selected in-silico their relevant pathways (p38, TNFα and NFKB) and validated them by determining the miRNA effects on MAP3K8, MAPK-APK2, MAPK11 and TRAF6 by qPCR. Two of them (466q, 467f) strongly reduced MAPK11 mRNA expression, thus inhibiting TNFα formation. Our results suggest that the amelioration of the reactive phenotype of spinal cord SOD1G93A astrocytes, brought about by in-vivo MSC treatment, operates through exosome-shuttled specific miRNAs.

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

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The C-Jun N-terminal kinase (JNK) pathway participates in several physiological and pathological mechanisms by phosphorylation of downstream effectors. JNK1 exerts pleiotropic roles during brain development such as control of regional apoptosis, of microtubule dynamics during dendrite morphogenesis and cortical interneuron migration. Moreover, JNK1 KO mice show alterations of the corpus callosum suggestive of myelin defects. Therefore, we investigated the role of JNK1 in oligodendrocyte (OL) development. The somatosensory cortex of JNK1 KO mice was stained with anti-PDGFRalpha antibodies to label oligodendrocyte precursor cells (OPCs), and with anti-myelin basic protein (MBP) antibodies to label the mature, myelinating OL. Immunohistochemical and quantitative analyses revealed a significant increase in the density of OPCs at P7 and P15 in KO mice compared to WT. Furthermore, JNK1 KO mice at both early postnatal and adult ages showed a lower extent of MBP expression, both in infragranular and in supragranular layers, indicative of abnormal myelin deposition. Inspection of the staining suggested that MBP expression was also altered in the corpus callosum. Based on these data, we analysed more deeply the structure of myelinated axons and examined the nodes of Ranvier by labelling for contactin associated protein 1 (CASPR), one of the proteins of the adhesion complex that mediates their assembly. We found that JNK1 KO mice display a higher density of nodes and that the nodes are longer compared to the WT. With the aim to assess cell autonomous defects of JNK1 KO OLs, we performed in vitro cultures of rat OPCs treated with DJNKi (a specific inhibitor of JNK that partly mimics JNK1 KO). Results suggest alterations in proliferation rate and cell morphology of OL treated with the inhibitor, compared to the non-treated ones. Our findings suggest for the first time that JNK1 takes part in oligodendocyte development and in the axo-glial interplay.

25. PD-L2 (CD273) immunomodulatory effects in neuroinflammation

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Extracellular Vesicles (EVs) are small (100-1000nm) membrane particles released by all cell types. EVs of myeloid origin are reported to be significantly increased in number in the cerebrospinal fluid (CSF) of patients with neuroinflammatory disorders. However, a clear understanding of EVs composition and surface markers expression is yet to be defined. We have previously observed that the majority of myeloid EVs in the CSF of EAE mice express PD-L2 (CD273) on their surface, a key inhibitor in the immune response.The aim of the present work is to investigate the expression and the function(s) of PD-L2 on myeloid EVs during experimental and human neuroinflammatory disorders. We isolated and analyzed EVs from the CSF of EAE mice and from the CSF of neurological patients. We then engineered a murine microglial cell line (BV2) with a PD-L2 lentiviral vector, resulting in a stable source of PD-L2+ EVs. We analyzed EVs immunomodulatory effects on T cells, exploiting the splenocytes from 2d2 mice, a transgenic model for the myelin oligodendrocyte glycoprotein (MOG)-specific TCR. Here we report that Ib4+PD-L2+ EVs isolated from the CSF of EAE mice mirror disease progression and correlate positively with the clinical score. Similarly, we found that PD-L2+ EVs are increased in the CSF of Multiple Sclerosis patients, particularly in patients with an active form of the disease. In vitro, BV2 cells stimulated with IL-4 increased the surface expression of PD-L2 and release EVs that reflect the polarization phenotype. The treatment of splenocytes from 2d2 mice with PD-L2+ EVs slightly restrains reactive T lymphocytes proliferation, but remarkably decreases CD25 expression on T lymphocytes and MHC II on CD11b+ antigen-presenting cells. These findings suggest a role for PD- L2+ EVs in modulating neuroinflammation and as an innovative marker for disease activity in patients with MS.

26. Microglial extracellular vesicles as therapeutic therapy in neuroinflammation

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Microglia is considered an eligible target against the progressive multiple sclerosis (MS), but current available therapies do not allow its efficient targeting. Recently my group described that microglia derived-extracellular vesicles (EVs), engineered to encapsulate IL4, are taken up by microglia itself, mediating a phenotype switch to a protective phenotype. In vivo studies suggest that these EVs can ameliorate established neuroinflammation, thus making them a promising drug-delivery tool to target CNS in MS. My project focuses on understanding the mechanism of action and the signalling pathway of EVs delivery and to exploit this knowledge to specifically deliver different potential therapeutic molecules.The EVs analysis with TRPS identify differences that can be consistent with the different pathway formation of exosomes and microvesicles. We demonstrated in vivo the strong phenotypic change induced by our EVs to resting microglia in a dose- and time-dependent effect. Then impairing the physiological procedure of the endosome acidification, the effect of our EVs on recipient cells is higher. Thus, suggesting an endocytic pathway for the internalization of the vesicles mediated by the recipient cells. We further demonstrate with a gradient ultracentrifugation the capability of our formulation to vehicle endogenous IL4 inside the vesicles. Even if some protein is co-purified in the procedure, we know that the half-life of this cytokine is too short to elicit a strong in vivo response. Consequently, we assume that the anti-inflammatory effect of our EVs in vivo is a result of the IL4 internalized in our formulation. These data help us understand more in detail the process of internalization and phenotype change mediated by these EVs. Our next goals are discriminate between different internalization pathways and further validate the efficacy of our therapy on the EAE mouse model.

27. Tristetraprolin/ZFP36 regulates the neuroinflammatory cascade

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Multiple Sclerosis (MS) CNS-restricted inflammation contributes to the complex pathogenesis of the disease. It involves several cell players and molecular checkpoint. Among them, RNA Binding Proteins (RBPs), like Tristetraprolin (TTP) encoded by Zfp36 gene, are endogenous brakes that avoid prolonging inflammation. TTP in microglia (MG) and macrophages acts like a feedback control avoiding over-activation of cells and excessive release of pro-inflammatory cues. The aim of the study is to investigate the role of TTP in myeloid cells of the inflamed CNS using Experimental Autoimmune Encephalomyelitis (EAE) mice as experimental paradigm of MS. We used CX3CR1-CreERT2 TTP flox/flox transgenic mice (CX3CR1-TTP mice) bearing a tamoxifen-depended Cre recombinase under the control of Cx3cr1 regulatory regions to conditionally inactivate TTP in cells. While MG-restricted TTP inactivation was done treating transgenic mice with tamoxifen, we used bone marrow chimeric mice to achieve TTP inactivation in macrophages/monocytes. Both models were used in the EAE experimental paradigm to study the role of TTP during acute neuroinflammation. Mice were evaluated for clinical outcomes and sacrificed at the peak of the disease to investigate demyelination and axonal damaging.
TTP deficiency in CNS-resident MG slightly increased demyelination without affecting clinical progression of the disease. On the other hand, the inactivation of TTP peripheral CX3CR1+ myeloid cells substantially worsened the clinical progression of EAE mice, increasing demyelination and axonal damage. A modulation of myeloid cells in MS is conceptually relevant to reduce tissue damage. RBPs are important players in the immune response, regulating the decay of mRNAs encoding pro-inflammatory cytokines and chemokines. Our approach allows the identification of TTP as crucial modulator of the inflammatory cascade in infiltrating macrophages/monocytes in EAE. Therefore, our study opens new avenues for therapeutic strategies aimed to reinforce TTP functionality during neuroinflammation.

28. Reactive Glia-Derived Neuroinflammation: a Novel Hallmark in Lafora Progressive Myoclonus Epilepsy That Progresses with Age

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Lafora disease (LD) is a rare, fatal form of progressive myoclonus epilepsy. The molecular basis of this devastating disease is still poorly understood and no treatment is available yet, which leads to the death of the patients around 10 years from the onset of the first symptoms. The hallmark of LD is the accumulation of insoluble glycogen-like inclusions in the brain and peripheral tissues, as a consequence of altered glycogen homeostasis. In addition, other determinants in the pathophysiology of LD have been suggested, such as proteostasis impairment, with reduction in autophagy, and oxidative stress, among others. In order to gain a general view of the genes involved in the pathophysiology of LD, in this work we have performed RNA-Seq transcriptome analyses of whole brain tissue from two independent mouse models of the disease, namely Epm2a-/- and Epm2b-/- mice, at different times of age. Our results provide strong evidence for three major facts: first, in both models of LD, we found a common set of upregulated genes, most of them encoding mediators of inflammatory response; second, there was a progression with the age in the appearance of these inflammatory markers, starting at three months of age; and third, reactive glia was responsible for the expression of these inflammatory genes. These results clearly indicate that neuroinflammation is one of the most important traits to be considered in order to fully understand the pathophysiology of LD, and define reactive glia as novel therapeutic targets in the disease

29. Set up of an in vitro model of quadripartite synapse to study synaptic injury

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Synaptic dysfunction is the first common neurodegenerative event in many brain diseases, composed by an initial reversible phase, in which synaptic function is impaired, but can be reverted; this phase evolves in a second irreversible stage with synaptic loss. The synapses are composed by stable elements, pre and post-synaptic terminals plus the end-feet of astrocytes, but it is now clear that there is also a 3rd cell-type, the microglia, that is in dynamic contact with synapses and participates to the synaptic function/dysfunction. To understand cellular interactions, molecular mechanisms, key-modulators and intracellular pathways governing the "quadripartite synapse", we are characterizing step-by-step a new in-vitro model starting with a co-culture composed by hippocampal astrocytes and neurons at DIV14 and DIV21. We found that neurons in the co-culture show lower membrane resting potential, but we did not observe any changes in mEPSCs and mIPSCs, except for reduced amplitude of mIPSCs compared to neurons alone. The co-cultures were studied also by biochemical analysis of total homogenate and TIF fraction, representing the PSD-region. The stress pathways, JNK and Caspase3, were less activated in co-culture's total homogenate and TIF fraction compared to neurons alone. TIF analysis revealed important changes in PSD biochemical markers: AMPA, NMDA-receptors and scaffold proteins levels were lower at DIV14 compare to neurons alone, but the co-culture reached normal levels at DIV21, suggesting a delayed maturation of the post-synaptic elements. We are now performing patch-clamp analysis at DIV21 to clarify this point. In parallel to the 2D co-culture system we are also setting-up the model in an innovative device, called Nichioid, in which cells can growth in a 3D scaffold. The next step will be adding Aβ−oligomers to the system inducing synaptopathy and lastly microglia. The final aim is to develop a new model defining critical cellular interactions and key intracellular pathways regulating the "quadripartite synapse" in physiological/pathological conditions, which will allow designing new therapeutic strategies against synaptopathy.

30. Neurogenic activation and lineage progression of striatal astrocytes following excitotoxic lesion.

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

31. Characterization of roles of Notch signaling in the generation of neural stem cells derived from lesion-reactive astrocytes

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Astrocytes in the mammalian brain play important roles in neuronal support and brain homeostasis. Although the production of new neurons in the adult brain is restricted to neurogenic regions or niches, astrocytes become activated by injuries to the central nervous system and acquire an undifferentiated, progenitor-like state, characteristic of neural-stem cells (NSCs). In vitro astrocyte-derived NSC-like cells regain multipotency and undergo long-term self-renewal. The activation and reversion of astrocytes to a NSC-like state could be potentially useful to generate new neurons in the brain after lesion. However, the molecular factors involved in astrocyte dedifferentiation are poorly understood. In this context, we developed an in vitro lesion system to investigate the role of the Notch signaling in astrocyte dedifferentiation by conditionally deleting Notch signaling component genes from cultured astrocytes prior to a scratch lesion. We deleted Rbpj and Notch2 genes by Cre-mediated recombination and monitored the fate of the ablated cells by following GFP expression from the Cre-activated CAG-STOP::GFP. The lesion-activated, reactive astrocytes were then assessed for self-renewal in neurosphere assays. The number and size of the neurospheres were quantified as an indication of self-renew ability and proliferative capacity. We performed further analysis to unravel the differentiation potential of reactive astrocytes-derived NSC and the role Notch signaling components play in the process.

32. Deltorphine derivatives for bbb targeting

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Central Nervous System (CNS) compartments is one of the most difficult districts for drug delivery due to the presence of the Blood Brain Barrier (BBB) that hampers the passage of 90% of drugs. Here we describe for the first time the use of deltorphin-derived peptides (DP) and glycosylated derivatives (Glu-DP) to deliver biodegradable and biocompatible polymeric poly-lactide-co-glycolide (PLGA) nanomedicines across the BBB. Molecular Dynamic (MD) studies to control the 3D structure of the peptide were used to analyze the most favored conformation of each modified peptide (DP and GluDP), which resulted to be α-helix-shaped. The DP and GluDP were then conjugated onto PLGA using peptide coupling chemistry and formulated into nanoparticles (NPs). Physico-chemical and technological characterization of the DP-NPs and Glu-DP-NPs showed no significant difference in comparison with plain control PLGA NPs. When IP injected into wild-type mice, both DP- and Glu-DP-NPs were observed to cross the BBB, unlike un-targeted NPs, and furthermore Glu-DP-NPs displayed a higher BBB penetration and co-localization with neurons.
Finding new ligands that chaperone large nanoparticulate systems through the BBB is one of the crucial challenges of nanomedicine. In this work, two deltorphin derivatives were shown to be effective in targeting brain tissues, and they could be chemically linked to PLGA stable nanoparticles. Most importantly, targeted nanoparticles were shown, in vivo, to cross the BBB and to be co-localized mostly with neurons, even though up to now poor is known about the intercellular destiny of these particles. Additionally, the recent growing knowledge of Tunnelling Nanotubes (TNTs) as a novel way of cells to communicate and exchange material puts a light on a possible intracellular trafficking of therapeutic nanoparticles.
Future experiments will help to better understand both the area/cell localization and the possibility to exploit TNTs to spread therapeutics beyond the recipient cells in brain tissues.

33. ADAM10 expression within the central nervous system

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Even though ADAM10 plays a key role in multiple cellular processes and in the central nervous system (CNS) development, its protein distribution in the CNS has not been fully addressed. Therefore, we investigate by immunofluorescence (i) ADAM10 expression profile in different areas of the CNS in C57Bl/6 adult mice and (ii) ADAM10 subcellular expression in different cell types as neurons, astrocytes, oligodendrocytes in primary cultures. Regarding the different brain regions explored, we showed a strong expression of ADAM10 in the hippocampus and piriform cortex in the brain, in the Purkinje and granular cell layers in the cerebellum and in the spinal cord to a lower extend. We show that ADAM10 is mainly expressed in neurons, but it can also be found in oligodendrocytes. Using primary cell cultures, we show that ADAM10 is found in the nuclei and cytoplasm of oligodendrocytes and astrocytes while in neurons it is mainly expressed in the membrane. Overall, this work highlights for the first time ADAM10 protein expression in different regions and cell types of the mice CNS. The nuclear localisation of ADAM10, probably due to its intracellular domain, emphasises its role in cell signalling in oligodendrocytes and astrocytes in physiological and pathological conditions. Further investigations are required to better elucidate the role of ADAM10 in glial cells.

34. Glucocorticoid receptor activity alters neuron and microglia plasticity in an animal model of Alzheimer's Disease

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Chronic exposure to high dose of glucocorticoids (GC) represents a key risk factor for the development and progression of Alzheimer's Disease (AD). In a triple transgenic mouse model of AD (3xTg-AD mouse), chronic stress, through hyperactivation of glucocorticoid receptors (GR), drives the appearance of the typical neuropathological hallmarks of this dementia. Considering the ability of GC to regulate, also, the neuron and microglia plasticity, we, firstly, investigated the effects of GR agonists and antagonists on dendritic spine density and microglia activity in the CA1 region of hippocampus of 3xTg-AD mice. Thus, through an innovative combined Golgi Cox and immunofluorescence technique, we found that 5 days of treatment with 8mg/kg of dexamethasone (DEXA), an agonist of GR, reduced significantly the dendritic spine density in CA1 region of 3xTg-AD mice, both at 6 and 10 months of age. Contextually, the same treatment enhanced, by 50%, the density of microglia and their levels of activation. On the contrary, the blockade of stress response, through the treatment with 20mg/kg of mifepristone (MIFE), an antagonist of GR, vigorously, increased the dendritic spine density in CA1 region, at both ages, as verified also by electron microscopy analysis. The antagonist improved, also, the 3xTg-AD mice performance in Y-maze task and reduced microglia density in CA1 hippocampal region. Secondly, we observed that the hyperactivation of GR, through DEXA treatment, enhanced the proximity of microglia processes to neuron dendrites, increasing, significantly, the portion of microglia filaments closer than 10μm from neuron dendrites. This result indicate that microglia could, directly, contribute to stress-induced dendritic spine degeneration in the 3xTg-AD mice. In conclusion, these data demonstrated that stress can exacerbate the AD pathology, while the use of GR antagonist, like MIFE, could represent a promising therapeutic strategy to slow down the progression of this disease.

35. Neuronal expression of E2F4DN modulates the immune response observed in the cerebral cortex of 5xFAD mice

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Alzheimer's disease (AD) is a neurodegenerative disorder in which altered immune response is an important etiological factor. The transcription factor E2F4 participates in both cellular quiescence and tissue homeostasis, and regulates gene networks affected in AD, thus constituting a potential target for intervention. We have studied whether neuronal expression of a dominant negative form of E2F4 (E2F4DN), unable to become phosphorylated in a Thr motif necessary for the induction of cell cycle re-entry in neurons, can modulate the immune response observed in AD. To this aim, we generated Mapt:E2F4DN knock-in mice (E2F4DN mice) that were crossed with 5xFAD mice, a known murine model of AD, and the cerebral cortex/hippocampus of the hybrid descendants was subjected to analysis. In parallel, descendants from 5xFAD mice crossed with control Mapt:EGFP knock-in mice (EGFP mice) were also evaluated. We first performed a temporary study with 5xFAD/EGFP mice of 2, 3, 6 and 12 months of age to define the distribution pattern of GFAP (reactive astrocytes) and Iba1 (microglia) throughout the different cortical layers. This analysis demonstrated a time-dependent increase of GFAP in the cerebral cortex of 5xFAD/EGFP mice, while neuronal-expression of E2F4DN led to a reduction in astrogliosis. In addition, Iba1-positive cells showed marked morphological changes in 5xFAD/E2F4DN mice, suggesting that microglia activation is altered by the presence of neuronal E2F4DN. In the cerebral cortex of 5xFAD/E2F4DN mice, most Iba1-positive cells were associated to Aβ deposits, which were increased in size, but not in number. We speculate that the crosstalk between E2F4DN-expressing neurons and microglia favors the aggregation of oligomeric Aβ, thus reducing its toxicity. Altogether, our data are consistent with a beneficial immune response in 5xFAD mice expressing neuronal E2F4DN, which we propose as a therapeutic agent against AD.

36. Novel combined gene/cell therapy strategies to provide full rescue of brain pathology in a severe lysosomal storage disease.

Francesca Ornaghi1, Davide Sala1,2, Francesco Morena2, Manuela Valsecchi3, Giorgia Serena Gullotta4, Marco Bacigaluppi4, Massimo Aureli3, Sabata Martino2, Angela Gritti1

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Sandhoff disease (SD) is a Lysosomal Storage Disease (LSD) caused by genetic deficiency of the β-N-acetylhexosaminidase (Hex) enzyme. The resulting accumulation of GM2 ganglioside preferentially in neurons has devastating effects on the central nervous system (CNS). The combination of intracerebral gene therapy and hematopoietic stem/progenitor cell (HSPC)-based approaches provides variable benefit in other LSDs. However, the impact of similar therapeutic strategies in halting SD pathology has been poorly investigated. Here, we show the therapeutic advantage of coupling neonatal intracerebral injection of lentiviral vectors coding for the functional Hex enzyme and HSPC transplant in SD mice. In addition, we clarify the respective contribution of treatments to disease correction, with a focus on the modalities of resident CNS microglia replacement by the transplanted HSPC progeny. We found that HSPC alone delays onset of symptoms and moderately increase the survival of SD mice, counteracting neuroinflammation but failing to clear GM2 storage. In contrast, SD mice treated with the combined treatment show a significant increase of lifespan and normalization of the pathological phenotype. This benefit correlates with increased Hex activity and remarkable reduction of GM2 storage in neural tissues. Our results suggest that the early enzymatic supply provided by intracerebral gene therapy is crucial for the proficient engraftment of HSPC progeny, which then reduce neuroinflammation and function as a stable reservoir of functional enzyme, delaying the progression of the disease. Results of the ongoing phenotypic and functional characterization of HSPC-derived microglia engrafted in the brain - in comparison with the endogenous counterpart - will clarify the impact of the primary neurological disease on the microglia replacement, paving the way to the identification of strategies to enhance and optimize this process. We anticipate that this study will boost the development of refined gene/cell-based approaches for treating the brain disease in SD and possibly other LSDs.

37. Do glial cells have a role in binge-eating disorder?

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Binge eating disorder (BED) is the most common eating disorder worldwide. Patients show recurrent episodes of eating large quantities of food very quickly, to the point of discomfort. BED is associated with obesity and diet cycling increases the prevalence of binge eating disorders. Similarly to drugs of abuse, consumption of palatable food, high in sugar and in fat content, stimulates the mesolimbic dopamine pathway to mediate motivated behaviors. Moreover, stress represents a powerful trigger for binge-eating episodes. In order to understand the neurobiology of BED, studies conducted so far have been focused on the neuronal circuits involved. However, in the brain, glial cells have been recognized as key players in the orchestration and fine regulation of neural signaling. They exert heterogeneous and complex functions, whose alterations, occurring in a context-dependent and disease-specific way, have been linked to a variety of neurological disorders. Therefore, the present study was aimed at characterizing glial cells in a rodent model of BED. Female rats exposed to sessions of restriction/re-exposure to food (mimicking diet cycling) and to a session of frustrations stress showed binge-like eating behavior. Control rats were included and did not show this behavior. Immunofluorescence experiments were carried out in regions of the frontal cortex, which were found involved in BED patients. Results indicate alterations of glial cells in subregions of the orbitofrontal cortex, confirming their key role in quickly responding to perturbations of brain homeostasis. Further tests are needed to deeply understand their contributions in binge-like behavior as novel possible pharmacological targets. Indeed, few pharmacological treatments exist for patients affected by BED, which pose serious limitations due to their severe adverse reactions.

38. The effect of aging on the spatial distribution of glycogen in Layer I somatosensory cortex of mice

Maria Fernanda Veloz Castillo1, Rana Alrabeh1, Kalpana Kare1, Daniya Boges1, Markus Hadwiger2, Marco Agus2,3, Pierre J Magistretti1 and Corrado Calì1

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

39. A chemical screening approach targeting new autophagy modulators in the context of ischemic stroke

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Autophagy is a self-eating process involved in the maintenance of neuronal homeostasis, both in physiological and in pathological conditions. A growing number of studies show that ischemia is associated with the activation of autophagic flux. However, the role of autophagy in regulating neuronal survival is not fully elucidated. The identification of selective modulators of the autophagic flux should help to discover novel therapeutics as well as functional outcomes of this biological process. We develop a sensitive and robust assay to explore novel autophagy modulators in brain ischemia. We use Microtubule-Associated Protein 1A/1B-Light Chain 3 (LC3) coupled with pHluorin to monitor the autophagic flux in mammalian cells. This chimeric protein offers the pH-sensitivity of the GFP variant pHluorin to sense the acidic environment of autolysosomes. We established cell clones constitutively expressing this sensor. We used a well-known autophagy inhibitor (Bafilomycin A1) and an activator (Torin-1) to characterize these cell clones. Through quality control experiments that allowed calculating fold variations, Z-factor, and Strictly Standardized Mean Difference, we set up conditions for a High Content, Medium-Low Throughput Screening by semi-automated flow cytometry. We introduced in our setting Oxygen-Glucose Deprivation to stress cells, mimicking the toxic environment that features the ischemic brain. We prioritized a library of 6839 bioactive compounds through an in silico screening based on physically significant descriptors and pharmaceutically relevant properties. We ended up with 901 molecules that are currently under evaluation. Selected molecules will be further validated using aged hiPSC-derived neurons as well as in the Middle Cerebral Artery Occlusion (MCAO) mouse model.

40. Effects of exosomes derived from IFNγ-primed mesenchymal stem cells in astrocytes cultured from late symptomatic SOD1G93A mice.

Roberta Arianna Zerbo1, F. Provenzano1, C. Torazza1, M. Balbi1, C. Marini2, B. Parodi2, M. Milanese1,3, D. Giunti2, N. Kerlero de Rosbo2, C. Usai4, A. Uccelli1,3,5, G. Bonanno1,3,5.

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Amyotrophic lateral sclerosis (ALS) is a rare and progressive neurodegenerative disease characterized primarily by the death of upper and lower motor neurons. The progression of the disease is mostly mediated by altered intercellular communication between neurons and glial cells and has been linked to microglia and astrocyte-mediated neuroinflammation. We previously demonstrated that the intravenous administration of mesenchymal stem cells (MSC) in the SOD1G93A mouse model of ALS prolonged survival, improved motor skills and reduced reactive gliosis. These beneficial effects were not associated with MSC differentiation, being possibly mediated through paracrine mechanisms. We postulated that MSC-derived exosomes can be a mode to sustain the paracrine effects of MSCs.

We studied here the activity of mouse IFNγ-primed MSC-derived exosomes on spinal cord astrocytes primary cell cultures prepared from late symptomatic 120 day-old SOD1G93A mice. GFAP, S100 β and vimentin expression was increased in 120 day-old SOD1G93A astrocytes compared to age-matched WT astrocytes. The in-vitro exposure to MSC-derived exosomes significantly reduced the overexpression of the three astrocyte activation markers. The pro-inflammatory cytokines IL-1β, TNF-α and IL-6 were significantly more expressed and more efficiently released in SOD1G93A astrocytes and the exposure to significantly decreased their overexpression and release. Conversely, the expression of the anti-inflammatory cytokine IL-10 was decreased in SOD1G93A astrocytes and normalized after exposure to exosomes. Also, the expression of the inflammation complex NLRP3 was increased in SOD1G93A astrocytes and the increase was reversed by exosomes. In addition, the viability of MNs seeded on exosome-treated late-symptomatic SOD1G93A astrocytes was significantly increased when compared to co-cultures with non-treated astrocytes.

Our results suggest that the reactive phenotype and the neuroinflammatory pathways in ALS-astrocytes are ameliorated by exosomes derived from IFNγ-primed-MSCs. Spinal MN viability also improved. These results open the possibility for in vivo translational preclinical studies in SOD1G93A mice.

Poster sessions will be held on the first and third day of the workshop. Max poster size: 200cm x 80cm (hxl)

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LOCATION

Lectures will take place at the Institute of Human Anatomy, C.so Massimo D'Azeglio 52, Turin
The theoretical-practical sessions (day 2) will be held at the Neuroscience Institute Cavalieri Ottolenghi, Regione Gonzole 10, Orbassano (Turin). A bust transfer will be organized.

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