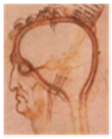
IN CNR Annual Retreat 2018



Bergamo,Seminario Vescovile Giovanni XXIII
26-28 September 2018

Oral communications and posters: schedule and abstracts

IN CNR Annual Retreat 2018

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INVITED LECTURES and ORAL COMMUNICATIONS: SCHEDULE AND ABSTRACTS

N	When	Speaker	Title

Plenary lecture

	W 20-21	**Luigi Naldini** Director, SR-TIGET, San Raffaele Telethon Institute for Gene Therapy	*Genetic Engineering of Hematopoiesis to Treat Human Disease*

Invited lectures

IL1	W 11-11.30	**Maria Passafaro** IN-MI	*The role of X-linked intellectual disability genes in synapse functions*
IL2	W 14.30-15.15	**Micaela Morelli** **SINS president** (IN-CA associate)	*How models of Parkinson's disease can help to understand the disease and aid to find new therapies*
IL3	F 8.30-9.00	**Enrica Strettoi** IN-PI	*Inflamaging the eye*
IL4	F 14.30-15.00	**Roberta Benfante** IN-MI	*Ion channels as putative drug targets in congenital central hypoventilation syndrome (CCHS)*

Oral communications

Session 1	***Brain cell physiology and pathology***		Chairs: **Giorgio Carmignoto** (IN-PD) **Bice Chini** (IN-MI)
OC1	W 11.30-11.55	**Marco Mainardi** IN-PD	*Purification and proteomic profiling of PSD-95 interactors at in vivo potentiated synapses*
OC2	W 11.55-12.20	**Laura Gerosa** IN-MI	*Activity –dependent translocation of PCDH19 C-terminus in the nucleus concurs to IEGs homeostasis*
OC3	W 12.20-12.45	**Cristina Spalletti** IN-PI	*Robotic rehabilitation and neuromodulation after stroke:novel approaches in a mouse model*

Session 2	***Neurodegenerative diseases***		Chairs: **Matteo Caleo** (IN-PI) **Carlo Sala** (IN-MI)
OC4	W 15.15-15.40	**Angelo Iannielli** IN-MI	*Establishing patterned neuronal circuits with human iPSC-derived neurons in microfluidics*
OC5	W 15.40-16.05	**Martina Gabrielli** IN-MI	*Role of microglial extracellular vesicles in early synaptic dysfuntion in Alzheimer's disease*
OC6	W 16.05-16.30	**Caterina Peggion** IN-PD	*Defective differentiation of primary myocytes in an ALS mouse model*

Session 3	***Sensory systems***		**Chairs: Alessandro Sale** (IN-PI) **Claudia Lodovichi** (IN-PD)
OC7	F 9.00-9.25	**Gabriele Sansevero** IN-PI	*Running towards a new therapy for amblyopia*
OC8	F 9.25-9.50	**Andrea Maset** IN-PD	*OPHN1 regulates the migration of newly generated cells in the olfactory system*
OC9	F 9.50-10.15	**Nicola Origlia** IN-PI	*In memory of Luciano Dominici*

Session 4	***Miscellanea***		**Chairs: Anna Lisa Muntoni** (IN-CA) **Giorgia Pallafacchina** (IN-PD)
OC10	F 15.00-15.25	**Claudia Sagheddu** IN-CA	*N-acylethanolamine acid amidase as a novel therapeutic target for nicotine addiction*
OC11	F 15.25-15.50	**Francesca Grisan** IN-PD	*PKA and EPAC crosstalk in HT29 migration: the importance of signal compartimentalization*
OC12	F 14.30-15.00	**Ludovico Arcuri** IN -MI	*Novel gene therapy approaches for whole brain delivery of the lysosomal GCase enzyme for wide protection from alpha-synuclein toxic aggregates*

THE ROLE OF X-LINKED INTELLECTUAL DISABILITY GENES IN SYNAPSE FUNCTIONS

Maria Passafaro

CNR -Institute of Neuroscience, Milan (Italy)

Intellectual Disability (ID) is a common and highly heterogeneous cognitive disorder with a very severe social impact. Although in the last 10 years a number of genes have been discovered whose mutations cause intellectual disability, we are still far away from the identification of the consequence of these mutations on brain functions. One of these genes is TM4SF2 that is located on Xp11.4 and encodes tetraspanin 7 (TSPAN7). Tetraspanins are a class of evolutionarily conserved transmembrane proteins that have the peculiar ability to organize membrane domains, tetraspanin enriched microdomains (TEMs).

We reported that, in cultured neurons, shRNA-mediated down-regulation of TSPAN7 affects AMPAR trafficking by enhancing PICK1–GluA2 interaction, thereby increasing the intracellular retention of AMPAR. Moreover, we found that loss of TSPAN7 function in mice causes alterations in hippocampal excitatory synapse structure and functionality as well as cognitive impairment.

These changes occurred along with alterations in AMPAR expression levels. We also found that interfering with PICK1–GluA2 binding restored synaptic function in Tm4sf2–/y mice. Moreover, potentiation of AMPAR activity via the administration of the ampakine CX516 reverted the neurological phenotype observed in Tm4sf2–/y mice, suggesting that pharmacological modulation of AMPAR may represent a new approach for treating patients affected by TM4SF2 mutations and intellectual disability.

HOW MODELS OF PARKINSON'S DISEASE CAN HELP TO UNDERSTAND THE DISEASE AND AID TO FIND NEW THERAPIES

Micaela Morelli

CNR Institute of Neuroscience, Cagliari and Dpt. Biomedical Sciences, University of Cagliari, Italy

Parkinson's Disease (PD) is a complex disease caused by protein aggregation, mitochondria dysfunction, neuroinflammation and aging. Moreover, some juvenile forms of PD may have a genetic origin. This complexity should be reflected in models of the disease in order to achieve effective therapies. So far, in fact, we only have symptomatic therapies and in addition they are not satisfactory in the long-term.
The present talk will browse the several PD models utilized to study the mechanisms that underlie the disease and to evaluate the effectiveness of new therapies. The models will include the most commonly used that utilize rodents and primate (6-hydroxydopamine, MPTP, rotenone) together with emerging models in multicellular organisms (c-elegans, zebrafish, drosophila). These animal models usually utilized drugs or toxins to induce the degeneration of the dopaminergic neurons that characterize the disease and some of them also reproduce a slow progression of the disease starting from the periphery and ending in the brain. In addition, genetic models that recapitulate sporadic forms of the disease or models reproducing the hallmark of PD as overexpression of α-synuclein, will be presented. Finally, the models utilized to assess the presence of motor complications associated to dopamine-replacement therapy, as evaluation of abnormal involuntary movement (AIMs) will be described. The talk will critically describe the symptoms that the different models may reproduce better, together with the advantages and disadvantages of the models.

INFLAMAGING THE EYE

Enrica Strettoi[1], Martina Biagioni[1,2], Viviana Guadagni[1,3], Antonia Stèfanov[1,2], Antonio Falsconi[1,4],Elena Novelli[1], Paolo Aretini[5], Chiara Maria Mazzanti[5]

[1]CNR Neuroscience Institute, Pisa; [2]Regional Doctorate School in Neuroscience, University of Florence; [3]Department of Biology, University of Pisa; [4]Scuola Superiore Sant'Anna, Pisa; [5]Pisa Science Foundation, Pisa, ITtaly

The eye is a self-contained, immune privileged organ, within which the CNS portion, the retina, is shielded by a blood-retinal barrier constituted by macroglia, blood vessels and the retinal pigment epithelium. Yet, inflammation and immune response are directly implicated in the pathogenesis of invasive ocular diseases, such as diabetic retinopathy, retinopathy of prematurity, age-related macular degeneration. Moreover, inflammatory molecular effectors are considered to contribute to dysfunction and neuronal death in retinal diseases for which inflammation and immune response are not the initiating factors. Microglia/macrophage activation and recruitment favor a microenvironment detrimental to cell survival, creating a vicious circle supporting further neuronal damage.

In Retinitis Pigmentosa (RP), a genetic defect leads to progressive photoreceptor degeneration and vision loss. RP can be caused by any among hundreds different mutations in genes which might control any aspect of photoreceptor biology, from development to metabolism, converging on common cell death pathways. Although initially only rods (genetically hampered) die out and night blindness occurs, later cones also undergo a gradual demise, causing loss of visual acuity and chromatic discrimination up to legal blindness. Hence, the most severe clinical signs of RP are not directly caused by the primary genetic defect but by the secondary degeneration of cones; for their fundamental role, preservation of even a small fraction of these cells would ensure relatively normal lives to RP patients.

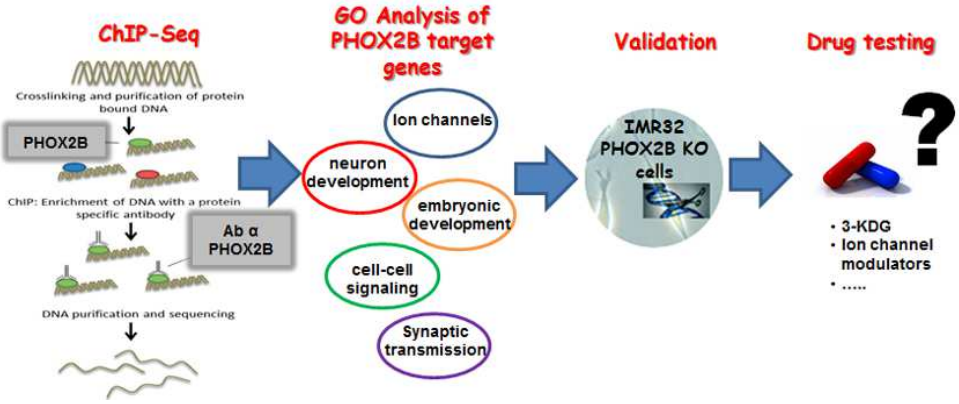
Decrease of survival factors, alteration of glucose metabolism, oxidative stress consequent to locally increased oxygen levels are all implicated in cone degeneration. We postulate that retinal inflammation consequent to the primary death of rods creates a local micro-environment detrimental to cone survival, contributing to the so-called "by-stander effect". We provide experimental evidence based on a mouse model of RP (the rd10 mutant mouse) showing that cone death and visual loss can be selectively delayed using oral steroids, commonly employed to treat various forms of ocular inflammations in humans. Steroids treatment reduces retinal microglial activation and local cytokine levels, concomitantly rescuing cone morphology and visual function. A rationale exists for inhibiting general versus local components of inflammation, using drugs that antagonize the efficacy of CCl2/CCR2 binding and lower monocyte recruitment from the blood stream.

Altogether, current literature and our data support the notion that selective inhibition of late component of retinal inflammatory response in RP prolongs cone survival and delays visual impairment, suggesting clinical applications for human patients.

ION CHANNELS AS PUTATIVE DRUG TARGETS IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

Silvia Cardani[1], Simona Di Lascio[1], Flavia Antonucci[1], Diego Fornasari[1,2], Roberta Benfante[1,2]

[1]BIOMETRA, University of Milan, Milan, Italy; [2]CNR -Institute of Neuroscience, Milan, Italy



Congenital Central Hypoventilation Syndrome (CCHS, MIM 209880) is a rare neonatal disease characterized by abnormal ventilatory response to hypoxia and hypercapnia, owing to failure of autonomic respiratory control. Frameshift mutations (5%) and polyalanine triplet expansions (95%) have been detected in the coding region of the transcription factor PHOX2B, responsible for the proper development and function of the ANS. Consistent with its role as transcriptional regulator, transcriptional dysregulation might be an important mechanism of CCHS pathogenesis. Stemming from the fortuitous observation that progestin Desogestrel can relief some symptoms of the disease, that the progestin-induced down-regulation of the expression of PHOX2B and of its target genes may be part of the molecular mechanism sustaining the clinical effect, and that desogestrel showed the same effect on wild type as well as mutant protein, it has strong proof of concept value in the perspective of a pharmacological intervention in CCHS, at least for ameliorating respiratory symptoms. These observations lead us to identify new PHOX2B target genes, by ChIP-seq analysis, as potential pharmacological targets for alternative molecules without contraceptive effects. Among the newly identified PHOX2B target genes, validated by comparing wild-type and CRISPR-CAS9 Knocked-down PHOX2B expressing IMR32 cells, we focused on genes encoding potassium and sodium channels since they have a central role in neuronal excitability and regulation of respiratory rhythm. Here we show that PHOX2B mutant protein increases the expression of these genes, leading to a possible detrimental effect on the excitability of these cells, opening up the possibility to screen for modulator of their activity or expression in order to restore a quasi-normal chemosensitivity of those neurons affected by PHOX2B mutations in CCHS.

PURIFICATION AND PROTEOMIC PROFILING OF PSD-95 INTERACTORS AT IN VIVO POTENTIATED SYNAPSES

Marco Mainardi[1], Francesco Gobbo[1], Ajesh Jacob[1], Lorena Zentilin[2], Cinzia Caterino[1,3], Alessandro Cellerino[1,3], Alessandro Ori[3], Antonino Cattaneo[1]

[1]Bio@SNS, Scuola Normale Superiore, Piazza dei Cavalieri 7, Pisa, Italy; [2]ICGEB, Trieste, Italy; [3]Franz-Lipmann Institut, Jena, Germany

The acquisition of new memories is accompanied by long-lasting modifications in the strength of information transmission between neurons i.e., synaptic plasticity. The structural substrate for synaptic plasticity is a reorganization of the protein content of the synapses. This includes changes in the subunit composition of pre-existing complexes, synthesis or accumulation/relocalization of new proteins, and the formation of new interactions, which can be induced or stabilized by posttranslational modifications (Herring & Nicoll, Ann Review Physiol 2016;78, 351–65). The postsynaptic density (PSD) is a hub of these processes; indeed, PSD-95 interacts with many structural proteins (Shank3, PSD-93) and effectors (AMPARs, NMDARs, CaMKII) (Okabe, Mol Cell Neurosci 2007;34, 503–18). While the set of PSD-95 interactors in the forebrain has been defined by means of proteomic analysis (Fernández et al., Mol Syst Biol 2009;5, 269), to date it has not been possible to describe how the interactome of PSD-95 changes in response to synapse potentiation. To fill this gap, we exploited the SynActive toolbox, which we recently developed to achieve specific expression of proteins of interests at potentiated synapses (Gobbo et al., Nat Comm 2017;8, 1629), to selectively purify the interactors of PSD-95 from in vivo potentiated synapses. A SynActive-controlled, FLAG-tagged version of PSD-95 was delivered via AAV to the hippocampus of mice, which were subsequently challenged with contextual fear conditioning. PSDs were then isolated by affinity purification and their protein content analysed by mass spectrometry. As a reference set, we analysed the interactomics of FLAG-tagged PSD-95 constitutively expressed in home caged animals. Our proteomics data, validated by Western Blot, are the first report of an unbiased comparison of the protein content of the PSD between unstimulated and potentiated synapses. Our data provide a deeper understanding of the mechanisms acting in concert at the synapse during learning. This approach can be applied also to mouse models for neurodegenerative pathologies, like Alzheimer's disease, to look for activity-dependent structural alterations at early stages of the disease, thus helping in the search for early therapeutic interventions.

ACTIVITY-DEPENDENT TRANSLOCATION OF PCDH19 C-TERMINUS IN THE NUCLEUS CONCURS TO IEGS HOMEOSTASIS

Laura Gerosa[1], Francesco Rusconi[2], Luca Murru[1], Alessandra Longaretti[2], Sara Mazzoleni[1], Nael Nadif Kasri[3], Elena Battaglioli[2], Maria Passafaro[1], Silvia Bassani[1]

[1]CNR Institute of Neuroscience, Milan, Italy; [2]BIOMETRA, Department of Medical Biotechnologies and Translational Medicine, University of Milan, Italy; [3]Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition, and Behaviour, Department of Cognitive Neuroscience, Nijmegen Netherlands

PCDH19 gene (Xq22) encodes protocadherin-19 (PCDH19), a transmembrane protein that belongs to the cadherin superfamily of calcium-dependent cell-adhesion molecules. *PCDH19* mutations cause female epilepsy (PCDH19-FE), a pathology characterized by early-onset epilepsy, intellectual disability and autism. PCDH19 consists of 6 extracellular cadherin domains, a transmembrane region and a cytoplasmic C-terminal tail (CT) harbouring a nuclear localisation signal.

By biochemical and immunofluorescence assays in primary rat hippocampal neurons we showed that PCDH19 is cleaved by the gamma-secretase upon sustained NMDA receptor (NMDAR) activation and that the resulting fragment corresponding to the CT enters the nucleus.

We found that CT overexpression in cultured neurons decreases immediate early genes (IEGs) expression levels. Conversely, PCDH19 shRNA-mediated downregulation increases IEGs transcripts.

IEGs activation represents the first and most relevant transcriptional event in response to stimuli that modulate neuronal excitability and synaptic plasticity. Among the epigenetic regulators of IEGs there is the CoREST complex and we showed that PCDH19 CT is able to associate with key components of this corepressor complex, namely LSD1, CoREST and SRF.

PCDH19 cleavage occurs also *ex vivo* upon epileptogenic stimuli, as demonstrated by CT generation in mouse hippocampal slices stimulated with Magnesium-free ACSF.

Further, ChIP analyses performed on hippocampal slices showed that PCDH19 CT is associated with the chromatin, especially after epileptogenic stimuli.

We therefore hypothesize that PCDH19 cleavage might represents a homeostatic mechanism in response to strong neuronal activation that prevents IEGs overactivation via the modulation of the CoREST complex.

According to our working model, PCDH19 CT exerts a negative feedback that controls neuronal excitability. PCDH19 loss of function might expose PCDH19-FE patients to runaway excitation and epileptic seizures.

Ongoing experiments aim at validating these data on human hippocampal neurons derived from iPS in order to get insights into PCDH19 biological roles and highlight new therapeutic targets..

ROBOTIC REHABILITATION AND NEUROMODULATION AFTER STROKE: NOVEL APPROACHES IN A MOUSE MODEL

Cristina Spalletti[1], Claudia Alia[1,2], Stefano Lai[3], Alessandro Panarese[3], Maria Pasquini[3], Sara Conti[1,3], Maria Arena [5], Silvestro Micera[3,4], Matteo Caleo[1]

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Background: Stroke is one of the leading causes of chronic motor disabilities and re-acquisition of motor skills is crucial for stroke survivors. Innovative therapeutic approaches combining physical rehabilitation and neuromodulating interventions represent a promising approach. Unfortunately, solid results in clinical trials are missing and there is a pressing need for appropriate animal models of these novel strategies.
Techniques: We use optogenetics, electrophysiology, behavioral tests and a novel kinematic analysis of reaching movement to investigate spontaneous evolution of motor deficits after stroke in mice forelimb Primary Motor Cortex (Caudal Forelimb Area, CFA, Lai et al., 2015, Alia et al., 2016). We also test the effectiveness of different therapeutic strategies to improve forelimb motor function after stroke.
Results: We already demonstrated that stroke induces long lasting deficits in forelimb motor function and kinematics and that physical training with robotic device or the manipulation of the inhibitory system are able to induce task-specific improvements (Spalletti et al., 2014, Alia et al., 2016). Importantly, we recently studied post-stroke electrophysiological alterations in spared Premotor Cortex (Rostral Forelimb Area, RFA) by means of Field Potentials (FP) and Multi Unit Activity (MUA) following optogenetic stimulation in the homotopic area on the healthy hemisphere. We found a significant decrease of MUA, an increase of the hyperpolarizing component of the FP and of the Paired Pulse Inhibition after stimulation of the contralateral RFA (Spalletti et al., 2018). These alterations were specific for the ipsilesional hemisphere, indicating changes in interhemispheric functional connectivity after stroke and an increased inhibition exerted by the healthy hemisphere over the injured one. Accordingly, we tested the efficacy of a rehabilitative strategy based on the combination of robotic training and transient inhibition of the healthy hemisphere with Botulinum Neurotoxin E (BoNT/E), intracortically injected in the homotopic contralesional CFA (Spalletti et al., 2017). We found that coupling robotic rehabilitation with transient inhibition of the healthy hemisphere results in a functional improvement in general motor tasks and in kinematics of grasping, with re-establishment of pre-lesion movement patterns and interhemispheric balance (Spalletti et al., 2017).
Ongoing experiments: These data demonstrated the effectiveness of combined therapy in promoting true motor recovery. We are now improving the rehabilitative treatment on the robotic platform with a real-time control of friction and isometric measure of forces (Pasquini et al., in revision). We're coupling the treatment with other neuroplastic treatments: (i) enhancement of endogenous serotonin release in a chemogenetic model for controlled serotonin release via systemic administration of clozapine-N-oxide (CNO) in transgenic mice expressing DREADD receptors specifically in serotonergic neurons; (ii) induction of gamma oscillation during rehabilitative treatment in transgenic animals expressing ChR2 in Parvalbuminergic interneurons.

ESTABLISHING PATTERNED NEURONAL CIRCUITS WITH HUMAN iPSC-DERIVED NEURONS IN MICROFLUIDICS

Angelo Iannielli[1,2], Alicia Rubio[1,2], Giovanni Stefano Ugolini[3], Tommaso Cabassi[1], Serena Giannelli[1], Marco Rasponi[3], Vania Broccoli[1,2]

[1]CNR Neuroscience Institute Milano, Italy; [2]San Raffaele Scientific institute Milano, Italy; [3]Department of Electronics, Information & Bioengineering, Politecnico di Milano, Italy

Patient-derived iPSCs can yield unlimited number of affected cells providing a superior in vitro system for deciphering the pathological mechanisms underlying neurodegenerative diseases. We have recently generated iPSC-derived neurons from Parkinson's disease (PD) patients with mutations in the OPA1 gene, encoding for a crucial mitochondrial protein (*Iannielli et al., Cell Rep. 2018*). OPA1 mutant neurons showed an altered mitochondrial dynamics, metabolic profile, oxidative stress and limited survival. However, conventional in vitro culturing of neurons failed to reconstitute the physiological connectivity between neurons and hampers manipulation of single neuronal compartments. In collaboration with the Bioengineering Department of *Politecnico di Milano*, we have fabricated microfluidic devices for the patterned and compartmentalized growth of human iPSC-derived neurons. We employed these devices to establish an in vitro model of the nigral-striatal connection, which selectively degenerates in PD. In fact, the demise of nigral dopaminergic neurons occurring in PD might be the final result of the dysfunctions of the dopaminergic terminals contacting the striatal medium spiny (MS) neurons. Thus, establishing an in vitro model of the dopaminergic-striatal connection will provide a superior system for properly addressing mechanisms of neurodegeneration in PD. To establish this model, we optimized differentiation of PD and control iPSCs into both MS and midbrain DA neurons. In this system, DA neurons extended their axons within the microchannel on the larger side to meet within the central chamber the SM neuronal dendrites and generate synaptic contacts. Thus, we were able for the first time to generate the nigral-striatal circuit in a compartmentalized and accessible in vitro system. We are validating the activity of the synapses and the functional connectivity of this circuit by stimulating the DA neurons and evaluate the downstream effects into SM neurons. We will develop similar cultures in microchips starting from OPA1 mutant iPSC-derived neurons for generating a PD nigral-striatal neuronal circuit, which will enable us to study how the mitochondrial defects impact on this system and lead to neuronal dysfunctions. For the first time, this patterned circuit will allow us to easily visualize organelles within different neuronal districts to assess regional-specific differences. The design of the microfluidic chamber can be tailored to model many other neuronal circuits creating the possibility to establish in vitro systems of brain connectivity using patient specific human iPSC-derived neurons.

ROLE OF MICROGLIAL EXTRACELLULAR VESICLES IN EARLY SYNAPTIC DYSFUNTION IN ALZHEIMER'S DISEASE

Martina Gabrielli[1], Pooja Joshi[1], Grazia Rutigliano[3], Giulia D'Arrigo[4], Marta Lombardi[1], Ottavio Arancio[5], Nicola Origlia[2], Claudia Verderio[1]

CNR Institute of Neuroscience, [1]Milan and [2]Pisa, Italy; [3]Scuola Superiore Sant'Anna, Pisa, Italy; [4]SISSA, Trieste, Italy; [5]Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University, New York, USA.

Alzheimer's disease (AD) is a progressive degenerative encephalopathy characterized by loss of synapses and neurons, extracellular amyloid-beta (Aβ) deposition, and intraneuronal tau aggregation. Extensive literature implicates synaptic dysfunction as an early mechanism affected in AD, that involves progressively larger areas of the brain over time. However, how synaptic dysfunction starts and propagates throughout the brain is largely unclear. This study aims at investigating whether and how extracellular vesicles (EVs) released from Aβ-treated microglia (Aβ-EVs) may induce synaptic dysfunction and propagate it through the brain. EVs are membrane vesicles of endosomal (exosomes) or plasma membrane (microvesicles) origin, which are released extracellularly by most cell types. EVs can interact with specific cells and deliver bioactive molecules to target cells, thus altering state and function of recipient cells. Several lines of evidence suggest a role for EVs in AD: among all, EVs isolated from the cerebrospinal fluid (CSF) of sporadic late-onset AD patients have elevated amounts of A*6* and trigger cell death (Joshi et al, 2014). More particularly, we have found that extracellular vesicles (EVs) released by glia exposed *in vitro* to high concentration of synthetic Aβ fibrils, carry neurotoxic soluble Aβ (Aβ-EVs) and dysregulate synaptic proteins (Joshi et al, 2014) and that microglia-derived EVs efficiently move along neurites of cultured hippocampal neurons. These data let us hypothesize that Aβ-EVs may move from neuron-to-neuron through their projecting axons, spreading synaptic dysfunction in AD.

To explore this possibility, we first analyzed the morphology and the strength of synaptic transmission in basal conditions and after induction of chemical long-term potentiation (LTP) in cultured hippocampal neurons exposed to Aβ-EVs or control EVs (ctrlEVs), finding that Aβ-EVs cause early alteration of dendritic spine morphology and density and impair LTP in culture. Given that the specific vulnerability of the entorhinal cortex (EC) during AD initiates the spreading of degeneration along the neuronal network (Harris et al, 2010), we measured LTP in EC slices through extracellular electrophysiological recordings after exposure to Aβ-EVs or ctrlEVs. LTP was impaired in EC slices treated with Aβ-EVs, while it was reliably elicited in slices exposed to ctrlEVs, thus revealing that Aβ-EVs are capable of interfering with mechanisms of synaptic plasticity of EC intrinsic circuitry. Then, using cortico-hippocampal slices, we measured LTP in the EC and in the dentate gyrus (DG), its main target region, after *in vivo* injection of Aβ-EVs, ctrlEVs or vehicle into mouse EC, in order to test the possibility that Aβ-EVs may mediate spreading of synaptic dysfunction. Extracellular recordings from the EC revealed a block of LTP 1h after Aβ-EV injection, whereas a stable LTP was recorded in the EC injected with ctrlEVs. Recordings at the synapse between the medial perforant pathway (PP) and the DG showed normal LTP 1h after Aβ-EVs (or ctrlEVs) injection. However, 24 hours after Aβ-EV injection, LTP was blocked both in the EC and at the PP-DG synapse, thus suggesting that Aβ-EVs specifically propagate LTP defects along the cortico-hippocampal connections.

Our data indicate that the amyloid cargo of microglial EVs causes synaptic dysfunction both in culture and in slice and propagate LTP impairment among the connected brain regions primarily affected in AD. These results provide strong evidence of the involvement of EVs released by microglia exposed to amyloid plaques in the rise and propagation of synaptic dysfunction at early stage of the disease

DEFECTIVE DIFFERENTIATION OF PRIMARY MYOCYTES IN AN ALS MOUSE MODEL

Caterina Peggion[1], Maria Catia Sorgato[1,2], Roberto Stella[3], Kelly Nies[1,4], Tito Calì[1], Alessandro Bertoli[1,5], Maria Lina Massimino[2]

[1]Dept. of Biomedical Sciences, University of Padova, Italy; [2]CNR Neuroscience Institute, Padova, Italy; [3]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy; [4]University of Maastricht, The Netherlands; [5]Padova Neuroscience Center, University of Padova, Italy

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by motor neuron (MN) degeneration, muscle atrophy, paralysis and, ultimately, death. While the great majority of ALS cases are sporadic (sALS), 10% are inherited (fALS), and approximately 20% of fALS are caused by mutations in the Cu/Zn superoxide dismutase 1 (SOD1) gene.
Accumulating evidence suggests that non-cell-autonomous processes involving the interaction with neighbouring cells, such as astrocytes and skeletal muscle cells, contribute to motor neuron (MN) pathology in ALS. In addition, numerous studies indicate that skeletal muscle is one of the primary targets in both sporadic and familial ALS and in mutant SOD1 (mSOD1) transgenic (Tg) mice. Little is known, however, about muscle fibre regeneration capacity in ALS. Muscle skeletal regeneration is an important homeostatic process that guarantees maintenance of muscle integrity and plasticity. Normally, satellite cells became activated, proliferate and differentiate in response to tissue damage to regenerate muscle fibers. Satellite cells impairment, with consequent less efficient regeneration of skeletal muscles, has been recently demonstrated in an ALS mouse model expressing the SOD1(G93A) mutant. Such a reduced regenerative potential could limit the efficacy of compensatory processes, thereby contributing to the progression and/or severity of muscle atrophy and weakness.
To determine if mSOD1 perturbs the myogenic program, we carried out an *in vitro* study using primary cultured myocytes from neonatal Tg mice expressing human (h)SOD1(G93A) or the wild-type (WT) hSOD1 counterpart. We found that, 4 days after switching to a differentiative culture medium, hSOD1(G93A) cells showed less differentiated myotubes, and reduced expression of embryonic myosin heavy chain (a late marker of *in vitro* myocyte differentiation), compared to hSOD1(WT) cultures. The finding that the expression of Pax7 and MyoD (early markers of myocyte differentiation), and the proliferation of myogenic precursor cells, are reduced in cultured mSOD1 myocytes suggests that hSOD1(G93A) results in defective proliferation of myoblasts in the early stages of the myogenic program, while leaving their differentiative potential in later stages intact.

We also found that the delayed myogenic process was accompanied by reduced expression of proteins involved in the regulation of Ca^{2+} homeostasis, including the sarco-endoplasmic reticulum Ca^{2+} ATP-ase (SERCA), which in turn resulted into defective mobilization of Ca^{2+} from intracellular stores, and uptake of the ion by mitochondria. This suggests that compromised Ca^{2+} handling may contribute to the muscular phenotype in ALS.

Finally, co-culturing of SOD1(G93A) myoblasts and the SOD1(WT) counterpart by means of trans-well cell culture systems resulted in an almost complete rescue of the observed mSOD1-associated phenotypes of skeletal muscle cells. This observation suggests that some still unknown soluble factor (e.g., cytokines, growth factors and/or miRNAs) may be involved in skeletal muscle dysfunctions, and – possibly – in noxious myocyte-MN cross-talk, in ALS. Studies for the identification of such soluble factor(s) are ongoing.

RUNNING TOWARDS A NEW THERAPY FOR AMBLYOPIA

Gabriele Sansevero[1,2], Claudia Torelli[1,2], Manuela Scali[1], Nicoletta Berardi[1,2], Alessandro Sale[1]

[1]CNR Neuroscience Institute, Pisa, Italy; [2]University of Florence, Italy

Visual cortex plasticity is high during a critical period of early postnatal development, but rapidly vanishes with the transition to adulthood. Accordingly, visual disorders such as amblyopia (lazy eye), can be treated early in life by functional penalization of the non-amblyopic eye, but may become irreversible in adults, because of the dramatic decline in brain plasticity. Recent research has provided evidence that non-invasive procedures, such as environmental enrichment and also one of its most effective components, voluntary physical activity, can rescue visual functions in animal models of amblyopia past the end of the critical period, acting through a reduction of the intracortical inhibitory/excitatory balance. Despite the high translational value of these results, however, several key open questions remain: i) are these active environmental procedures able to rescue visual functions when performed in subjects with unrestricted binocular sight? ii) Which is the impact on recovery of depth perception abilities? iii) Are the effects long-lasting? Which is the specific role of distinct sub-populations of GABAergic interneurons? Here we show, using a model of experimental amblyopia in adult rats, that physical training promotes a permanent recovery of visual acuity and depth perception abilities, possibly by modulating the activity of VIP+ and SOM+ interneurons in the primary visual cortex.

OPHN1 REGULATES THE MIGRATION OF NEWLY GENERATED CELLS IN THE OLFACTORY SYSTEM

Andrea Maset[1,2], Luisa Galla[1,2], Claudia Lodovichi[1,2,3]

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Oligophrenin1 (OPHN1), a X-linked gene associated to intellectual disability, encodes a Rho-GTPase activating protein that is thought to regulate several developmental processes including axon outgrowth, dendritic maturation and cell migration. How OPHN1 could affect circuit formation and function leading to cognitive dysfunction remain obscure.

Neuronal migration is one of the fundamental process that underlies proper assembly and function of neuronal circuits. Migration occurs mostly during embryonic life although it persists in the sub-ventricular zone (SVZ), in adulthood. Neuronal precursors generated in the SVZ, migrate along the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they become mature interneurons. To understand the impact of OPHN1 on cell migration, we employed a line of mice expressing a null mutation of OPHN1.

We found that loss-of-function mutation of OPHN1 did not affect the generation of new neuronal precursor cells in the SVZ. However the number of adult-born cells (i.e. GCs) that reached the OB was dramatically reduced in OPHN1$^{-/y}$ mice, suggesting that the migration from the SVZ to the OB was perturbed.

By combining birthdating experiments and lentiviral vectors to labels progenitors in the SVZ, we found that the progression, the morphology and the directionality of migrating cells is deeply perturbed in OPHN1$^{-/y}$ mice. To investigate the mechanism underlying altered cell migration, we performed two photon time-lapse imaging of migrating neuroblasts, *in vivo*, testing molecular cues known to modulate cell migration, such as GABA and modulators of GABA signalling. GABA is abundantly present in the rostral migratory stream. It is produced by migrating neuroblasts and is known to modulate rate of migration acting on neuronal precursor in a paracrine/autocrine manner.

The polarity change (i.e. depolarization versus hyperpolarization) exerted by GABA on neurons is not univocal but depends critically on the intracellular $[Cl^-]_i$ concentration, $[Cl^-]_i$. The latter is finely regulated by two co-transporters, whose expression is developmentally regulated. NKCC1, predominantly expressed in immature neurons, favors high $[Cl^-]_i$, thus leading to depolarizing GABA. On the contrary, KCC2, widely present in mature neurons, favors low $[Cl^-]_i$, promoting hyperpolarizing GABA response.

Our preliminary data, from time-lapse imaging performed in presence of inhibitors of the Cl co-transporters, suggest that the polarity of GABA response could be subverted in migrating neuroblasts in OPHN1$^{-/y}$ mice with respect to controls. This alteration is likely to hamper the migration of newly-generated cells along the RMS in OPHN1$^{-/y}$ mice. How Ca^{2+} signalling modulates the migration from the SVZ to the OB remains poorly understood and data in the literature are controversial. To understand the contribution of Ca^{2+} kinetics in the migration process and to dissect the mechanisms that link GABA signalling, Ca^{2+} dynamics and altered migration in OPHN1$^{-/y}$ mice, we are currently study Ca^{2+} dynamics by means of 2-photon imaging on migrating neuroblasts.

N-ACYLETHANOLAMINE ACID AMIDASE AS A NOVEL THERAPEUTIC TARGET FOR NICOTINE ADDICTION

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Tobacco smoking is a leading cause of preventable diseases and associated deaths, but most smokers fail to quit despite their purpose.Addiction to tobaccois sustained by activation of $\alpha_4\beta_2$ nicotinic cholinergic receptors in reward-associated brain regions, such as the ventral tegmental area (VTA). Therefore, the development of new drugs that act on nicotinic receptorsis considered a promising strategy to facilitate smoking cessation and to prevent linked pathologies. Activation of intranuclearperoxisome proliferator-activated receptor-alpha (PPARα) was shown to regulate $\alpha_4\beta_2$ nicotinic receptors through a non-genomic mechanism. Accordingly, PPARαsynthetic or endogenous agonistssuppress nicotine-induced activation of mesolimbic dopamine neurons, release of dopamine in the shell of the nucleus accumbens (shNAc), and nicotine-seeking behavior in rats and monkeys.

In this study, we indirectly activated PPARα signalling in the brain via inhibition of the hydrolytic degradation enzyme N-acylethanolamine-hydrolyzing acid amidase (NAAA), one of the major hydrolyzing enzyme for its endogenous agonists palmitoylethanolamide and oleoylethanolamide.We took advantage of a novel potent and selective NAAA inhibitor, AM11095, which showed high stability and exceptional CNS permeability.

In adult male Sprague Dawleyrats, we carried out a functional observational battery (FOB) to assess toxicity, *in vivo* electrophysiological recordings from VTAdopamine neurons, brain microdialysis in the shNAcand behavioral experiments to assess its effect on nicotine-induced conditioned place preference (CPP).AM11095 (5 and 25 mg/kg, i.p.) was devoid ofneurotoxic and behavioral effects. Moreover, AM11095 (5 mg/kg i.p.), prevented nicotine-induced activation of dopamine neurons, nicotine-induced elevation of dopamine levels in the shNAcand decreased the expression of nicotine CPP.

Our data provide evidence to support the role of NAAA in modulating PPARα signalling, in turnregulating the activity of nicotinic receptors. Hence,pharmacotherapies based on NAAA inhibitors appear to be promising against nicotine/tobacco addiction and complicationsfor health.

PKA AND EPAC CROSSTALK IN HT29 MIGRATION: THE IMPORTANCE OF SIGNAL COMPARTMENTALIZATION

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The multifunctional second messenger cAMP is involved in several cellular processes including transcription, cell metabolism and migration. The effects of cAMP on motility are debated as this messenger can both inhibit and promote cell migration. Two cAMP effectors, Protein Kinase A (PKA) and Exchange Protein Activated by cAMP (EPAC) have been involved in the regulation of cell migration, however their individual and combined effects in this process are not well established. To investigate the role(s) of these proteins in cellular migration we used the metastatic human colon adenocarcinoma cell line HT29.

In wound healing assays, inhibition of PKA drastically accelerated cell migration while inhibition of EPAC completely block this phenomenon. On the other hand simoultaneus inhibition of both didn't induce changes in the migration patterns of HT29 cells. These experiments suggest a functional crosstalk between the two cAMP effectors.

To further investigate the underlying signalling mechanism we used single cell imaging and biochemical approaches.

FRET-based PKA activity sensors showed that in HT29 cells PKA-dependent phosphorylation is strictly compartmentalized with low PKA activity in the cytosol and high at the endoplasmic reticulum, outer mitochondria membrane and plasma membrane.

Cytosolic PKA activity is kept low due to a great concentration of soluble phosphatases that, on the other hand, are less able to dehosphorilate phospho-proteins tethered on the other compartments.

In parallel experiments we found that HT29 cells highly express EPAC1. Interestingly, in response to increases in cAMP concentration EPAC translocates from cytosol to the mitochondria opening the possibility that coordinated PKA and EPAC signals regulate migration in HT29 cells. In order to dissect the actions of the two proteins we differentiated HT29 cells by replacing glucose with galactose in their culture media. After 30-50 days, HT29s lost their colonic nature and acquired the phenotype of mature intestinal cells.

Differentiated HT29 cells showed a significant reduction in EPAC1 protein levels, while at a functional level these cells migrated at significantly lower speeds. Interestingly, contrary to EPAC, differentiated HT29 cells show no difference in the expression of PKA subunits.

Our future plan is to investigate if this signal compartmentalization has a specific role in migration and which are the EPAC and PKA targets that regulate this phenomenon.

NOVEL GENE THERAPY APPROACHES FOR WHOLE BRAIN DELIVERY OF THE LYSOSOMAL GCase ENZYME FOR WIDE PROTECTION FROM alpha-SYNUCLEIN TOXIC AGGREGATES

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Alpha-synuclein (alpha-Syn) toxic aggregates spread over time throughout large brain areas in Parkinson's disease (PD), MSA and Lewy body dementia and are responsible for cortical functional decline leading to severe cognitive deficits. Approximately 5–8% of PD patients are carriers of a heterozygous GBA1 mutation, causing a detectable reduction in GCase global activity. Conversely, stimulating GCase activity has been shown to fuel lysosomal activity to stimulate degradation of alpha-Syn deposits. We have established two novel gene therapy approaches to widely express GCase in the whole brain and, thereby, reaching a global protection from alpha-Syn-dependent dysfunctions. First, we showed that a single intravenous injection of the brain penetrant GBA1 expressing AAV-PHP.B is sufficient to provide robust and long-lasting protection from alpha-Syn deposits in a mouse model of synucleinopathy. AAV-PHP.B delivered GCase is targeted to the lysosome and acquires functionality, which resulted in significantly diminished accumulation of insoluble alpha-Syn species in all the forebrain regions. However, it remains to be shown whether this AAV serotype will maintain the capability to efficiently permeate the blood-brain barrier in humans. Alternatively, we conceived a different approach based on the cell-to-cell spreading of the GCase enzyme. In fact, we showed that GCase can be released by brain endothelial cells and reuptaken by surrounding neurons and astrocytes. Thus, we generated GBA1-expressing AAV-BR1 particles able to specifically target the brain microvasculature and allow GCase to diffuse throughout the central nervous system. Remarkably, hA53T-alpha-Syn transgenic mice subjected to this treatment exhibited a widespread reduction of alpha-Syn deposits in the cerebral cortex. We propose that the AAV-BR1-based gene therapy is a crucial strategy to convert the brain microvasculature in a stable source of supplemental GCase enzyme for the long-term protection of neural tissue from accumulating alpha-Syn aggregates.

POSTER SESSION 1: SCHEDULE AND ABSTRACTS

N	When	Speaker	Title

Session 1

P1.1	W	Maria Nicole Colombo	*VAPB downregulation delays neurite elongation and alters phosphoinositide balance in motor neuron-like NSC34 cells: implications for mutant VAPB - linked ALS*
P1.2	W	Sara Francesca Colombo	*Role of rare missense variants of the β4 nicotinic receptor subunits in intracellular trafficking*
P1. 3	W	Giuliana Fossati	*The innate immune molecule, PTX3, enhances the synaptic content of AMPA receptors via extracellular matrix remodeling and β1 integrin*
P1.4	W	Anna Longatti	*ARHGAP22 disruption affects RAC1 signaling pathway and results in altered formation and function of glutamatergic synapses in mouse hippocampus*
P1.5	W	Francesca Santini	*Oxytocin receptors in neurodevelopmental disorders: innovative approaches for the quantification of sopramolecular receptor complexes*
P1.6	W	Luca Murru	*Tm4sf2 knock-out mice display ASD-related behaviors and LHb hypo-function*
P.1.7	W	Emanuela Dazzo	*Loss-of-function reelin mutations linked to autosomal dominant lateral temporal epilepsy*
P.1.8	W	Marco Mainardi	*A triheptanoin-supplemented diet rescues hippocampal hyperexcitability and seizure susceptibility in Foxg1$^{+/-}$ mice*
P1.9	W	Sara Mazzoleni	*Characterization of a new conditional mouse model for the PCDH19 gene involved in PCDH19 female epilepsy (PCDH19-FE)*

P1.10	W	Alia Claudia	*Neurons derived from mESCs extend projections into lesioned brain: a strategy for stroke recovery*
P1.11	W	Chiara Adriana Elia	*Advanced MRI imaging reveals microstructural features of the corticospinal tract in stroke patients. Correlations with biohumoral markers and with rehabilitation outcome?*
P1.12	W	Mirko Luoni	*Effective and safe AAV-based gene therapy for Rett syndrome*
P1.13	W	Francesca Tozzi	*3-Iodothyronamine ameliorates ischemia-induced synaptic dysfunction in the mouse entorhinal cortex*
P1.14	W	Silvia Penati	*Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline*
P1.15	W	Luisa Galla	*Mitochondrial dysfunctions as an early event in the pathogenesis of familial Alzheimer's disease?*
P1.16	W	Simone Bido	*A new mouse model for Parkinson's disease recapitulating the major neuropathological hallmarks of the human pathology*
P1.17	W	Ludovico Arcuri	*AAV.PHP.EB-mediated-OPA1 gene expression in a mouse model of Parkinson's disease as a valuable strategy for neuroprotection*
P1.18	W	Monica Angelini	*Perspective-dependent reactivity of sensorimotor mu rhythm in alpha and beta ranges during action observation: an EEG study*
P1.19	W	Arturo Nuara	*An interactive home-based platform promoting child-to-child interaction improves hand function in unilateral cerebral palsy*
P1.20	W	Maria Del Vecchio	*The missing piece of somatosensory evoked potentials: difference between activation and phase resetting according to a stereo- EEG perspective.*
P1.21	W	Maddalena Fabbri Destro	*The neurological motor deficits as an endophenotype of autism shared by affected and unaffected siblings*
P1.22	W	Giovanni Vecchiato	*Electroencephalographic correlates of braking and acceleration events during simulated car driving*
P1.23	W	Valentina Gizzonio	*Story-telling and story-acting: their effects on development of prescholer children*

P1.24	W	Doriana De Marco	*Gestures and words: new perspectives on the relation between abstract language and action*
P1.25	W	Roberto Bizzotto	*Insulin clearance and its determinants in double incretin receptor knockout and wild-type mice*
P1.26	W	Andrea Mari	*The different mechanisms of action of GIP AND GLP-1 explain their different efficacy as therapeutic agents*
P1.27	W	Gabriele Losi	*GABA tonic currents in Dravet syndrome*

VAPB DOWNREGULATION DELAYS NEURITE ELONGATION AND ALTERS PHOSPHOINOSITIDE BALANCE IN MOTOR NEURON-LIKE NSC34 CELLS: IMPLICATIONS FOR MUTANT VAPB-LINKED ALS

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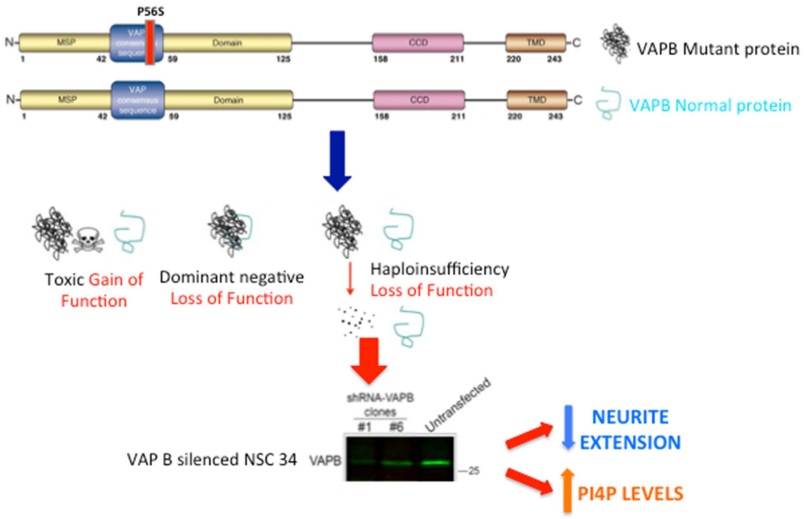
VAPB, and its homologue VAPA, are conserved and ubiquitously expressed tail-anchored transmembrane proteins of the Endoplasmic Reticulum (ER). The VAPs function as receptors for cytosolic proteins bearing FFAT motifs and play key roles in the genesis of membrane contact sites and in the exchange of lipids between organelles at these sites. A mutant form of VAPB (P56S) is linked to a dominantly inherited form of Amyotrophic Lateral Sclerosis (ALS8). Mutant VAPB, expressed in cultured cells, forms aggregates, which have been thought to cause motor neuron degeneration either by a toxic gain of function and/or by a dominant negative effect, by sequestering VAPB, VAPA, and other interacting proteins into non-functional aggregates. However, we have shown that not only is mutant VAPB unstable but, when expressed at moderate levels, it interferes neither with proteostasis nor with secretory pathway function. Thus, we have hypothesized that simple haploinsufficiency may underly the dominant inheritance of the P56S-VAPB allele. To investigate this possibility, we generated motor neuron-like cell lines (NSC34) either partially or nearly completely silenced for VAPB. Although VAPA levels were normal in these cells, the VAPB-depleted cells showed a significant reduction in the rate of neurite extension when induced to differentiate, thus suggesting that selective VAPB downregulation may interfere with motor neuronal viability and contribute to the development of ALS. In the same cells, changes in the intracellular distribution of phosphatidylinositol-4-phosphate (PI4P) were observed, such as an increase in the content in the Golgi complex and acidic LAMP1-positive vesicles. Given the importance of this phosphoinositide molecule in both Golgi and endosome function, we are characterizing the dynamics of PI4P positive structures to disclose the relationship between PI4P imbalance and delayed neurite elongation in VAPB-depleted cells. Towards this goal, we are investigating the effect of the pharmacological inhibition of the PI4 Kinase III beta, which is responsible for the generation of PI4P in the Golgi complex and lysosomes, on the differentiation of NSC34 cells. Treatment with the PI4 Kinase III beta inhibitor PIK 93 accelerated neurite outgrowth in VAPB-downregulated and control NSC34 cells, suggesting that VAPB deficit negatively affects neurite elongation at least in part by altering intracellular PI4P levels.



ROLE OF RARE MISSENSE VARIANTS OF THE β4 NICOTINIC RECEPTOR SUBUNITS IN INTRACELLULAR TRAFFICKING

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Neuronal nicotinic acetylcholine receptors (nAChRs) are a large family of cationic channels consisting of nine α (α2-α10) and three β subunits (β2-β4) which assemble in pentamers with different subunit composition. The α3β4 receptors may be present in two alternative stoichiometries: 2α/3β and 3α/2β that have similar agonist sensitivity but different antagonist sensitivity, and markedly different single-channel conductance.
In order to dissect the mechanism by which the receptor can reach plasma membrane, we generated a dimeric construct (consisting of the two subunit β4-α3) that, when co-transfected with a monomeric subunit (α3 or β4), allows to study a specific population of pentameric receptors with fixed stoichiometry. By means of morphological, biochemical and functional assays, we found that only the receptors with three β4 subunits are recruited to plasma membrane while pentamers with three α3 subunits are retained in the endoplasmic reticulum. Indeed, the type of accessory subunit present in the fifth position in the pentamers determines the trafficking of the receptor to the cell surface. This study demonstrates a novel function of the accessory subunit in the α3β4 receptor that may be relevant also for other pentameric receptors (Crespi et al., 2018).
Recently, some rare missense variants of the β4 nicotinic receptor subunit have been identified and the role of these single nucleotide polymorphisms (SNPs) in CHRNB4 (the gene coding for the β4 nicotinic receptor subunit) have been linked to altered risk of nicotine dependence (Slimak et al, 2014). Habenular expression of these β4variants in mice revealed a critical role of these subunits in nicotine consumption. In particular they identified a new variant, β4D447Y that, when co-expressed, in hippocampal neurons, with the α3 subunit significantly increases the amplitude of nicotine-evoked currents. On the contrary the co-expression of the α3 subunit with the variant β4R348C, the mutation most frequently encountered in sporadic amyotrophic lateral sclerosis (sALS), leads to reduced nicotine-evoked currents.
Taking advantage of the system we developed to express in cells nAChRs pentamers with a defined subunit composition, we investigated the intracellular trafficking and plasmamembrane localization of α3β4 nAChRs having in the fifth position the human β4 variant D444Y or R349C (corresponding to the mice variant D447Y and R348C respectively).

THE INNATE IMMUNE MOLECULE, PTX3, ENHANCES THE SYNAPTIC CONTENT OF AMPA RECEPTORS VIA EXTRACELLULAR MATRIX REMODELING AND β1 INTEGRIN

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In the developing central nervous system, the control of synapse number and function is critical to the formation of neural circuits. Astrocytes play a key role in this process by releasing factors which promote the formation of excitatory synapses. Astrocyte-secreted thrombospondins (TSPs) induce the formation of structural synapses, which are however post-synaptically silent, suggesting that completion of early synaptogenesis may require a double-step mechanism. Here we show that the humoral innate immune molecule PTX3 is expressed in the developing rodent brain and is able to bind the CNS specific TSP-1 and -2, but not the peripheral TSP-4. PTX3 plays a key role in promoting functionally active CNS synapses, by increasing the surface levels and synaptic clustering of the AMPA glutamate receptors, through the remodeling of the perineuronal network via a β1 integrin, MAPK dependent process. These data unveil a fundamental crosstalk between the immune and nervous systems to establish the first wave of synaptogenesis and the organization of the early functional circuits in the developing brain.

ARHGAP22 DISRUPTION AFFECTS RAC1 SIGNALING PATHWAY AND RESULTS IN ALTERED FORMATION AND FUNCTION OF GLUTAMATERGIC SYNAPSES IN MOUSE HIPPOCAMPUS

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ArhGAP22, a member of RhoGTPase-activating protein (GAP) family, has been demonstrated to be expressed in post-synaptic compartment of excitatory synapses *in vitro* where is thought to regulate actin-cytoskeleton and dendritic spine formation through its inhibitory activity on Rac1.
The aim of this project was to define the molecular and functional mechanisms underlying biological properties of ArhGAP22 *in vivo* using an animal model knock-out (KO) for Arhgap22.
First, we analyzed the levels of active Rac1 and its downstream effectors in hippocampal synaptosomes derived from both genotypes. Rac1-GTP and effectors levels were significantly increased in KO mice. We therefore performed morphological analyses of excitatory synapses in hippocampus and we found that KO mice presented increased dendritic spines density.
To explore functional consequences of these alterations, we studied electrophysiological properties of hippocampal neurons of WT and KO mice. KO mice showed a reduction in amplitude and frequency of mEPSCs while mIPSCs were not altered. Moreover, LTP induction at the CA3–CA1 synapse was impaired in KO mice compared to WT.
We also analyzed the effect of ArhGAP22 disruption on the expression of several synaptic markers in hippocampal synaptosomes by Western blot: KO mice had a significative reduction of GluA1 and GluA2/3 AMPAR subunits.
Furthermore, we observed significant alterations in anxiety and learning/memory behavior in mutant compared to normal mice.
Overall, these data suggest that ArhGAP22 silencing leads to deficits in hippocampal-dependent cognitive functions in mice. These alterations could be explained by abnormal dendritic spines number and maturation together with altered AMPA receptors composition.

OXYTOCIN RECEPTORS IN NEURODEVELOPMENTAL DISORDERS: INNOVATIVE APPROACHES FOR THE QUANTIFICATION OF SUPRAMOLECULAR RECEPTOR COMPLEXES

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The neuropeptide oxytocin (OT) is able to regulate many different social and non-social functions via activation of its receptor (OTR). Alterations in the oxytocinergic system are present in several neurodevelopmental disorders and psychiatric disorders, such as autism, Prader-Willi syndrome and schizophrenia. By consequence, several studies highlighted the use of OT to rescue pathological phenotypes linked to social behavior, making OT a very promising agent for new and effective therapeutic approaches.

The OTR is a G protein coupled receptor able to form homo and/or heterodimers with different pharmacological properties. However, it is still unknown whether these dimeric structures are involved in the emergence of social deficits observed in neurodevelopmental and psychiatric disorders. The identification and the characterization of GPCR (and OTR in particular) dimers are a real challenge, because current techniques used to study dimeric complexes in cells and native tissues are unable to prove “real” dimerization and can only show molecular proximity between two protomers of a dimer. This is the case of Proximity Ligation Assay (PLA), an antibody-based technique commonly used to study the interaction between two proteins of interest in cells and tissues: PLA estimated resolution (20-40 nm) is not compatible with direct contact between two protomers (and therefore with GPCR dimerization).

To fill this gap, we are developing a new and innovative assay, based on a DNA “nanoruler” technology. This system is composed by *ad hoc* designed hairpin oligonucleotides conjugated with a selective OT analog. When the conjugated ligands bind to the two protomers of a dimeric receptor, the two oligonucleotides are brought in a very close proximity (<10 nm); the addition of a third DNA “activating sequence” then triggers their hybridization and the complex that is formed can be recognized by fluorescently tagged reporter hairpins, which generate a branched fluorescent DNA molecule by means of a Hybridization Chain Reaction (HCR). The Nanoruler assay will be at first tested in transfected HEK293 cells, to analyze the formation of both OTR homodimers and then in brain sections. In general, with our new Nanoruler technique we hope to provide an innovative and powerful tool to study receptor-receptor interactions in native tissues.

TM4SF2 KNOCK-OUT MICE DISPLAY ASD-RELATED BEHAVIORS AND LHB HYPO-FUNCTION

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Mutations in many genes have been linked to increased risk of developing autism spectrum disorders (ASD) so far, including Tm4sf2 that encodes for tetraspanin7 (TSPAN7) protein. We previously demonstrated defects in hippocampal function and related behaviors in Tm4sf2 knock-out ($Tm4sf2^{-/y}$) mice. The aim of the project is to study a possible link between ASD-related behavior and lateral habenula (LHb) function in $Tm4sf2^{-/y}$ mice. In preliminary results, $Tm4sf2^{-/y}$ mice showed a minor sociability, increased self-grooming and altered marble burying behaviors. Moreover, functional experiments showed a hypo-excitability, an increased quinpirole-evoked D2DR-GIRK currents together with altered potassium conductance in LHb neurons of $Tm4sf2^{-/y}$ mice.
Since LHb regulates VTA dopaminergic neurons activity and dopaminergic signaling is reported to be altered in ASD patients, with our data we suggest that an hypo-function of the LHb could be causative for alteration in the VTA function that leads to ASD-related behavioral phenotype.

LOSS-OF-FUNCTION REELIN MUTATIONS LINKED TO AUTOSOMAL DOMINANT LATERAL TEMPORAL EPILEPSY

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Autosomal dominant lateral temporal epilepsy (ADLTE; OMIM 600512) is a genetic epileptic syndromes clinically characterized by focal seizures with prominent auditory symptoms, likely originated from the temporal lobe lateral region, and negative MRI results. It is inherited with an autosomal dominant pattern with reduced penetrance (about 70%).
Three genes are involved in the etiology of ADLTE: LGI1 and RELN (Reelin), which encode two secreted proteins, and MICAL-1, encoding an intracellular protein with oxidoreductase activity that depolymerizes actin filaments. The functional test that has been used for a long time to establish the effects of LGI1 variants on its function is the secretion test, while in the case of RELN functional tests are not available. In order to verify the effects of the RELN variants we developed a secretion test using transfected cells.
We evaluated the effect on protein secretion of seven Reelin mutations identified in families with ADLTE. Our tests showed that all but one variants completely inhibit or impair Reelin secretion, demonstrating a loss-of-function effect of these mutations.
In addition, since both Lgi1 and Reelin are extracellular proteins, and their relationship, as well as the pathogenic mechanism by which their mutations lead to ADLTE, are unknown, we asked if they could interact with each other. We performed immunofluorescence and immunoprecipitation experiments that use conditioned media. The preliminary results of this analysis suggest that the two proteins interact extracellularly.

A TRIHEPTANOIN-SUPPLEMENTED DIET RESCUES HIPPOCAMPAL HYPEREXCITABILITY AND SEIZURE SUSCEPTIBILITY IN FOXG1$^{+/-}$ MICE

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The *Forkhead Box G1* (*FOXG1*) gene encodes a transcription factor, essential for the mammalian telencephalon development. *FOXG1*-related disorders, caused by deletions, intragenic mutations or duplications, are usually associated with severe intellectual disability, autistic features, and, in 87% of subjects, epilepsy. In at least a subset of the patients with *FoxG1* mutations, seizures remain intractable, prompting the need for novel therapeutic options. To address this issue, we took advantage of a haploinsufficient animal model, the *Foxg1*$^{+/-}$ mouse. Hippocampal activity was monitored in freely moving mice using implanted electrodes. The electrophysiological analysis detected hippocampal hyperexcitability in *Foxg1*$^{+/-}$ mice. Moreover, behavioral analysis revealed that *Foxg1*$^{+/-}$ mice showed higher susceptibility to kainic acid (KA)-induced epileptic seizures than wild-type littermates. These functional alterations were associated with decreased expression of the chloride transporter KCC2 and impaired phosphorylation of ribosomal protein S6 (rpS6).
We next tested whether these phenotypes could be impacted by treating *Foxg1*$^{+/-}$ mice with a triheptanoin-based ketogenic diet. This manipulation abated altered neural activity and normalized the enhanced susceptibility to KA-induced seizures, in addition to rescuing the levels of KCC2 and phospho-rpS6.
In conclusion, our data show that *Foxg1* haploinsufficiency causes altered activity of hippocampal circuits and increases the susceptibility to proconvulsant-induced seizures, which are rescued by triheptanoin dietary treatment.

CHARACTERIZATION OF A NEW CONDITIONAL MOUSE MODEL FOR THE *PCDH19* GENE INVOLVED IN PCDH19 FEMALE EPILEPSY (PCDH19-FE)

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PCDH19 Female Epilepsy (PCDH19 – FE), also known as early infantile epilepticencephalopathy-9 (EIEE9), is a debilitating neurological condition, mainly characterized by early seizure onset and a varying degree of intellectual disability and autism.
This disease is due to mutations in the *PCDH19* that encodes for protocadherin – 19 (PCDH19) and is localized on chromosome Xq22.1.
PCDH19 is a calcium-dependent cell-cell adhesion molecule of the cadherin superfamily, which is highly expressed in the brain, especially in the cortex and in the hippocampus.
PCDH19 - FE is considered a particular X – linked disorder due to its peculiar mechanism of inheritance, as it affects females, while sparing males, with the exception of mosaic males. To explain this peculiar inheritance pattern, a cellular interference or mechanism has been postulated: the coexistence of two neuronal populations (PCDH19-positive and PCDH19-negative cells), which are not able to communicate properly, would lead to epilepsy, cognitive impairment and autism.
By exploiting the Cre – LoxP technology, our laboratory has generated a conditional knock out (KO) model for PCDH19 (PCDH19 floxed mouse), which could help in identifying the possible pathophysiological mechanisms behind the disorder.
The breeding of PCDH19 floxed mouse with hSyn1-Cre mice generated males and females KO mice with a mosaic expression of PCDH19 in their brains, evaluated by RT-PCR, WB and IHC.
Notably, PCDH19 KO mice showed a reduction of GABA α1 expression compared to controls both in the forebrain slices and in the hippocampus, confirming the interplay between PCDH19 and GABAAR that we recently reported (Bassani et al., 2018).
Ongoing experiments aim at characterizing PCDH19 mosaic mice from a morphological, functional and behavioural point of view.

NEURONS DERIVED FROM MESCS EXTEND PROJECTIONS INTO LESIONED BRAIN: A STRATEGY FOR STROKE RECOVERY

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Ischemic injuries within the motor cortex result in functional deficits that may profoundly impact activities of daily living in patients. The brain reorganizes spontaneously after injury, but in a limited manner. For this reason, effective therapeutic strategies have to be determined in order to enhance recovery. Transplantation of stem cells has been employed in several studies to improve functional recovery after ischemic damage. Recent studies have shown that murine embryonic stem cells (mESC) derived neurons transplanted into the adult brain can integrate into the host tissue, send long distance projections and make functional synapse. In this context, our group has recently developed a specific differentiation protocol that allows to produce mESC-derived neurons with the identity of cortex or hippocampus.

Here, we co-injected cortical and hippocampal mESC-derived neurons, labelled with lentiviral vectors carrying GFP and mCherry respectively, in the hippocampus and primary motor cortex of adult healthy mice by using a micropump. Then, the integration and projection patterns of these cells have been analyzed in a mouse model of phothrombothic stroke in the primary motor cortex. In this case, cells are injected three days after stroke and the assessment of recovery is based on well established motor tasks (Schallert Cylinder test and Gridwalk Test) (Spalletti et al, 2014). Four-eight weeks after the grafting, the survival and projection patterns of cortical and hippocampal mESC-derived neurons have been evaluated by histological analysis.

Our data show that both hippocampal and cortical mESC-derived neurons are able to survive several weeks in the host tissue. Specifically, hippocampal-like cells transplanted in the hippocampus sent specific projections to the CA3 area and were able to elongate fibers even when transplanted in the motor cortex, independent of the presence of the ischemic lesion. In contrast, cortical-like cells showed a remarkably different projection patterns in healthy and stroke animals. In particular, the quantification of fibers demonstrate that cortical-like neurons transplanted in ischemic lesion display a higher number of projections with respect to the healthy, naïve brain. Importantly, the motor tasks showed evidence for behavioral recovery after stroke and transplantation of the cortical-like cells.

In this study, we demonstrate that the neural precursor cells carry intrinsic signals regulating their axonal extension in different regions and damaged adult brain provides signals supporting axonal projections by cortical cells. Moreover, the results obtained from ischemic animals could set the stage for an effective cell-based therapy in stroke recovery.

ADVANCED MRI IMAGING REVEALS MICROSTRUCTURAL FEATURES OF THE CORTICOSPINAL TRACT IN STROKE PATIENTS. CORRELATIONS WITH BIOHUMORAL MARKERS AND WITH REHABILITATION OUTCOME?

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Stroke is worldwide the second leading cause of death, after ischemic heart failure and the second leading cause of long-term disability, with one third of stroke survivors having motor impairment or motor-loss of the entire left or right side of the body, often accompanied by visual loss associated with motor deficits and sensory impairment, or emotional and cognitive disturbances such as dementia. Neuronal death in stroke is accompanied and sustained by inflammation, edema and tissue remodeling events eventually leading to axonal degeneration of connected brain regions. The corticospinal tract (CST) is the most affected white matter tract, since it is the motor pathway emanating from the cortex and terminating at the motor neurons in the spinal cord, whose integrity is responsible for the voluntary motor control of the body and limbs

Objective of the study was to analyze a cohort of 30 stroke patients (age 40-85) hospitalized in the Stroke Unit of Humanitas Hospital in order to identify multiple parameters characterizing the subacute and the chronic condition and the functional outcome.

The study has focused on

1) analysis of lesional and contra-lesional areas of the brain by means of advanced DTI (FA and NODDI) at 3T, allowing to investigate microstructural characteristics of the CST downstream of the lesion compared to the unlesioned side at 10-15 days and at 6-12 months after stroke.

2) analysis of plasma samples early (5 days) and at two later stages (30 days and 6-12 months) in order to investigate the inflammatory profile, investigating multiple markers (cytokines, chemokines and growth factors).

The long-term goal is to identify multiple biomarkers, based on different approaches (imaging, plasma) and to investigate which parameters correlate with a better rehabilitation outcome in order to identify novel therapeutic perspectives for those who have a bad rehabilitation score.

EFFECTIVE AND SAFE AAV-BASED GENE THERAPY FOR RETT SYNDROME

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Rett syndrome (RTT) is severe neurological disorder mainly caused by loss-of-function mutations in *MECP2* gene. Despite the severity of the disease, recent studies have provided a strong proof-of-concept that the reactivation of *Mecp2* in mutant mice resulted in a robust reversal of phenotype. Given this prospective, a gene therapy approach aimed at restoring *Mecp2* expression through viral-based transduction *in vivo* has become a promising strategy to treat RTT. Recent studies have been exploited the Adeno-Associated Virus (AAV) serotype 2/9 as a gene transfer system into *Mecp2-null* mice. Unfortunately, the brain transduction efficiency of this strain after intravenous injection is lower than in liver. Indeed, mostly of the symptoms were not reversed, in addition administration of high viral doses led to significant adverse effects. For these reasons, the application of novel strains is necessary to establish the potential of the gene therapy approach. In this project we have exploited the AAV-PHP.eB, a new enhanced variant of the AAV-PHP.B generated to efficiently transduce the mouse brain after intravenous. Thus, we have tested three different viral doses (1×10^{11}; 1×10^{12}; 1×10^{13} vector genomes) of *Mecp2* expressing AAV-PHP.eB by tail administration in adult *Mecp2-null* mice observing severe adverse effects only with the higher one. Both the other two doses induced a significant beneficial effect on the disease progression. Indeed, we have confirmed by immunofluorescence and Western Blot analysis a wide transfer of MeCP2 in the brain respect to the liver. Finally, we have assessed the safety of these viral doses in *wild-type* mice and conceived a new strategy to improve the regulation of MeCP2 expression exploiting the shRNA system. This study will contribute to tailor a safe and efficient approach of gene therapy for MeCP2 mutant mice by improving vector design and viral capsid choice and delivery.

3-IODOTHYRONAMINE AMELIORATES ISCHEMIA-INDUCED SYNAPTIC DYSFUNCTION IN THE MOUSE ENTORHINAL CORTEX

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Abnormalities in thyroid hormone (TH) availability and/or metabolism have been hypothesized to contribute to Alzheimer's disease (AD) and to be a risk factor for stroke. Recently, 3-iodothyronamine (T1AM), an endogenous amine putatively derived from TH metabolism, gained interest for its ability to promote learning and memory in the mouse. In the present work we investigated the effect of T1AM on ischemia-induced synaptic dysfunction in the entorhinal cortex, a brain area crucially involved in learning and memory and early affected during AD.

Field excitatory post-synaptic potential (fEPSP) were recorded in EC/hippocampal horizontal slices obtained either from WT mice (C57bl) or mice overexpressing a human mutant form of amyloid precursor protein (mhAPP). Slices were exposed to an oxygen-glucose deprivation protocol (OGD) for 10 min and then recorded for 50 min after reperfusion. T1AM was perfused to slices at the concentration of 5µM for 10min during the application of OGD. The effect of T1AM was compared to that of RO5166017 (250nM), a specific agonist of trace amine-associated receptor 1 (TAAR1), which is considered as the chief molecular target of T1AM.

A long-lasting synaptic depression was induced by OGD in WT slices. As previously reported, OGD effect was enhanced in EC slices form mhAPP mice (mean fEPSP amplitude in the last 10min of recording was of 59±4% of baseline *n=6 slices,* 5 mice). However, T1AM perfusion was capable of preventing the long lasting synaptic depression after OGD either in WT slices or mhAPP slices (mean fEPSP ampl. in mhAPP+T1AM was 104±2% of baseline; $p<0,001$ vs mhAPP untreated slices; n=4, 3 mice). A similar protective effect was achieved by the perfusion with RO5166017 (mean fEPSP ampl. was 108%±3% of baseline in mhAPP+RO5166017 , $p<0,001$ vs. mhAPP untreated slices; n=4, 3 mice).

T1AM ameliorates ischemia-induced synaptic dysfunction in the EC. This effect was confirmed in an amyloid enriched environment. RO5166017 demonstrated a similar efficacy, suggesting the involvement of TAAR1 in T1AM-mediated neuroprotection.

MOLECULAR AND CELLULAR MECHANISMS UNDERLYING THE RELATIONSHIP BETWEEN METABOLIC ALTERATIONS AND COGNITIVE DECLINE.

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Increasing evidence suggests an association between metabolic disorders, notably insulin-resistance (IR), type 2 diabetes (T2D), and Alzheimer's Disease (AD). Clinical and epidemiological studies indicate that diabetic patients have increased risk of developing AD and vice versa. Moreover AD brains exhibit defective insulin signaling and insulin resistance. Recent studies have shown that diet-induced changes in peripheral insulin sensitivity contribute to alterations in brain insulin signaling and cognitive functions. IR could in fact be the common pathogenetic mechanism underlying AD and T2D affecting glucose metabolism in different organs, including the brain. In addition chronic low grade inflammation is a condition accompanying AD, T2D and the condition of IR, typical of pre-diabetes. Moreover elevated fatty acids levels in combination with hyperglycemia determine prolonged exposure of cells to gluco-lipotoxicity, which has been shown to induce pancreatic beta cell functional impairment and death.
The aims of this study are:
1. to determine how high fat diet-induced insulin resistance can lead to cognitive impairment prodromal to Alzheimer's disease;
2. investigate the effect of IR on Central Nervous System neurotransmission and myelination
3. replicate some aspect of animal model in in vitro cellular models
4. identify the molecular mechanisms responsible for the effect of IR

Mice were fed with 45% and 60% high fat diets (HFD) for several weeks and the effect of diet was tested on body weight, glucose-, pyruvate- and insulin-tolerance. Moreover the cognitive status of mice has been investigated by behavioural tests. Biochemical analyses of different brain areas show that HFD determines modifications in neuronal receptor proteins level. Western blotting analysis revealed that mice fed for 8 weeks with HFD have lower levels of PLP in the hippocampus and prefrontal cortex (~20% or ~25% less upon 45% or 60 % HF respectively) compared to controls suggesting that oligodendrocyte maturation and myelination are altered in animal exposed to elevated fatty acids.
To mimic in vitro the metabolic condition determined by HFD on neuronal, astrocyte and microglia cell cultures, cells were treated with palmitic acid (PA). Hippocampal and cortical neurons exposed to different concentrations of PA and Oleic Acid (OA), as a control, show that higher concentrations of PA induce significative cell death, while OA does not.
The biological framework for explaining the effects of high fat diet on brain is very complex and probably involves different cell types and mechanisms. We hope with this work to be able identifying the contribution of individual factors and to eventually translate this knowledge into the clinic.

MITOCHONDRIAL DYSFUNCTIONS AS AN EARLY EVENT IN THE PATHOGENESIS OF FAMILIAL ALZHEIMER'S DISEASE?

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Alzheimer's disease (AD) is the most common age-related progressive neurodegenerative disorder in which learning, memory and cognitive function decline simultaneously, dramatically and permanently. The most prominent lesions in AD brains are extracellular neuritic plaques formed by amyloid beta (Aβ) aggregation and intracellular neurofibrillary tangles of hyperphosphorylated tau. Other pathological dysfunctions include cell cycle abnormalities, inflammatory processes, oxidative stress, as well as mitochondrial and neurovascular dysfunction that cause synaptic damage, synaptotoxicity and neurotransmitter disturbances. The pathogenesis of AD is known to be associated with significant dysfunction in the cholinergic and glutamatergic neurotransmitter systems, including altered levels of these neurotransmitters and the massive degeneration of neuronal networks. However, the progression of the disease is likely far more complex, with the involvement of additional neurotransmitter systems and molecular components.

From a genetic point of view, AD is a heterogeneous disorder including both familial and sporadic forms. In AD in particular, the role of the genetic lesions in the pathogenesis is firmly established. Up to now, the mutations identified as causative in the familial forms of AD (FAD) are mutations in the Aβ precursor protein (APP) gene, in the presenilin 1 (PS1) gene and in the presenilin 2 (PS2). PS1, and its homologue PS2, are the catalytic core of the γ-secretase complex that, by cleaving APP in concert with β-secretase, produces the neurotoxic Aβ peptides. However, the mechanism linking these mutations to neuronal dysfunction and eventually cell death is still largely obscure.

We have demonstrated that FAD-PS2 mutants, localized on the ER surface, is a positive modulator of ER-mitochondria tethering and of the Ca^{2+} transfer between the two organelles, despite it lowers the ER Ca^{2+} content. Although strongly suggested by experimental data obtained in cultured cells, the impairment of mitochondrial Ca^{2+} dynamics in the early stages of FAD pathogenesis has never been directly tested in more complex systems such as brain slices. To address this issue, we are evaluating FAD-PS2 and FAD-APP mutations on mitochondrial functions and Ca^{2+} homeostasis in hippocampal slices of wild type (wt), PS2-N141I/APPswe and PS2KO mice at different time points before amyloid plaque deposition, at the onset of Aβ accumulation. Animals KO for PS2 are included in the study, since the group of Raymond J. Kelleher III, recently, raised the provocative hypothesis of a loss-of-function phenotype associated to FAD-PS1 mutations.

In order to study mitochondrial Ca^{2+} dynamics in brain slices, we are employing newly-developed mitochondria-targeted Genetically Encoded Ca^{2+} indicators (mtGECI): FRET-based quantitative biosensors (Cameleons) and Single FP ultrasensitive biosensors (GCaMP). To study Ca^{2+} signals, we apply pharmacological stimuli of glutamatergic system but also cholinergic and GABAergic systems in order to have a global consideration of neurotransmitter dysfunctions in the diseased brain. This functional study will be complemented with biochemical and genetic data that will clarify the mechanism behind the possible phenotype we will identify, allowing a comprehensive understanding of AD pathogenesis with a view towards the development of novel treatment strategies.

A NEW MOUSE MODEL FOR PARKINSON'S DISEASE RECAPITULATING THE MAJOR NEUROPATHOLOGICAL HALLMARKS OF THE HUMAN PATHOLOGY

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Parkinson's disease (PD) is the most common age-related movement disorder affecting to date about 1.3 million people only in Europe. PD is characterized by progressive degeneration of dopaminergic (DAergic) neurons in the *substantia nigra* (SN) and the presence of intraneuronal inclusions, known as Lewy Bodies, constituted by alpha-Synuclein (α-Syn) protein aggregates. Despite the intensive research in the last decade and the significant gaining of knowledge on the disease mechanisms, this has not yet translated in novel effective therapies. An important barrier for that has been related to the fundamental limitations in the cellular and animal models available for research in PD over the years. In fact inactivation in mice of the Parkin, PINK1 and DJ1 genes, associated to the genetic forms of PD in humans, has not resulted in developing overt PD-like neuropathological signs in these animals. On the other side, the use of toxins like MPTP and 6-OHDA has been extremely helpful to induce a preferential loss of nigral DAergic neurons either in mouse or rats, respectively. However, these toxins trigger a very rapid neurodegeneration process occurring in 2 weeks, but without any sign of α-Syn protein accumulation either. A growing body of evidence has implicated α-Syn as a crucial determinant in PD pathogenesis. A number of animal models expressing the human mutant α-Syn gene have been generated where these altered processes have been highlighted. However, these mice normally develop limited amount of α-Syn aggregates in few restricted brain areas and without a detectable degeneration of nigral DAergic neurons. An important step-forward resulted the use of adeno-associated viruses (AAV) to express α-Syn at high levels. α-Syn expressing AAVs delivered in adult rat SN induces α-Syn protein deposits, DA release impairment followed by severe loss of nigral DAergic neurons and accompanied by profound deficits in motor functions in about 2 to 4 months. Surprisingly, a similar treatment in adult mice with comparable AAV serotypes and titers induces only a modest neuronal loss (20-40%) in about 4 months and with mild motor behavioral defects. This robust resistance of mice to neurodegeneration has limited the use of the genetic tools particularly informative and effective available in these animals. We have been working in improving the AAV system to obtain a more efficient and rapid neurodegeneration in adult mice. Through a different choice of the promoter, the WPRE sequence removal and the use of a new AAV9 synthetic strain, named AAV-PHP.B we could obtain massive nigral DAergic neurodegeneration and profound behavioral alterations in about 5 weeks. Our model represents a unique tool in PD research able to recapitulate the distinctive features of the disease (α-Syn toxicity, massive DAergic neuronal loss, strong inflammation and behavioral motor deficits) circumscribed in a practical amount of time for experimental investigation.

AAV.PHP.EB-MEDIATED OPA1 GENE EXPRESSION IN A MOUSE MODEL OF PARKINSON'S DISEASE AS A VALUABLE STRATEGY FOR NEUROPROTECTION

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Parkinson's disease (PD) has been linked to defects in the mitochondrial function; indeed, reduced activity in the mitochondrial complex I impairs mitochondrial respiration and is associated with the selective degeneration of the dopaminergic neurons of the Substantia Nigra. More recently, the mitochondrial involvement in PD has been extended to perturbations to fusion/fission. OPtic Atrophy protein (OPA1), a dynamin-related GTPase of the inner mitochondrial membrane, participates in mitochondrial fusion and apoptotic mitochondrial cristae remodeling. Complex I inhibition leads to the disruption of OPA1 oligomeric complexes that are crucial for healthy mitochondria. Similar deficiencies have been observed in postmortem PD Substantia Nigra samples. Strikingly, these mitochondrial changes can be reverted if the levels of OPA1 are increased. These observations are prompting us to speculate whether the increase of OPA1 activity might affect alpha-synuclein accumulation and have beneficial effects in terms of neurodegeneration.

To answer this question, we investigated whether the overexpression of OPA1 is a viable therapeutic option against the neurodegenerative process in a mouse model of PD. Thus, we delivered OPA1 unilaterally in the mouse substantia nigra, using an AAV-PHP.eB vector in order to selectively increase the expression of OPA1. Consequently, we bilaterally injected AAV-PHP.B-SCNA-A53T, to trigger parkinsonian-like neurodegeneration and alpha-synuclein accumulation in five weeks. Mice were checked for general motor function, neuronal survival, a-Syn accumulation and phosphorylation, gliosis and astrocytosis. Importantly, to modulate OPA1 gene expression levels, two distinct promoters with different expressivity were cloned upstream the OPA1 gene and directly compared for their neuroprotection efficacy. With this project we aim at discovering a potential novel target for gene therapy to prevent neurodegeneration and disease progression.

PERSPECTIVE-DEPENDENT REACTIVITY OF SENSORIMOTOR MU RHYTHM IN ALPHA AND BETA RANGES DURING ACTION OBSERVATION: AN EEG STUDY

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During action observation, several visual features of observed actions can modulate the level of sensorimotor reactivity in the onlooker. Among possibly relevant parameters, one of the less investigated in humans is the visual perspective from which actions are observed. In the present EEG study, we assessed the reactivity of alpha and beta mu rhythm subcomponents to four different visual perspectives, defined by the position of the observer relative to the moving agent (identifying first-person, third-person and lateral viewpoints) and by the anatomical compatibility of observed effectors with self- or other individual's body (identifying ego- and allo-centric viewpoints, respectively). Overall, the strongest sensorimotor responsiveness emerged for first-person perspective. Furthermore, we found different patterns of perspective-dependent reactivity in rolandic alpha and beta ranges, with the former tuned to visuospatial details of observed actions and the latter tuned to action-related parameters (such as the direction of actions relative to the observer), suggesting a higher recruitment of beta motor rhythm in face-to-face interactions. The impact of these findings on the selection of most effective action stimuli for "Action Observation Treatment" neurorehabilitative protocols is discussed.

AN INTERACTIVE HOME-BASED PLATFORM PROMOTING CHILD-TO-CHILD INTERACTION IMPROVES HAND FUNCTION IN UNILATERAL CEREBRAL PALSY

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Background: By engaging the Mirror Neuron System and brain networks shared with action execution, Action Observation Therapy (AOT) proved effective in improving hand motor function in children with unilateral cerebral palsy (UCP). The aim of this study is to assess the effectiveness of an AOT-based platform combining video stories with interactive child-to-child remote sessions in improving hand function in children with UCP due to perinatal stroke.
Methods: Twenty UCP children (age 5-10) with mild-to-moderate hand impairment underwent 20 sessions structured as follows: first, they had to observe and imitate a wizard performing dexterity demanding magic tricks, a child-to-child live video session aimed at practicing the same exercises. During sessions, affected limb movement was accompanied by real-time positive feedbacks thanks to a Kinect 3D camera. The paretic hand motor function was evaluated through Besta Scale: a composite scale assessing global hand motor skills (Besta G), quality of grasp (Besta A), hand involvement in specific bilateral tasks (Besta B), hand recruitment in daily activities (Besta C). Moreover, Fugl Meyer Upper Extremity, Modified Ashworth Scale, segmental strength, mood VAS and Global Impression of change (GIC), were collected. Evaluations have been performed 1 month before (Tpre), at baseline (T0) and at the end of treatment (T1).
Results: Subjects showed a T1-T0 improvement relative to T0-Tpre in paretic hand global motor function (Besta G, 53%$\pm$41 vs 57%$\pm$41, $p<0.01$), recruitment of paretic hand in bimanual activities (Besta B, 58%$\pm$25 vs 63%$\pm$24, $p<0.01$) and a trend to significant improvement in Grasping (Besta A, 57%$\pm$31 vs 60%$\pm$36, $p=0.056$). Moreover, a significant improvement was perceived (average GIC 0.50$\pm$0.65) by families, contrarily to the pre-treatment period. Noteworthy, a correlation between motor improvement and the difference in hand motor skills relative to the peer ($r=-0.519$, $p=0.019$) was found: in other words, the better is my peer, the better is the outcome of my treatment.
Conclusion: Our results evidenced that AOT associated with child-to-child interaction is effective in improving hand motor functioning in UCP. Peer-to-peer difference in hand motor ability is associated to the improvement, suggesting that it is preferable for a child to observe a leading fellow with higher motor skills than his own. In conclusion, our platform showed a potential helpful role in hand rehabilitation programs for children with UCP, opening traditional AOT approaches to novel social-enriched scenarios, through which children could be at the same time beneficiary and provider of motor learning processes.

THE MISSING PIECE OF SOMATOSENSORY EVOKED POTENTIALS: DIFFERENCE BETWEEN ACTIVATION AND PHASE RESETTING ACCORDING TO A STEREO-EEG PERSPECTIVE.

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In the present study, we mapped the spatio-temporal dynamics of cortical responses to ipsilateral median nerve stimulation using intracerebral recordings (stereo-EEG) in 38 drug-resistant epileptic patients by examining the increase of power in gamma band. Overall, 50 hemispheres have been explored (28 right, 22 left) including 5872 cortical sites of which 4466 were localized in the grey matter according to the anatomical reconstruction (2783 in the right hemisphere, 1683 in the left hemisphere). The 37 responsive leads were almost exclusively located in the parietal operculum and in particular in its dorso-caudal part corresponding to area OP1. Active leads were found, at a smaller extent, bilaterally in the frontal operculum and in the long gyri of the right insular cortex while only residual activity was found in right inferior parietal cortex (2 leads), left short gyri of insular cortex (2), right premotor dorsal cortex (1) and in primary somatosensory cortex (2). Since previous findings are mostly based on SSEPs recording, and identified active clusters in primary somatosensory cortex, but inconsistently across subjects and topography, inter-trial coherence (5-145 Hz) was computed for all leads to bridge the gap with existing EEG/MEG literature about somatosensory processing. Results indicated a weak broadband gamma phase-resetting in SI and PMd within 50 ms from the stimulus, while opercular and insular regions showed a diffuse response in sub-gamma frequencies. The phase synchronization in gamma band cannot be considered as a measure of neuronal recruitment in absence of a significant gamma power increase, thus suggesting that it reflects a transcallosal echo originating from the activation of the contralateral homologue cortical area. This point was hindered so far by methodological constraints. The use of stereo-EEG, instead, allows one to distinguish power increase from phase synchronization phenomena, offering a valuable insight for interpreting non invasively recorded findings and thus complementing the classical view about the somatosensory system organization.

THE NEUROLOGICAL MOTOR DEFICITS AS AN ENDOPHENOTYPE OF AUTISM SHARED BY AFFECTED AND UNAFFECTED SIBLINGS

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A large body of evidence reports that, beyond the core socio-communicative deficits, autism is accompanied by an impairment of motor functions, including abnormalities in coordination, gait, praxis, action preparation and imitation. However, at present it is still an open question whether motor abnormalities may be found also in unaffected siblings. The aim of our study was to profile the neuro-motor abilities of typically development children (TD), autistic children (ASD) and their unaffected siblings (SIB), thus, providing an overall picture of neurological impairment of ASD and, possibly, identifying motor endophenotypes typical of autism. Seventy children were enrolled: 24 TD, 27 ASD and 19 SIB. The neurological motor picture was obtained by administering the Physical and Neurological Examination of Subtle Signs (PANESS) test. The test is composed by two main subscales, namely Gaits-Station and Timed Movements, further composed by several trials. The statistical analysis was conducted according to a top-down approach following the hierarchical structure of PANESS test: starting from the top (total score), we moved backward in search of the domains distinguishing ASD from all other groups and, most interestingly, of the domains in which SIB assumed an intermediate position in between TD and ASD. Statistical analysis on total score revealed a triple significant contrast, with ASD exhibiting the worst scores (27.9±11.5), TD the lowest (10.8±5.2) and SIB located in between (15.7±6.1). Also for the two subscales separately, we obtained significant main effect of Group. However, while post-hoc analysis on Gaits-Stations indicated only a difference between ASD and the other groups (both $p<0.001$), the analysis on Timed Movements scores revealed an additional difference between SIB and TD ($p<0.05$). Moving to single timed-trial contrasts SIB proved similarly to ASD, and worse than TD, in finger apposition speed ($p<0.01$) and in adiadochokinesia ($p<0.05$). As expected, our results confirmed the presence of a diffuse motor impairment in all domains for ASD children. Most interestingly, while TD and SIB performance is equivalent in Gaits and Station, when required to perform timed movements (where speed and accuracy have to be properly combined), SIB performed worse than TD, indicating an overall weakness in timed movements as a possible motor endophenotype of autism. If confirmed, this finding would represent a key step for the definition of biomarkers accessible in children at risk for autism at an early onset, by far preceding the speech and cognitive impairments onset, thus facilitating the early diagnosis of autism and a consequent early intervention.

ELECTROENCEPHALOGRAPHIC CORRELATES OF BRAKING AND ACCELERATION EVENTS DURING SIMULATED CAR DRIVING

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Driving is a daily complex task which involves several motor and cognitive abilities whose temporal and spatial neurophysiological dynamics are still to be clearly depicted [1]. Many of these motor and cognitive elements have been studied separately in i) non-ecological fMRI paradigms without attempting to analyze temporal dynamics of driving [2], [3], and in ii) EEG experiments with the final aim to instruct a brain-computer interface [4]–[6]. The goal of this study is to highlight electroencephalographic features which could ultimately predict human intention in a semi-ecological driving scenario. To this aim, we analyzed the high density EEG activity collected by 30 subjects while they were driving a car simulator on a realistic track, without pedestrians and other vehicles. Pedals position was used to identify and segment the neural activity within the time window of [-1500, 1000] ms around the foot action onset from which to extract the neurophysiological features able to discriminate braking from acceleration events. We performed the Adaptive Mixture Independent Component Analysis followed by a cluster analysis to group significant and reliable activations. This led to the identification of a recurrent pattern of activation localized in mesial frontal areas for which the EEG source localization confirmed to be generated by mesial and dorsal motor cortices. Time frequency analysis returned a significant synchronization of theta rhythm around 800ms before the upcoming braking events, when compared with gas pedal pressure. These findings show the activation of the mesial premotor areas in the preparation for movement, which have been showed to be involved in low level motor control in program planning and execution [7], as well as in higher level motor control processes regarding the detection and transmission of conflicts as indicators of adapted processing in the sensory, motor and attention systems [8]. These evidences could be the basis for further research on driving behavior prediction.

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STORY-TELLING AND STORY-ACTING: THEIR EFFECTS ON DEVELOPMENT OF PRESCHOLER CHILDREN

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Two groups of preschooler children played with plush toys or construction toys telling and acting stories under the guidance of a psychologist. The aim of the study was to evaluate if the relation between story-telling and -acting improved cognitive and motor development of preschooler children respect to a control group. Both experimental groups, regardless the type of toy, improved their non - verbal fluid reasoning competence, narrative abilities and, surprisingly, also imitating hand positions skills. Most interestingly, children who played with construction toy have marked improvement in their visuospatial abilities.

GESTURES AND WORDS: NEW PERSPECTIVES ON THE RELATION BETWEEN ABSTRACT LANGUAGE AND ACTION

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Strong embodiment theories claim that action language representation is grounded in the sensorimotor system, which would be crucial to semantic understanding. However, there is a large disagreement in literature about the neural mechanisms involved in abstract (symbolic) language comprehension. In two TMS studies we demonstrated how context variability influenced motor cortex involvement in semantic processing of language related to symbolic manual gestures.
The results of the first study showed that a prior presentation of a gestural posture facilitated the comprehension of a semantically related word, involving the activation of hand primary motor cortex (M1) in the early stage of word lexical processing; this suggested that the gestural motor representation was integrated with corresponding word meaning in order to accomplish (and facilitate) the lexical task.
In the second study, we found M1 modulation as the effect of a specific associative-learning paradigm, even in the absence of a contextual prime. Indeed, coupling abstract words presentation with manual gestures execution caused changes in motor activation during a lexical task performed before and after the training. Interestingly, M1 excitability was specifically modulated by the semantic congruence between the word and the associated motor-schema.
Data are discussed in the framework of a multimodal representation of abstract concepts, where sensorimotor areas could be activated at different degrees depending on contextual variables and lexical flexibility; following this way, learning mechanism could have a central role in language development and representation, as in the gradual transition from transitive actions to speech.

INSULIN CLEARANCE AND ITS DETERMINANTS IN DOUBLE INCRETIN RECEPTOR KNOCKOUT AND WILD-TYPE MICE

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It has been recently shown that incretin hormones affect insulin clearance at physiological, non-stimulated levels. This was done assessing insulin clearance in mice with double incretin receptor knockout (DIRKO) and wild-type (WT) mice. The aim of this analysis is the identification of possible determinants of insulin clearance in DIRKO vs WT mice, via the investigation of the relationships of insulin clearance with parameters of insulin sensitivity, insulin secretion, beta-cell function, and glucose effectiveness.

DIRKO (n=31) and WT C57BL6J mice (n=45) were injected with D-glucose (0.35 g/kg) and blood was sampled for 50 minutes and assayed for glucose, insulin and C-peptide. C-peptide kinetics was established after human C-peptide injection, assuming a two-compartment distribution model with linear elimination. Then, insulin secretion rate following glucose injection was assessed by mathematical deconvolution based on the derived C-peptide kinetics parameters. Insulin kinetics parameters, including insulin clearance, were estimated from the obtained insulin secretion rate assuming a one-compartment distribution model with linear elimination. Finally, glucose effectiveness, total insulin secretion and beta-cell glucose sensitivity (AUC of C-peptide to that of glucose) were calculated.

With regard to the first phase clearance (which proved to be higher in DIRKO than WT: 0.68±0.06 *vs* 0.54±0.03 mL/min, $p<0.05$), in univariate linear regression analysis we found a significant relationship only with the acute insulin response, *AIRg* ($R_2=0.43$, $p<0.0001$; inverse relationship). However, in multivariate analysis, we found that insulin clearance was related to both parameters of insulin sensitivity and insulin secretion/beta-cell function: specifically, we observed $p<0.05$ for insulin sensitivity, *Si*, $p<0.01$ for total insulin secretion, $p<0.0001$ for beta-cell glucose sensitivity, $p<0.0001$ for *AIRg*; all inverse relationships except for glucose sensitivity, adjusted $R_2=0.71$.

In WT, similar results were found in univariate analysis. However, in multivariate analyses some differences emerged, since insulin clearance was related only to total insulin secretion and *AIRg* (adjusted $R_2=0.57$; $p<0.05$ and $p<0.0001$, direct and inverse relationships, respectively).

This analysis suggests that mice where incretin receptors have been knocked out undergo not only variations in insulin clearance, but also possible differences in the way clearance is affected by its determinants. However, this should be further investigated, e.g. to exclude possible artefacts related to group size (more WT than DIRKO mice). The results suggest that possible pharmacological treatments augmenting insulin sensitivity and/or insulin secretion may also affect insulin clearance, and such effects may depend on the action of incretins.

THE DIFFERENT MECHANISMS OF ACTION OF GIP AND GLP-1 EXPLAIN THEIR DIFFERENT EFFICACY AS THERAPEUTIC AGENTS

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Background and aims: The reduced incretin effect in type 2 diabetes (T2D) represents an important cause of postprandial hyperglycaemia, but the different pharmacologic efficacy of its major players, GLP-1 and GIP, remains unexplained. At cellular level the mechanisms activated by the two hormones and the defects of T2D are still poorly understood. In this study, we have extended a recently developed mathematical model of the β-cell to 1) investigate the role of incretins at the cellular level on Ca^{2+} signalling and on the glucose mediated amplifying pathway (AP); 2) characterise incretin action *in vivo* in subjects with normal glucose tolerance (NGT) or T2D; 4) provide an explanation for the different insulinotropic activity of GIP and GLP-1 in T2D subjects. Materials and methods: We used in vivo data from: A) two studies with constant infusions of GIP or GLP-1 at basal glucose; B) four hyperglycaemic clamp studies with boluses or constant infusion of GIP or GLP-1; C) a graded glucose infusion test with constant infusion of GLP-1; D) two OGTT or isoglycaemic intravenous glucose infusion studies with GIP or GLP-1 infusion. In the β-cell model, we hypothesize that GIP and GLP-1 increase insulin secretion rate (ISR) by a transient increase in Ca^{2+} levels (the first 15-30 min after the incretin stimulus) and by potentiating the AP; the Ca^{2+} and glucose-dependent refilling function representing the AP is multiplied by a time-dependent factor (K_{incr}); K_{incr}=1 without and K_{incr}>1 with incretin stimulation.
Results: A transient Ca^{2+} increase is necessary to reproduce the transient ISR increase observed with GIP infusion at basal glucose in NGT subjects (Study A). This mechanism also accounts for the increase in early ISR during the OGTT (Study D). The amplification of the refilling function through the factor K_{incr} accounts for the sustained ISR potentiation in all studies. The estimated effect on transient Ca^{2+} increase was similar for GIP and GLP-1 and was preserved in T2D compared to NGT. In contrast, the effects of GIP and GLP-1 on K_{incr} had markedly different patterns: K_{incr} increased linearly with GLP-1 over a wide dose range, while with GIP K_{incr} reached a plateau already at low GIP concentrations (Figure). K_{incr} sensitivity to GLP-1 was reduced by ~30% in T2D subjects compared to NGT, while for GIP the maximal K_{incr} was reduced by ~50%.
Conclusion: By modelling a variety of *in vivo* protocols, the following cellular mechanisms of incretins emerge: 1) a transient rise in intracellular Ca^{2+}, which underlies the early effects of incretins; and 2) a potentiation of the AP, which mediates the sustained ISR. Our analysis suggests that in T2D the incretin effect on Ca^{2+} is preserved while the amplification of the AP is impaired, though not abolished. Finally, we found that saturation of GIP effects, more than impaired sensitivity, underlies the lack of insulinotropic activity of pharmacological doses of GIP in T2D.

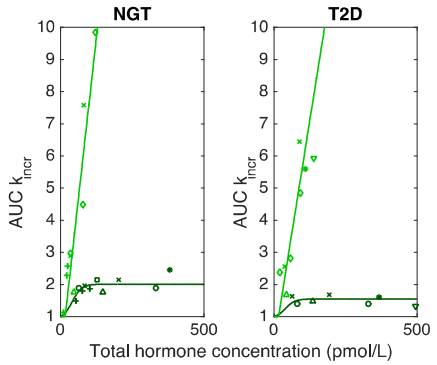

Figure: Relationship between K_{incr} and total GIP (light green lines) or GLP-1 (dark green lines) concentration. The analysis reveals saturation of the effect of GIP but not of GLP-1. Markers represent the different studies.

GABA TONIC CURRENTS IN DRAVET SYNDROME

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Dravet Syndrome (DS), also known as severe myoclonic epilepsy in infancy (SMEI), is a rare genetic epileptic encephalopathy. It begins in the first year of life and in 80% of patients it is linked to de novo loss of function mutations of the SCNA1 gene encoding for α subunit of Nav1.1 voltage-dependent sodium channels. These channels are mainly expressed on GABAergic interneurons that play a crucial role in different forms of epilepsy. In a mouse model of DS (Nav1.1 +/-), the reduced excitability of GABAergic interneurons been proposed to cause brain network hyperexcitability and seizures (Catterall et al 2010). Recently, in the same mouse model of DS (kindly provided by William Catterall), unaltered firing activity of interneurons was observed *in vivo* suggesting that other mechanisms may also be involved (DeStasi et al 2017). Notably GABAergic inhibition can be achieved not only at synapses but also outside them. GABA tonic (persistent) currents are indeed activated by low levels of GABA spilling over from synaptic sites and activating non desensitizing extrasynaptic $GABA_A$ receptors. Of note, no specific study has addressed the role of GABA tonic inhibition in DS. Preliminary data from our group suggest a reduced GABA tonic inhibition in DS mouse model. We here propose that reduced GABA tonic inhibition may increase brain circuit excitability leading to seizure generation in DS. Accordingly our aim is to characterize the molecular and cellular mechanisms that are responsible for the altered GABA tonic inhibition in DS animals. Patch-clamp recordings from pyramidal neurons of entorhinal cortex from DS mice revealed a significant reduction of GABA tonic currents evoked by THIP, a selective agonist of extrasynaptic $GABA_A$ receptors. This result suggests a reduced expression or function of GABA extrasynaptic receptors, compared to controls. Similar experiments are now being performed in dentate gyrus granule cells (DGGC) and preliminary data suggest, as opposed to entorhinal cortex, an increased response to the selective agonist THIP. Since these experiments are performed before the onset of spontaneous seizures, the increased GABA tonic currents, if confirmed, suggests a compensatory modulation that may protect from seizure onset. It will be important to evaluate if this increase is lost later during development, when full seizures start to occur. Future experiments will also address the role of astrocyte GABA transporters and the possibility to interfere with astrocytic function to prevent seizures in vivo.

Characterization of GABA tonic signaling in DS will help to clarify the mechanisms at the basis of seizure generation, possibly unveiling new targets for human therapy.

POSTER SESSION 2: SCHEDULE AND ABSTRACTS

N	When	Speaker	Title

Session 2

P2.1	F	Irem Kilicarslan	*Changes in retinal bipolar cell wiring in Cav1.4 mutant mouse models*
P2.2	F	Martina Biagioni	*Targeting inflammation to rescue cones in inherited retinal degeneration*
P2.3	F	Antonia Stefanov	*Inner retinal readjustment in a photo-inducible, rhodopsin mutant mouse model of Retinitis Pigmentosa*
P2.4	F	Laura Baroncelli	*Cortical processing of visual inputs in Retinitis Pigmentosa*
P2.5	F	Antonio Falasconi	*Retinal phenotype in rd9 mutant mice, a model of X-linked Retinitis Pigmentosa*
P2.6	F	Marco Righi	*Preliminar analyses of retinal OCT-scans and automatic quantification of healthy ocular angioarchitectures*
P2.7	F	Simona Francia	*A mouse model carrying a null mutation for a odorant receptor ligand exhibits an altered organization of the sensory map*
P2.8	F	Giorgia Pallafacchina	*SIGMA-1 receptor mutations cause distal hereditary motor neuropathy by impairing ER-mitochondria tethering and Ca^{2+} signalling*
P2.9	F	Valentina Giorgio	*The inhibitor protein IF1 modulates the permeability transition pore in a human tumorigenic cell model*
P2.10	F	Elisa Lidron	*A zebrafish model to study the role of mitochondrial calcium uptake in physiological and pathological conditions*
P2.11	F	Giulietta Di Benedetto	*Phosphatases control PKA-dependent microdomains at the outer mitochondrial membrane*

P2.12	F	Marina Campione	*Myocardial overexpression of ANKRD1 results in sinus venosus defects and leads to adult diastolic dysfunction*
P2.13	F	Samuele Negro	*CXCL12α is a key factor in the regeneration of the damaged neuromuscular junction by acting on the CXCR4 receptor*
P2.14	F	Nina Kaludercic	*Genetic ablation of monoamine oxidases impairs cardiomyocyte differentiation from hIPSC*
P.2.15	F	Ilaria Rossetti	*Differential expression of calcitonin gene-related peptide (CGRP) in extended amygdala system of sardinian alcohol-preferring and -non preferring rats*
P2.16	F	Paola Maccioni	*Reducing effect of saikosaponins A and D, active ingredients of bupleurum falcatum, on alcohol and chocolate self-administration in rats*
P2.17	F	Paola Maccioni	*Further investigation of the anti-addictive profile of COR659*
P2.18	F	Federica Fara	*The positive allosteric modulator of the $GABA_B$ receptor, CMPPE, suppresses alcohol self-administration and reinstatement of alcohol seeking in alcohol-preferring rats*
P 2.19	F	Mauro Congiu	*Exploring the role of astrocytes in regulating ventral tegmental area dopamine neurons and in a model of binge alcohol drinking*
P2.20	F	Liana Fattore	*Methoxetamine induces neurological, sensorimotor and cardiorespiratory alterations in mice and persistent behavioral abnormalities and neurotoxicity in rats*
P2.21	F	Carla Lobina	*Analgesic effects of a mixture of Zingiber officinale and Acmella oleracea extracts in rats*
P2.22	F	Luisa Ponzoni	*Serotonin 5-$HT_{2A/C}$ and arginine-vasopressin V_{1A} subtype receptors modulate rewarding, prosocial and anxiolytic effects induced by two synthetic phenethylamines in zebrafish*
P2.23	F	Valentina Satta	*Chronic administration of the synthetic cannabinoid WIN 55,212-2 induces cross-sensitization to cocaine behavioural effects in adolescent rats*

P2.24	F	Federica Scaroni	*Microglia versus macrophage effects on oligodendrocyte precursor cells: role of extracellular vesicles*
P2.25	F	Susanna Pucci	*New anti-glioblastoma agents by hybridizing the onium-alkyloxy-stilbene-based structures of a α7/α9-nAChR antagonist and of a pro-oxidant mitocan*
P2.26	F	Roberta Benfante	*The human-restricted duplicated form of the α7 nicotinic receptor, CHRFAM7A: expression and transcriptional regulation in inflammatory cells*
P 2.27	F	Antonio Di Carlo	*Prevalence of atrial fibrillation in the italian elderly population. Final results from the progetto FAI*
P2.28	F	Fiorella Tonello	*Secreted phospholipases A2 go inside cells: possible role in regulation of eicosanoid biosynthesis*
P2.29	F	Marzia Baldereschi	*The Tuscany stroke network:team is brain*
P2.30	F	Genni Desiato	*Role of the pro-inflammatory cytokine interleukin 6 in the control of the GABA switch in hippocampal neurons*

CHANGES IN RETINAL BIPOLAR CELL WIRING IN Cav1.4 MUTANT MOUSE MODELS

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Cav1.4 L-type calcium channels are predominantly expressed in retinal photoreceptor (PR) terminals at specialized ribbon synapses as well as also in bipolar cells (BC). They serve not only an important role for synaptic transmission but also for synapse formation and maturation. Mutations in the CACNA1F gene encoding for Cav1.4 channels are associated with congenital stationary night blindness type 2 (CSNB2), a disease characterized by impaired night vision, decreased visual acuity, nystagmus and myopia.

In this study we investigated two CSNB2 mouse models - one carrying a Cav1.4 gain-of-function mutation (Cav1.4-IT), the other comprising a loss-of-function due to Cav1.4 deficiency (Cav1.4-KO) - in comparison to wild type inbreed controls (WT) at the age of 11 to 14 weeks.

Retinal immunostaining with anti-PSD-95 showed mislocalization and retraction of Cav1.4-IT rod photoreceptor (PR) terminals. Of note, Cav1.4-KO retinas totally lacked PSD-95 immunoreactivity. As previously reported, mislocated rod PR terminals contained mainly elongated (immature) synaptic ribbons however some mature ones were still observed. Co-staining with PKCα revealed that rod bipolar cell (BC) dendrite invaginations to displaced rod PR terminals were still formed in Cav1.4-IT mice. Rod BC dendritic sprouting also occurred in Cav1.4-KO retinas but their ribbon structures never matured. Synaptic connections between PRs in the outer plexiform layer (OPL) seemed abnormal in both CSNB2 mutants. This might be a consequences of disturbed organization of rod PR terminals in the OPL.

In both mouse models, immunostaining with different cone BC markers demonstrated that also cone BC dendrites were sprouting into the outer nuclear layer (ONL) supporting the fact that both rod and cone pathways are affected by Cav1.4 altered expression. Preliminary analyses showed changes in the morphology of BC axonal endings in the inner plexiform layer (IPL) as well. This can be a consequence of the defective PR to BC transmission or a direct effect of the mutated Cav1.4 channels expressed at BC terminals.

Rod BC dendrites were completely lacking in the peripheral retina in both CSNB2 mouse models at the age of 28 weeks, likely due to the absence of photoreceptors in the corresponding area. This observation suggests that altered expression of Cav1.4 channels can cause not only congenital stationary night blindness but also a progressive, retinitis pigmentosa-like phenotype.

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TARGETING INFLAMMATION TO RESCUE CONES IN INHERITED RETINAL DEGENERATION

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In Retinitis Pigmentosa (RP), a mutation causes the primary degeneration of rods, followed by secondary death of cones and consequent blindness. We showed that in rd10 mice, a RP mouse model, the peak of cone death (P45) is associated with a strong retinal inflammatory response, which prevails on any other biological process. Our working hypothesis is that the breakdown of the eye "immune privilege" leads to retinal accumulation of inflammatory species, which eventually become toxic for cones, contributing to their death. Hence, we implemented a protocol based on Dexamethasone, a widely used steroid, which we administered to rd10 mice during the time window of maximum rod and cone degeneration.

Groups of rd10 mice received daily 4mg/kg Dexamethasone (DEXA) administered sub-cutaneously (4mg/ml), from P24 to P45 or to P60. Control mice received water. For histological studies, retinas harvested at P45 or P60 were fixed in 4% PFA and stained with cell-type specific antibodies. The number of cones and microglial cells/retina was estimated by cell counting on whole mount retinas after cone arrestin or CD11b antibody staining, respectively. Cone arrestin and Ccl2 (MCP-1) protein content was measured by Western blot in retinal homogenates from DEXA-treated and control mice aged P60. Visual acuity was tested at P45 and P60 with a Prusky water maze.

Dexamethasone treatment results in increased cone survival at P45. This occurs concomitantly to preservation of photopic visual acuity of treated mice and reduction of microglia/macrophages activation in the outer retina. At P60, Dexamethasone protective effects are also maintained, as indicated by higher persistence of cones and lower level of inflammatory species in the retina of treated mice. Western blot analysis detects decreased Ccl2 and higher cone arrestin levels in retinas of DEXA-treated rd10 mice, confirming higher cone survival associated to reduced inflammation.

Although the role of inflammation in RP is becoming progressively recognised, anti-inflammatory, pharmacological approaches specifically aiming at rescuing cones in this disease have never been tested. Our study reveals a very important link between retinal inflammation and cone degeneration, showing cones rescue with a commonly employed drug. This opens the possibilities to repurpose steroid treatment to slow down vision loss in human RP.

Funded by Fondazione Roma, Italy, Retinitis Pigmentosa call

INNER RETINAL READJUSTMENT IN A PHOTO-INDUCIBLE, RHODOPSIN MUTANT MOUSE MODEL OF RETINTIS PIGMENTOSA

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Common rodent models of Retinitis Pigmentosa (RP) exhibit a photoreceptor-degeneration phenotype while the retina is still developing, differing from what is usually observed in humans, where the disease manifests during early adulthood. This limits general applicability of animal model results. Studies on laboratory animals faithfully mimicking human RP are necessary. Tvrm4 mice bear a photo-sensitive rhodopsin mutation that can be induced at any age. Here we studied retinal phenotype in Tvrm4 mice induced in adulthood with the specific aim of gaining insight into the nature, time-course and severity of remodeling of bipolar cells. These are retinal neurons performing major parallel processing of visual signals and fundamental targets of retinal repair strategies, such as optogenetics. Preservation of bipolar cells is crucial for restoration of light sensitivity in RP.

Heterozygous Tvrm4 mice aged 3-5 months (n≥3/group) were photo-induced with 12k LUX white-neon light for 2 mins in one eye (HT), while the other served as control (WT). Eyes were explanted 3, 6 or 9 weeks post-induction (PI) and processed for immunohistochemistry or Transmission Electron Microscopy (TEM) for imaging bipolar cells and IPL synaptic connections. Quantitative image analysis was performed using MetaMorph software, followed by statistical analysis in SigmaPlot platform.

Rod bipolar cells (RBC) stained with anti-PKCα were counted in retinal whole mounts 3 and 6 weeks PI. No statistical difference was found in their number compared to WT controls ($p=0,295$). A significant shrinkage of axonal arborizations of these cells ($p\geq0,001$) appeared 3 and 6 weeks PI.

Anti-CtBP2/RIBEYE staining of ribbon synapses revealed a significant increase in the threshold area of RIBEYE+ puncta in the inner plexiform layer (IPL) ($p=0,010$), 3 weeks PI, with respect to controls. A similar analysis of anti-Cx36 positive gap-junctions showed significant increase of CX36+ threshold coverage in all experimental groups compared to WT controls ($p\geq0,001$).

Cone bipolar cells were studied using anti-secretagogin (SCGN) antibodies, 3 and 6 weeks PI. No statistical difference was found in the number of cell bodies, yet we observed a gradual, significant decrease in the thickness of SCGN+ profiles in the IPL ($p\geq0,001$).

TEM studies confirmed shrinkage of RBC axonal endings but preservation of IPL synapses, although more than 60% of ribbons displayed an abnormally globular shape 3 and 6 weeks PI.

High bipolar cell survival rate despite regressive dendritic and axonal remodeling found here coincide with previous findings in developmental models of RP. Hence, we conclude that these features are independent of age of disease onset and underlying genetic mutation. Increased threshold area of RIBEYE and CX36 immunostaining suggest alterations in IPL connections and should be studied further. Preservation of synaptic contacts in the IPL despite morphologic alterations indicate retinal resistance to secondary degeneration and likely attempt to maintain the flow of remnant visual signals. Altogether, these data provide promising information for retinal repair strategies targeting bipolar cells.

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CORTICAL PROCESSING OF VISUAL INPUTS IN RETINITIS PIGMENTOSA

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Retinitis Pigmentosa (RP) is a family of inherited disorders caused by the progressive loss of retinal photoreceptors. There is no cure for RP, but research aimed at preventing further photoreceptor loss, or substituting new light-responsive elements of biological or artificial nature, is generating hope for these patients. These strategies require that the visual system downstream of the photoreceptors is capable of elaborating visual signals. Anatomical and functional studies have shown that retinal and thalamic structure are well preserved with RP, but the effect of photoreceptor degeneration on the visual cortex is still unknown. Here, we studied how visual cortical processing changed during the course of progression of RP, and whether the visual cortex retained the capability of plastic remodelling. Binocularity is a key property of primary visual cortex (V1) neurons that is widely used to study synaptic integration in the brain and plastic mechanisms following an altered visual experience. We used visual evoked potential to test whether cortical processing of visual inputs is altered in RP. We found a robust shift in eye preference in RP animals, due to a selective increase of ipsilateral eye-driven responses. The disruption of the binocular properties of neurons in the primary visual cortex was associated with behavioural deficits in depth perception. We also performed in vitro electrophysiological recordings of field excitatory post-synaptic potentials in V1. Basic synaptic transmission, as assessed by response vs stimulus amplitude, showed a significant shallower response in RP mice. Biochemical analysis suggests that this synaptic deficit could be related to the alteration of absolute levels of inhibition and excitation in the visual corte. These results suggest that cortical changes occur in the visual cortex that might further compromise vision by downregulating or suppressing visual processing, as the retinal input progressively deteriorates.

RETINAL PHENOTYPE IN RD9 MUTANT MICE, A MODEL OF X-LINKED RETINITIS PIGMENTOSA

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Retinitis Pigmentosa (RP) comprises a group of genetic eye diseases characterized by progressive photoreceptor degeneration and vision loss. Hundreds different mutations in over 170 genes identified so far can lead to a convergent rod-cone degeneration phenotype but the time course can vary from a few years to decades before total vision loss occurs.
The *Retinitis Pigmentosa GTPase Regulator* (RPGR) gene, on the X chromosome, is one of the etiological determinants of RP. Noticeably, gene therapy for RPGR-ORF15 mutations is currently under development with a phase II clinical trial. Unfortunately, photoreceptor transduction efficiency of retinal gene therapy is not very high at the moment and the disease course can only be significantly improved in forms with slow rate of photoreceptor loss. Hence, assessing the severity of various forms of X-linked RP is crucial to evaluate the success possibilities of gene therapy and enhance our understanding of phenotype variability of this particular type of RP.
Here, we provide a morphological and functional characterization of the retinal phenotype of the rd9 mouse model, bearing a 32-bp duplication in RPGR-ORF15. Quantitative immunohistochemistry on retinal samples from 12-months-old mice showed a still confined photoreceptor loss sided by cone morphological dystrophy and evident remodelling of the outer plexiform layer (OPL), where the dendritic arbors of rod bipolar cells were partially atrophic, showing loss of fine dendritic tips; on the other side, sprouting of the same cells was also observed toward the outer retina, overall suggesting altered communications of photoreceptors and second order neurons. No relevant signs of retinal inflammations in glial cells were detected, except for a minor upregulation of GFAP immunoreactivity in astrocytes.
Functional analysis of one-year-old rd9 animals (and age-matched, wt controls) showed a reduction of the retinal activity. The amplitude of the a-wave of the scotopic ERG measured at 7ms and directly correlated with the number of light-responding photoreceptors was reduced; the b wave, giving information about signal transfer from photoreceptors to second order neurons, was also reduced. ERG responses following photopic stimulation showed a tendency (although non-significant) to a reduction in amplitude. The kinetics of the responses remains unchanged in both scotopic and photopic ERGs.
These results confirm the moderate morphological and functional degeneration phenotype of rd9 mutant mice and suggest that similar RPGR-ORF15 human mutations might lead to a likewise slow-progressing RP, opening up the possibility of successful gene therapy. The limited inflammatory/glial involvement, opposite to the strong response observed in faster mutants, make rd9 mice a useful model for the study of inflammation-independent dynamics in neurodegenerative retinal diseases.

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PRELIMINAR ANALYSES OF RETINAL OCT-SCANS AND AUTOMATIC QUANTIFICATION OF HEALTHY OCULAR ANGIOARCHITECTURES

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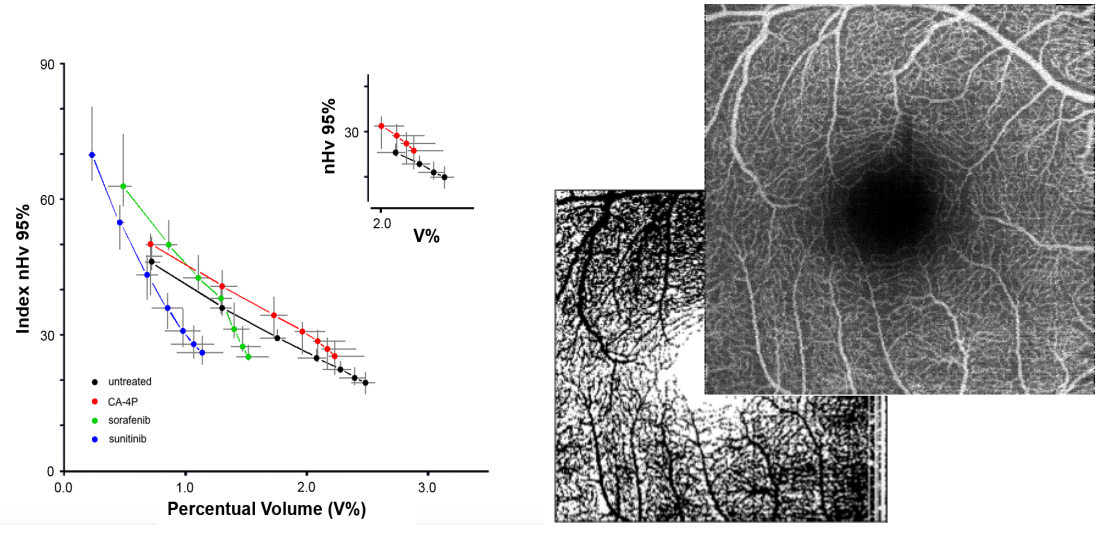
Several piece of evidence suggest that subtle alterations in retinal vascularization might betray cryptic pathological states, sometimes involving the brain vasculature and therefore a risk of dementia on a vascular basis. Automatic quantification of this information is mandatory to imagine for a widespread clinical screening but it is a difficult task since the only useful and accepted parameter in vascularization analysis is the Mean Vascular Density (MVD).
Nevertheless, the eye provides an unique access to body vasculature and can be analysed by low invasive techniques such as Optical Coherence Tomography Angiography (OCT-A). In this respect we conducted preliminary analyses to clarify whether we could apply to OCT scans an automatic method we developed and tested in an animal model using a xenotransplanted tumor cell line. In that model, the analyses of those angioarchitectures revealed that we could summarize the vessel layout as a near-linear relationship on the basis of the amount, dispersion and caliber of the observed vascular trees. The slope of the resulting lines dropped after antiangiogenic pharmacological treatments and thus correlated with the angiogenic potential of the tumors.
Turning back to retina analyses, OCT-A images of healthy individuals were obtained with a Topcon scanner and elaborated using the freely available ImageJ application. After a preliminar registration step, much effort was devoted to develop a rationale for an automatic application able to isolate the retinal vascular plexa. Isolated greyscale volumes representative of superficial and deep retinal plexa were then thresholded to binary signals using a combination of approaches and all of the 3D vascular information obtained from the volume. The resulting signals were then classified according to the minimal projection of vascular cross-sections observed on the 3 Cartesian planes and OCT-A artefacts removed assigning to vessels a depth not greater of their planar caliber. This classification step was repeated in parallel with several combination of classes given that vessels larger than the largest caliber considered were to be discarded and this step could alter the final result.
The final analysis was carried out on a set of partially reconstituted vascular trees, obtained combining vessels with different calibers but belonging to the same plexus. We tested the percentual vascular amount and the normalized dispersion of each tree with reference to the total volume of the plexus using a masking approach. The results obtained seem to support the feasibility of the analysis, although caliber classification turned out to be a critical step to obtain near-linear meaningful relationships. Analysis of controlateral eyes provided an internal control and in a case seemed to pinpoint a slightly different angioarchitecture in the deep retinal plexus of an healthy individual.



A MOUSE MODEL CARRYING A NULL MUTATION FOR A ODORANT RECEPTOR LIGAND EXHIBITS AN ALTERED ORGANIZATION OF THE SENSORY MAP

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It is known for more than 20 years that odorant receptors (OR) not only detect odors but also determine the convergence of sensory neurons to form glomeruli in specific locations in the olfactory bulb, giving rise to the sensory map. Odorant receptors are expressed specifically in two locations in the sensory neurons: at the cilia, where they detect odors, and at the axon terminal, a suitable location for axon guidance molecules. In this location the OR could direct OSN axonal convergence in specific loci of the OB, resulting in the sensory map of the OB, that exerts a critical role in odor coding.
In a previous work, we found that the OR at the axon terminal is functional and coupled to local increase of cAMP and Ca^{2+}. The critical question that remained to be addressed was the mechanism of activation of the OR at the axon terminus.
We hypothesized that a few molecules expressed in the olfactory bulb, could bind and activate the OR at the axon terminal of OSNs. We identify a ligand that locally applied, was able to induce Ca^{2+} rise and modulate OSN turning behaviour at the OSN axon terminus. We confirmed that this Ca^{2+} rise was due to OR activation, by expressing OR in HEK293T cells, loaded with the calcium indicator fura 2. Only HEK cells expressing distinctive OR exhibited a prompt Ca^{2+} rise in response to the identified ligand.
We then characterized the expression of the ligand in the olfactory system. We found that the ligand is mostly expressed in periglomerular cells in the OB, and it is not expressed in OSNs.
If the ligand is involved in the formation of the sensory map, mouse carrying a null mutation in the ligand should exhibit an altered sensory map. Indeed, we found that the sensory map was disrupted by the presence of additional heterogeneous glomeruli in mutant in respect to control mice.
All together our data demonstrated that the ligand of the axonal odorant receptor acts as an axon guidance molecules, that contribute in providing the olfactory sensory neurons with instructions to reach the proper target.

SIGMA-1 RECEPTOR MUTATIONS CAUSE DISTAL HEREDITARY MOTOR NEUROPATHY BY IMPAIRING ER-MITOCHONDRIA TETHERING AND CA^{2+} SIGNALLING

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Distal Hereditary Motor Neuropathies (dHMNs) are clinically and genetically heterogeneous neurological disorders characterized by degeneration of the lower motor neurons. To date, 19 dHMN genes have been identified but 80% of dHMN cases remain without genetic description.
We have recently identified two causative mutations in the *SIGMAR1* gene (E138Q and E150K) coding for the sigma-1 receptor (sigma-1R) in two Italian families affected by an autosomal recessive form of dHMN. Sigma-1R is a highly conserved 28 kDa chaperone protein of the endoplasmic reticulum (ER) which shares no homology with any mammalian protein. We demonstrated that sigma-1R substitutions behave as "loss-of-function" mutations affecting cell viability and altering Ca^{2+} homeostasis due to the derangement of ER-mitochondria tethering in neuroblastoma cells.
We describe here our study of the molecular mechanisms underlying sigma-1R role in the etiopathology of dHMN, using a more physiological model consisting of patient primary cells. We characterize human skin fibroblasts homozygous for one of the two σ1R mutations previously described, the E150K, by exploring global Ca^{2+} signalling and assessing the extent and distribution of ER-mitochondria contact sites, which are one of the main determinants of mitochondria Ca^{2+} signalling. Our data clearly show a significant disorganization of ER-mitochondria tethering, an upregulation of basal autophagy, and an altered global Ca^{2+} handling compared to controls. Importantly, patient fibroblasts display a reduced amount of the mutated sigma-1R protein compared to controls, similarly to a knock-down phenotype. This could explain the abnormal Ca^{2+} response recorded in E150K mutant cells compared to controls and, at least in part, explain the differences in Ca^{2+} dynamics that might be observed with the overexpression model.
Furthermore, we analyse the effect of sigma-1R mutation on cell metabolism and on intracellular organelle architecture. Our measurements of mitochondrial respiration indicate that E150Q mutant fibroblasts have an altered basal oxygen consumption rate. On the other hand, our electron microscopy analysis reveals a significant rearrangement of mitochondria architecture in mutant fibroblasts compared to controls, similarly to what reported in cells where sigma-1R has been downregulated.
Concluding, our data demonstrate the involvement of sigma-1R in the maintenance of cell homeostasis and highlight the crucial role of this protein in the establishment of functional ER-mitochondria contacts and in the modulation of global Ca^{2+} signalling. This suggests a correlation between *SIGMAR1* gene mutations and motor neuron dysfunction in dHMN and point to the deregulation of sigma-1R function as a critical aspect of neuronal degeneration in human neuropathies.

THE INHIBITOR PROTEIN IF1 MODULATES THE PERMEABILITY TRANSITION PORE IN A HUMAN TUMORIGENIC CELL MODEL

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The mitochondrial protein IF1 is the natural inhibitor of F-ATP synthase. IF1 is encoded in *ATPF1* gene which is located in chromosome 1 and 4 of human and mouse genomes. Alternative splicing of the human IF1 mRNA transcripts can generate three isoforms that differ in length and sequence. The longest mature protein consists of 84 amino acids, a N-terminus necessary for its inhibitory property and a C-terminus required for formation of its active dimeric form. It is well established that binding of the mature IF1 to the catalytic F1 domain of F-ATP synthase inhibits ATP hydrolysis and is optimal at low pH. It has also been suggested that IF1 binding may stabilize dimers of the F-ATP synthase. Our previous work has demonstrated that purified F-ATP synthase dimers added to a lipid bilayer form channels matching the electrophysiological features of the permeability transition pore (PTP), a mitochondrial mega-channel which is activated by matrix Ca^{2+} and ROS. Long-lasting openings of the channel induce cell death through the release of pro-apoptotic factors. We have found that silencing of IF1 in a human tumorigenic cell line decreases F-ATP synthase dimer/oligomer stability, promotes PTP opening and prevents tumorigenic capacity *in vitro.*

A ZEBRAFISH MODEL TO STUDY THE ROLE OF MITOCHONDRIAL CALCIUM UPTAKE IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Ca^{2+} is a second messenger that decodes and controls a variety of biological processes within the cell and mitochondria play active part in the regulation of intracellular Ca^{2+} signalling. The molecular identification of the mitochondrial Ca^{2+} uniporter (MCU) complex components has been instrumental for the biochemical and functional characterization of the mitochondrial Ca^{2+} contribution to cell homeostasis. Unexpectedly, the *MCU*$^{-/-}$ mouse model shows a relatively mild phenotype, its severity, however, depends on the specific genetic background, underlying a role for MCU during embryogenesis. Our work aims to explore the contribution of MCU and mitochondrial Ca^{2+} to the regulation of vertebrate development and organogenesis in the zebrafish animal model. We use the morpholino knock-down strategy in order to reduce MCU expression during zebrafish embryonic development. MCU-morphant embryos develop without gross morphological abnormalities. However, they display alterations in many tissues, such as skeletal muscle disorganization and impairment of motor neuron development. Skeletal muscle differentiation and motor neuron pathfinding processes are intimately connected during zebrafish embryogenesis and adaxial cells play a crucial role in driving motor neuron axon growth. Given that, we explore the distribution of these cells in MCU-down-regulated embryos. Our results indicate a remarkable mislocalization of adaxial cells together with a reduction in their number in morphant embryos. This deficit may be responsible for the defective skeletal muscle-motor neuron developmental axis of MCU morphants. In addition, we have generated a *mcu*$^{-/-}$ fish by CRISPR/Cas9 technology, which will represent the ideal system for studying the role of mitochondrial Ca^{2+} signals in vertebrate homeostasis also in the adult, and for dissecting the contribution of MCU gene dosage to physiological and, more interestingly, in pathological conditions.

PHOSPHATASES CONTROL PKA-DEPENDENT MICRODOMAINS AT THE OUTER MITOCHONDRIAL MEMBRANE

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Evidence supporting the heterogeneity in cAMP and PKA signaling is rapidly accumulating and has been largely attributed to the localization or activity of adenylate cyclases, phosphodiesterases, and A-kinase–anchoring proteins in different cellular subcompartments. However, little attention has been paid to the possibility that, despite homogeneous cAMP levels, a major heterogeneity in cAMP/PKA signaling could be generated by the spatial distribution of the final terminators of this cascade, i.e., the phosphatases. Using FRET-based sensors to monitor cAMP and PKA-dependent phosphorylation in the cytosol and outer mitochondrial membrane (OMM) of primary rat cardiomyocytes, we demonstrate that comparable cAMP increases in these two compartments evoke higher levels of PKA-dependent phosphorylation in the OMM. This difference is most evident for small, physiological increases of cAMP levels and with both OMM-located probes and endogenous OMM proteins. We demonstrate that this disparity depends on differences in the rates of phosphatase-dependent dephosphorylation of PKA targets in the two compartments. Furthermore, we show that the activity of soluble phosphatases attenuates PKA-driven activation of the cAMP response element-binding protein while concurrently enhancing PKA-dependent mitochondrial elongation. We conclude that phosphatases can sculpt functionally distinct cAMP/PKA domains even in the absence of gradients or microdomains of this messenger. We present a model that accounts for these unexpected results in which the degree of PKA-dependent phosphorylation is dictated by both the subcellular distribution of the phosphatases and the different accessibility of membrane-bound and soluble phosphorylated substrates to the cytosolic enzymes.

MYOCARDIAL OVEREXPRESSION OF ANKRD1 RESULTS IN SINUS VENOSUS DEFECTS AND LEADS TO ADULT DIASTOLIC DYSFUNCTION

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Aims: Increased ANKRD1 levels linked to gain of function mutations have been correlated to selective congenital heart disease onset and adult cardiomyopathy occurrence in humans. The link between increased ANKRD1 level and cardiac structural and functional disease onset is not understood. To get insight into this problem, we have generated a gain of function ANKRD1 mouse model by overexpressing ANKRD1 in the myocardium.
Methods and Results: We show that ANKRD1 delineates discrete sub-compartments in the developing mouse heart. ANKRD1 transgenic mice present impaired cardiac remodeling, which strongly affects the developing sinoatrial region and leads to sinus venosus defects. Transgenic mice survive to adulthood but develop left atrial enlargement accompanied by severe diastolic dysfunction. Myofibrils isolated from embryonic, neonatal and adult hearts present progressive and differential alterations of contractile parameters, which indicate a functional shift towards a stiffer and hyper-contractile phenotype in transgenic hearts: whereas early staged transgenic myofibrils are characterized by reduced compliance (i.e higher passive tension and maximal force) compared to wild type, adult transgenic myofibrils present impaired relaxation. At the molecular level, these changes are accompanied by dynamic alterations in titin isoforms ratio. Embryonic and neonatal transgenic cardiomyocytes present irregular shape and sarcomeric disorganization, which progresses into sarcomeric loss and mitocondrial damage in adult ventricular but not atrial cardiomyocytes.
Conclusions: Our data indicate that genetic mutations leading to increased ANKRD1 levels can lead both to congenital heart disease and adult cardiomyopathy by progressively affecting cardiomyocyte contractile and functional properties, resulting in *increased cardiomyocyte stiffness*. Increased ANKRD1 levels is sufficient to generate phenotype onset, which is exacerbated into a pathological response in the presence of high workload. ANKRD1 is a critical strain sensor-signaling molecule which finely modulates cardiomyocyte functional properties during development and postnatal life.

CXCL12α IS A KEY FACTOR IN THE REGENERATION OF THE DAMAGED NEUROMUSCULAR JUNCTION BY ACTING ON THE CXCR4 RECEPTOR

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The neuromuscular junction (NMJ) is one of the few human tissues capable of complete regeneration after major damages (1). We have set up a reliable model of acute degeneration of the motor axon terminals followed by complete recovery of function. We have performed a time resolved trascriptomic analysis of the NMJ and have found that the chemokine CXCL12α and other factors, that are currently under analysis, play a major role in NMJ regeneration after complete degeneration. CXCL12α acts via the CXCR4 receptor which is localized at the tip of the regrowingh motor axons (2).
We are currently testing the activity of CXCR4 agonists and have found a very active, non toxic, compound that strongly promotes the recovery of function of the NMJ completely degenerated by the spider neurotoxin α-latrotoxin. Moreover, we are extending the analysis of the pharmacological properties of the CXCR4 agonists to the sciatic nerve crush.

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GENETIC ABLATION OF MONOAMINE OXIDASES IMPAIRS CARDIOMYOCYTE DIFFERENTIATION FROM hIPSC

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Myocardial function depends largely on oxidative phosphorylation and mitochondria play a pivotal role during heart development. Monoamine oxidases (MAO-A and -B) are flavoenzymes located in the outer mitochondrial membrane, responsible for the degradation of neurotransmitters and biogenic amines. In pathological conditions, MAO activity contributes to cardiac damage, mainly due to the excessive formation of H_2O_2 and aldehydes. However, the role and contribution of MAOs during early development is still unknown. Here, we investigated the role of MAO-A in cardiomyocyte differentiation from human induced pluripotent stem cells (hiPSCs) in which MAO-A expression was either transiently knocked-down (siRNA) or genome edited (CRISPR/Cas9) to study its function when the protein is fully deleted.

MAO-A$^{-/-}$ hiPSCs cells showed a reduction in basal respiration and spare respiratory capacity. Transmission electron microscopy analysis showed that MAO-A$^{-/-}$ hiPSCs contained more mitochondria that appear more elongated in comparison with the isogenic control. In addition, MAOs knock-down during cardiomyocyte differentiation negatively affected contractile properties of hiPSC-derived cardiomyocytes, resulting in higher frequency and lower amplitude of calcium transients. Moreover, cells treated with siRNA against MAO-A showed sarcomere disorganization and a reduction in the α- to β-myosin heavy chain ratio, similarly to what occurs in failing hearts. Taken together, these results suggest a major role for MAO-A in regulating mitochondrial function and structure in hiPSCs. Furthermore, we show that MAO isoforms profoundly affect cardiomyocyte differentiation.

DIFFERENTIAL EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN EXTENDED AMYGDALA SYSTEM OF SARDINIAN ALCOHOL-PREFERRING AND -NON PREFERRING RATS

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Excessive alcohol consumption can lead, under a variety of circumstances, to alcohol abuse and dependence. Understanding the biological basis of excessive alcohol drinking can be useful to unravel signaling mechanisms and molecular signatures that underlie abnormal drinking behaviors. This could ultimately lead to the discovery of potentially effective therapeutic approaches. Sardinian alcohol-preferring (sP) and -non preferring (sNP) rats have been selectively bred for opposite alcohol preference and consumption under the standard, homecage, two-bottle "alcohol *vs* water" choice regimen with unlimited access. sP rats voluntarily consume large amounts of alcohol, resulting in significant blood alcohol levels and producing psychopharmacological effects (including anxiolysis and motor stimulation). Conversely, sNP rats avoid alcohol virtually completely. sP and sNP rats have been characterized for different phenotypes: in comparison with sNP rats, alcohol-naive sP rats displayed (1) more anxiety-related behaviors; (2) higher initial sensitivity to the locomotor stimulating and sedative/hypnotic effects of alcohol; and (3) lower sensitivity to the aversive effects of alcohol. Calcitonin Gene-Related Peptide (CGRP) is a pleiotropic neuropeptide involved in modulation of anxiety-related behaviors. We thus aimed at analyzing whether CGRP expression was differentially regulated in sP and sNP rats. The analysis focused on extended amygdala (AMY), a system influencing anxiety-related behaviors where differential gene expression were described between sP and sNP by microarray analysis. In particular, our analysis was carried out on CGRP-immunoreactive terminals present in bed nucleus of the stria terminalis (BNST) and AMY, as well as on their neurons of origin, CGRP-immunoreactive cell bodies present in parabrachial nucleus. Adult, male alcohol-naive sP and sNP rats were anesthetized and perfused by paraformaldehyde. Their brains were dissected, embedded in sucrose, frozen, and cut at cryostat. Sections (10 um thick, collected on chrome alum-coated slides) were processed for immunofluorescence and analyzed at confocal microscope. An analysis was conducted on CGRP terminals in BNST (mainly in its medial division, anterior part: BNST-MA, and ventral division: BNST-V; the two main CGRP areas in this rat strain) and AMY, as well as CGRP cell bodies in parabrachial nucleus (neurons of origin of CGRP projections to BNST and AMY). CGRP expression in this system was compared to a second CGRP system, the one consisting of neurons in peripeduncular nucleus and posterior intrathalamic nucleus and their projections to caudate/putamen. This system is not linked to anxiety-related behaviors, instead it seems to drive associative and acoustic information. Nomenclature as described in Paxinos and Watson (1986; Tre rat brain in stereotaxic coordinates; IInd edition) was used.

An automatic quantitative image analysis revealed that CGRP immunofluorescence intensity in terminals of BNST-MA was significantly lower in sP than sNP rats. In BNST-V subnucleus CGRP fluorescence intensity was similar in the two rat lines. A colocalization analysis between CGRP and synaptophysin showed that colocalization coefficient increased in sNP rats in both BNST-MA and -V. However, synaptophysin expression did not differ between the two rat lines. In AMY, CGRP intensity was unchanged. In the parabrachial nucleus, CGRP fluorescence intensity was lower in lateral aspect of parabrachial nucleus, but not in its medial aspect.

In the peripeduncular nucleus and posterior intralaminar thalamic nucleus CGRP fluorescence intensity in cell bodies was not different in the two rat lines. Similarly, terminals in the caudate/putamen region did not show any difference in CGRP intensity between the two lines.

These results suggested that sP rats (which display an "anxious" phenotype) exhibit lower CGRP expression in a system which is involved in the modulation of anxiety-related behaviors. In particular, CGRP expression was lower in the neuronal cell bodies of lateral parabrachial nucleus as well as in BNST-MA, its likely projection terminals. No intensity differences were present in medial parabrachial nucleus as well as in BNST-V or any AMY subnucleus. No differences were observed in a second CGRP system, which is not related to anxiety behaviors. It can thus be hypothesized that the lower CGRP expression in the parabrachial-BNST(-MA) system may be at the base of the differential anxiety-related behavior of sP rats and hence of their alcohol preference, in comparison to sNP rats.

REDUCING EFFECT OF SAIKOSAPONINS A AND D, ACTIVE INGREDIENTS OF BUPLEURUM FALCATUM, ON ALCOHOL AND CHOCOLATE SELF-ADMINISTRATION IN RATS

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Background: Recent work demonstrated that treatment with saikosaponin (SS) A (SSA) – an active ingredient of the medicinal herb, *Bupleurum falcatum* – suppressed i.v. self-administration of morphine and cocaine in rats *via* a $GABA_B$ receptor-mediated mechanism [Yoon et al., Neurosci. Lett. 529:97-101, 2012; Yoon et al., Neurosci. Lett. 555:198-202, 2013].
Study Aims: The present study was designed to address the following 5 research questions (RQs): RQ1 – Do the *anti*-addictive properties of SSA extend to oral alcohol self-administration? RQ2 – Is the suppressing effect of SSA on alcohol self-administration mediated by the $GABA_B$ receptor? RQ3 – Does SSA also inhibit the self-administration of a highly palatable food, such as a chocolate solution? RQ4 – Are the *anti*-addictive properties of SSA shared by other SSs contained in *Bupleurum falcatum* extracts? RQ5 – Do extracts of *Bupleurum falcatum* reproduce the pharmacological effects of SSA?
Results: RQ1 – Acute treatment with SSA (0.1-1 mg/kg i.p.) markedly reduced lever-responding for alcohol (15% w/v in water) and amount of self-administered alcohol in selectively bred Sardinian alcohol-preferring (sP) rats exposed to the fixed ratio (FR) 4 (FR4) schedule of reinforcement. Acute treatment with SSA (0.1-1 mg/kg i.p.) also reduced breakpoint for alcohol in sP rats exposed to the progressive ratio (PR) schedule of reinforcement. RQ2 – Pretreatment with a *per se* ineffective dose of the $GABA_B$ receptor antagonist, SCH50911 (2 mg/kg i.p.), partially blocked the reducing effect of 0.5 mg/kg SSA (i.p.) on lever-responding for alcohol in sP rats. Combination of *per se* ineffective doses of the $GABA_B$ receptor positive allosteric modulator, GS39783 (5 mg/kg i.p.) and SSA (0.1 mg/kg i.p.) synergistically reduced lever-responding for alcohol. RQ3 – Acute treatment with SSA (0.25-5 mg/kg i.p.) suppressed lever-responding for a chocolate solution (5% w/v Nesquik in water) and amount of self-administered chocolate solution in Wistar rats exposed to the FR10 schedule of reinforcement. Acute treatment with SSA (0.25-5 mg/kg i.p.) also suppressed breakpoint for the chocolate solution in Wistar rats exposed to the PR schedule of reinforcement. RQ4 – Acute treatment with SSD (an epimer of SSA) reduced lever-responding for alcohol in sP rats and lever-responding for the chocolate solution in Wistar rats. SSD displayed potency and efficacy comparable to those of SSA. RQ5 – Acute treatment with an extract of *Bupleurum falcatum*, containing SSA, SSB_2, SSB_3, SSB_4, SSC, and SSD and administered at 0.75-3 mg/kg i.p., effectively reduced lever-responding for alcohol in sP rats and lever-responding for the chocolate solution in Wistar rats.
Conclusions: Together, these results (a) extend to alcohol (RQ1) and a highly palatable food (RQ3) the *anti*-addictive properties of SSA, (b) suggest that the $GABA_B$ receptor system is part of the neural substrate underlying the *anti*-alcohol effects of SSA (RQ2), and (c) suggest that *Bupleurum falcatum* is a source of several compounds with *anti*-alcohol potential (RQ4 and RQ5).

FURTHER INVESTIGATION OF THE ANTI-ADDICTIVE PROFILE OF COR659

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Previous work from this lab found that COR659 (methyl-2-[(4-chlorophenyl)carboxamido]-4-ethyl-5-methylthiophene-3-carboxylate) reduced operant alcohol, sucrose, and chocolate self-administration in rats. COR659 apparently exerts its effects *via* a composite mechanism, including positive allosteric modulation of the $GABA_B$ receptor and an action at the cannabinoid CB_1 receptor. The present study was designed to further characterize the suppressing properties of COR659 on alcohol- and chocolate-motivated behaviors in rats. To this end, the present study investigated whether the reducing effect of COR659 on alcohol and chocolate self-administration was maintained after repeated treatment and if COR659 also affected cue-induced reinstatement of alcohol and chocolate seeking (models of human relapse into heavy drinking and disordered eating, respectively). Alcohol self-administration experiments employed selectively bred Sardinian alcohol-preferring (sP) rats trained to lever-respond for alcohol (15% v/v in water) under the fixed ratio (FR) 4 (FR4) schedule of reinforcement. Chocolate self-administration experiments employed Wistar rats trained to lever-respond for a chocolate solution (5% w/v Nesquik in water) under the FR10 schedule of reinforcement. In the reinstatement experiments, previously extinguished lever-responding for alcohol (or chocolate) was reinstated by the non-contingent presentation of complexes of cues previously associated to alcohol (or chocolate) and predictive of alcohol (or chocolate) availability. In each experiment, COR659 was administered at the doses of 0, 2.5, 5, and 10 mg/kg (i.p.). In the “repeated treatment” experiments, administration of COR659 for 10 consecutive days produced a dose-related reduction of both alcohol and chocolate self-administration, with limited loss of efficacy on continuing treatment. In the “reinstatement” experiments, acute treatment with COR659 suppressed reinstatement of both alcohol and chocolate seeking. The above effects occurred at doses of COR659 much lower than those producing hypolocomotion, suggesting the existence of a large separation between the doses of COR659 decreasing alcohol- and chocolate-motivated behaviors and those inducing sedation. Together, these results provide additional lines of experimental evidence on the *anti*-addictive profile of the novel chemical entity, COR659.

THE POSITIVE ALLOSTERIC MODULATOR OF THE $GABA_B$ RECEPTOR, CMPPE, SUPPRESSES ALCOHOL SELF-ADMINISTRATION AND REINSTATEMENT OF ALCOHOL SEEKING IN ALCOHOL-PREFERRING RATS

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Positive allosteric modulators (PAMs) of the $GABA_B$ receptor constitute a class of pharmacological agents gaining increasing interest in the alcohol research field because of their ability to suppress multiple alcohol-motivated behaviors in rats. CMPPE is a novel $GABA_B$ PAM, still limitedly characterized *in vivo*. It was therefore of interest to test its ability to affect operant, oral self-administration of alcohol (experimental procedure that measures the reinforcing and motivational properties of alcohol) and cue-induced reinstatement of alcohol seeking (experimental model of human relapse into heavy drinking) in rats. To this end, selectively bred female Sardinian alcohol-preferring (sP) rats were trained to lever-respond for alcohol (15% v/v in water) under the fixed ratio (FR) 5 (FR5) schedule of reinforcement. Once lever-responding had stabilized, rats were exposed to test sessions under the FR5 (Experiment 1) and progressive ratio (PR; Experiment 2) schedules of reinforcement, preceded by acute treatment with CMPPE (0, 2.5, 5, and 10 mg/kg; i.p.). In Experiment 3, once lever-responding had stabilized, rats underwent an extinction responding phase and then a single reinstatement session during which lever-responding was resumed by the non-contingent presentation of a complex of cues previously associated to alcohol availability; CMPPE (0, 2.5, 5, and 10 mg/kg; i.p.) was administered acutely before the reinstatement session. Selectivity of CMPPE action on alcohol-related behaviors was assessed by evaluating the effect of acute treatment with CMPPE (0, 2.5, 5, and 10 mg/kg; i.p.) on self-administration of a highly palatable chocolate solution (5% w/v Nesquik in water) in male Wistar rats exposed to the FR10 schedule of reinforcement (Experiment 4). In Experiments 1 and 2, treatment with 5 and 10 mg/kg CMPPE reduced lever-responding for alcohol, amount of self-administered alcohol, and breakpoint for alcohol in a dose-related manner. In Experiment 3, treatment with 5 and 10 mg/kg CMPPE completely suppressed reinstatement of alcohol seeking. In Experiment 4, no dose of CMPPE affected – even minimally – lever-responding for the chocolate solution and amount of self-administered chocolate solution. These results (i) extend to CMPPE the ability of all previously tested $GABA_B$ PAMs to selectively inhibit several alcohol-motivated behaviors in rodents and (ii) confirm that these effects are a shared feature of the entire class of $GABA_B$ PAMs. This conclusion is of relevance in view of the forthcoming transition of $GABA_B$ PAMs to clinical testing.

EXPLORING THE ROLE OF ASTROCYTES IN REGULATING VENTRAL TEGMENTAL AREA DOPAMINE NEURONS AND IN A MODEL OF BINGE ALCOHOL DRINKING

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Dopamine (DA) neurons in the ventral tegmental area (VTA) are essential for reward-driven behaviours, including drug seeking. Emerging evidence suggests that astrocytes can play an important and underestimated role in the development and maintenance of drug abuse by actively influencing, and be influenced by, many aspects of neuronal functions. Astrocytes respond to different neurotransmitters and/or drugs with intracellular Ca^{2+} elevations and the release of gliotransmitters that ultimately modulate neuronal activity and impact behaviour. Whether a similar cross-talk between astrocytes and neurons is present in the VTA, and whether this specific interaction contributes to the development of addictive behaviours, remain elusive.

Here we took advantage of a combination of tools, including inositol 1,4,5-trisphosphate receptor type 2 (IP3R2) knockout mice (in which astrocytic Ca^{2+} surges are absent), *in vivo* single-unit electrophysiological recordings, and a model of binge–alcohol drinking to address the following questions. Are astrocytes involved in the regulation of VTA DA neuron activity? Do astrocytes play a role in binge-like alcohol drinking?

While preliminary, our results indicate that removal of IP3R2, the predominant source of physiological Ca^{2+} elevations in astrocytes, does not significantly affect basal electrophysiological properties of VTA DA neurons and binge-like alcohol drinking in mice. We are currently investigating whether a more sophisticated approach, namely selective impairment of gliotransmission within the VTA, would allow to uncover astrocyte role in the modulation of mesolimbic DA circuitry and alcohol drinking.

METHOXETAMINE INDUCES NEUROLOGICAL, SENSORIMOTOR AND CARDIORESPIRATORY ALTERATIONS IN MICE AND PERSISTENT BEHAVIORAL ABNORMALITIES AND NEUROTOXICITY IN RATS

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Novel psychoactive substances (NPS) are intoxicating substances developed to mimic the effects of well-established drugs of abuse. They are not controlled by the United Nations drug convention and are posing serious health concerns worldwide. Among them, the dissociative drug methoxetamine (MXE) is structurally similar to ketamine (KET) and phencyclidine (PCP) and was created to purposely mimic the psychotropic effects of its “parent” compounds. We previously showed that MXE induces KET-like discriminative and rewarding effects, stimulates the mesolimbic dopaminergic transmission and alters emotional states and behavior in rats. In light of the increasing use of MXE and the renewed interest in KET and PCP analogs, we decided to deepen the investigation of MXE-induced effects by means of a battery of behavioral tests widely used in studies of "safety-pharmacology" for the preclinical characterization of new molecules.

In a first study, the acute effects of MXE on neurological and sensorimotor functions in mice, including visual, acoustic and tactile responses, thermal and mechanical pain, motor activity and acoustic startle reactivity, were evaluated in comparisons with KET and PCP to better appreciate its specificity of action. Cardiorespiratory parameters and blood pressure were also monitored in awake and freely moving animals. Results showed that acute intraperitoneal (i.p.) administration of MXE (0.01-30 mg/kg) induces *in vivo* effects qualitatively similar to PCP (1 and 10 mg/kg i.p.) and KET (1 and 30 mg/kg i.p.) on neurological and sensorimotor responses and cardiorespiratory functions in mice. Yet, quantitative differences were noted, with PCP typically producing more robust effects than MXE and KET, and MXE producing more long-lasting effects that the other two drugs.

In a separate study, to investigate its persistent effects on emotional states and behavior and its neurotoxic effects *in vivo,* MXE (0.1-0.5 mg/kg, i.p., × 5) was repeatedly administered to rats every other day, and 7 days later animals were challenged with MXE (0.1-0.5 mg/kg, i.p.). Emission of ultrasonic vocalizations and locomotor activity were measured after each administration. Thereafter, persistent behavioral effects were evaluated starting 8 days after the drug challenge through a battery of test including the elevated plus maze, spontaneous alternation task, novel object recognition and marble burying tests. Finally, neurotoxic effects of MXE were evaluated after completion of behavioral analysis, by measuring dopamine transporter, tyrosine hydroxylase, and serotonin transporter in various brain regions. Data showed that repeated treatment and challenge with MXE modified neither calling nor locomotor activity of rats. Conversely, rats previously treated with MXE exhibited behavioral alterations in the elevated plus maze, marble burying and novel object recognition tests, suggestive of increased anxiety and impaired non-spatial memory. Noteworthy, the same rats displayed dopaminergic damage in the medial prefrontal cortex, nucleus accumbens, caudate-putamen, substantia nigra pars compacta, and ventral tegmental area, along with accumbal serotonergic damage.

While the study in mice provided the first direct comparison of the *in vivo* effects of MXE with the two parental compounds, PCP and KET, findings from the second study in rats provided the first demonstration that MXE induces long-lasting behavioral abnormalities and neurotoxicity in rats. Altogether, our findings clearly indicate the need for more research in the field of dissociative drugs and for more information about the consequences of their use to make social and health professionals aware of their acute intoxicating effects.

ANALGESIC EFFECTS OF A MIXTURE OF ZINGIBER OFFICINALE AND ACMELLA OLERACEA EXTRACTS IN RATS

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Data from ethnopharmacological surveys and a few preliminary rodent studies suggest that preparations from the medicinal plants, *Zingiber officinale* and *Acmella oleracea*, may exert analgesic and anti-inflammatory effects. The present study was designed to assess the analgesic properties of a product made of the combination of *Zingiber officinale* and *Acmella oleracea* extracts in rats. This product – named Mitidol and composed by 11.4% and 2.4% *Zingiber officinale* and *Acmella oleracea* extracts, respectively – was tested in three different models of acute (Tail Flick test) and chronic, inflammatory (Dynamic Plantar Aesthesiometer test; Randall-Selitto Analgesy-Meter test) pain in male, adult Wistar rats. The Tail Flick test measures the rat response to a heat stimulus on the tail; the Dynamic Plantar Aesthesiometer test measures the rat response in terms of touch sensitivity on the plantar surface; the Randall-Selitto Analgesy-Meter test measures the rat response to a mechanical pressure on the paw. Mitidol was provided by Indena S.p.A. (Milan, Italy), a drug company with which this lab has collaborated for 25 years in the search for new, natural products for treatment of different ailments. Preliminary experiments indicated that combination of *Zingiber officinale* and *Acmella oleracea* extracts was more effective than each single extract given alone, suggesting that – when combined – *Zingiber officinale* and *Acmella oleracea* extracts may exert synergistic, analgesic properties. In the present study, acute pain was assessed in healthy rats; chronic, inflammatory pain was assessed in rats the left hind paw of which was made sore, one week before, by the intraplantar injection of 50 µl complete Freund's adjuvant (CFA). In each experiment, Mitidol was administered acutely and intragastrically at the doses of 0, 125, 250, 500, and 1000 mg/kg; the rat response to the noxious stimulus was recorded at baseline and 20, 60, 120, and 180 min after Mitidol administration. The nonsteroidal anti-inflammatory drug, diclofenac, was tested at the 5-mg/kg dose as reference compound. In healthy rats exposed to the Tail Flick test, treatment with Mitidol resulted in a dose-related increase – up to 100% at the 1000-mg/kg dose and in comparison to vehicle-treated rats – in response threshold to the noxious stimulus; Mitidol effect was observed at both 20- and 60-min recording times. In CFA-treated rats exposed to the Dynamic Plantar Aesthesiometer test, treatment with 500 and 1000 mg/kg Mitidol resulted in marked increases in response threshold at both 20- and 60-min recording times. The Randall-Selitto Analgesy-Meter test was even more sensitive in revealing the analgesic properties of Mitidol: treatment with 250, 500, and 1000 mg/kg Mitidol produced substantial increases – up to 10-fold at the 1000-mg/kg dose and in comparison to vehicle-treated rats – in response threshold; Mitidol effect was still evident at the 180-min recording time. In each experiment, Mitidol effect was of comparable magnitude to that produced by diclofenac. Together, the results of the present study indicate that a mixture of *Zingiber officinale* and *Acmella oleracea* extracts exerted remarkable analgesic effects in validated rat models of acute and chronic pain. Additional studies are now needed to possibly identify the active ingredients of the two extracts as well as how they synergistically interact when producing their analgesic effects. Notably, a preliminary clinical study headed by Indena S.p.A. has recently and successfully translated to humans the results of the present rat study.

SEROTONIN 5-HT$_{2A/C}$ AND ARGININE-VASOPRESSIN V$_{1A}$ SUBTYPE RECEPTORS MODULATE REWARDING, PROSOCIAL AND ANXIOLYTIC EFFECTS INDUCED BY TWO SYNTHETIC PHENETHYLAMINES IN ZEBRAFISH

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DOB and PMA are two phenethylamines sold openly through websites and associated with psychostimulant activity and toxicity. Both drugs and the classical phenethylamine 3,4-methylenedioxymethamphetamine (MDMA) have been found to interact with serotonin receptors in rodents. There is growing evidence that the neuropeptide oxytocin (OT) can modulate drug-related reward and may act as a pharmacological treatment of drug dependence. Aquatic models such as zebrafish have been recognized as useful models to test the toxicity of addictive drugs and to evaluate their potential clinical applications. The aim of our work was to investigate the role of serotonin 5-HT$_{2A/C}$ like- and of arginine-vasopressin V$_{1a}$ receptors on reward, social preference and anxiety-like behaviour induced by DOB and PMA, compared to MDMA, using ritanserin and SR49059, two antagonists of serotonin 5HT$_{2A/C}$ and the arginine-vasopressin V$_{1a}$ subtype receptor, respectively. MDMA and its derivatives dose-dependently induced rewarding, anxiolytic effect and an increase in social preference following a biphasic trend, being PMA the most potent. Both ritanserin and SR49059 significantly blocked all the effects, suggesting the involvement of serotonin 5-HT$_{2A/C}$ and arginine-vasopressin- like receptors. The current study demonstrated a rewarding, prosocial and anxiolytic effect of DOB and PMA in zebrafish and focused on the mechanisms of their action suggesting a potential clinical application in drug dependence.

The work was supported by Zardi-Gori Foundation

CHRONIC ADMINISTRATION OF THE SYNTHETIC CANNABINOID WIN 55,212-2 INDUCES CROSS-SENSITIZATION TO COCAINE BEHAVIOURAL EFFECTS IN ADOLESCENT RATS

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Cannabis is the most commonly used illicit drug, especially among adolescents. In recent decades, the effects of Cannabis use on mental health have received increasing attention also in close relationship with the implications for public health.

The "Gateway Hypothesis" postulates that early exposure to cannabinoids in adolescence could cause neurobiological changes that affect the cerebral maturation leading to a major risk of vulnerability to abuse other drugs during adulthood.

We investigated the potential gateway effect of the synthetic cannabinoid receptor agonist WIN55,212-2 (WIN) evaluating the drug's cross-sensitizing behavioural in both adolescence and adulthood. Adolescents (PND 40) and adults (PND 70) male Sprague Dawley rats underwent intraperitoneal (i.p.) administration sessions of increasing doses of WIN (2, 4 e 8 $mg \cdot kg^{-1}$), or vehicle, twice a day for 11 consecutive days. After a washout period of 7 days, rats were treated i.p. with cocaine (10 $mg \cdot kg^{-1}$) for 4 consecutive days, and the locomotor activity test was performed on day 1 and day 4. On day 1 adolescent WIN-pretreated rats showed a significant increase to the motor-activating effects of cocaine compared to vehicle-pretreated rats, whereas on day 4 they displayed a convergence of sensitization values reducing the gap. In adulthood on day 1 no difference in behavioural response to cocaine were found between groups, while on day 4 WIN pretreated rats showed a reduction of locomotor sensitization to the drug.

In conclusion these findings may reflect an alteration of susceptibility to substance abuse emphasizing the importance of adolescence in drug exposure.

MICROGLIA VERSUS MACROPHAGE EFFECTS ON OLIGODENDROCYTE PRECURSOR CELLS: ROLE OF EXTRACELLULAR VESICLES

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Neuroinflammation plays a central role in multiple sclerosis (MS) by impairing remyelination and causing neuronal injury. Brain resident microglia (MG) and infiltrating macrophages (MP) are among the main effector cells of the inflammatory response associated to MS. They contribute to MS onset and outcome, including secondary progressive phases, but also to the restorative phase of the disease. However, it is still unclear whether all inflammatory myeloid cells are detrimental in the disease and the possibility exists that the inflammatory activity of brain resident MG and peripheral MP may be distinct in MS, as reported in other brain diseases.

In line with this hypothesis, unexpected data of the laboratory show that inflammatory MG, through the secretion of extracellular vesicles (EVs), do not inhibit differentiation of cultured oligodendrocytes towards mature myelinating cells, nor prevent myelin deposition in oligodendrocytes-DGR neuron co-cultures. Given the production of EVs from myeloid cells contribute to EAE, these data suggest that MP, rather than MG, might be responsible for the block of oligodendrocyte differentiation and remyelination failure in progressive MS.

To assess this hypothesis, I explored the action of EVs shed from pro-inflammatory MP or pro-regenerative MP on the migration and the differentiation of oligodendrocyte precursor cells (OPC), the glial cell type able to generate myelinating oligodendrocytes, in respect to the action of MG-derived EVs.

OPC migration was assessed by a classical transwell-based chemokinesis assay. Quantification of migrating OPCs revealed that MP-derived EVs never attract OPCs, independent of the phenotype of donor MP. Specifically, EVs released by pro-inflammatory MP tend to limit OPC migration, albeit the difference doesn't reach a significant difference. Sphingosine phosphate (S1P), a known chemoattractant agent, was used as positive control. In fact, S1P promote OPC migration, while the OPC treatment with MP EVs and the S1P receptor antagonist (s-ene) do not influence OPC migration.

Immunocytochemistry analysis of the marker of mature oligodendrocytes (MBP) revealed that MP-EVs are not able to promote OPC differentiation as compared to MG-derived EVs. In particular, EVs released by pro-inflammatory MP significantly inhibit rather than promote in vitro OPC maturation into myelinating cells.

Collectively these results indicate that infiltrating macrophages, rather than resident microglia, block the pro-regenerative activity of OPCs in demyelinating disease.

NEW ANTI-GLIOBLASTOMA AGENTS BY HYBRIDIZING THE ONIUM-ALKYLOXY-STILBENE-BASED STRUCTURES OF A α7/α9-nAChR ANTAGONIST AND OF A PRO-OXIDANT MITOCAN

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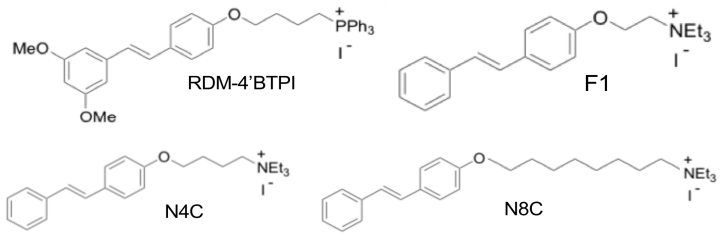
Neural nicotinic acetylcholine receptors (nAChRs) are a family of ligand-gated ion channels expressed not only by neuronal cells, but also by non-neuronal normal and cancer cells. Subtypes containing the α7 and/or the α9 subunit play a role in tumour angiogenesis, proliferation, survival, migration, invasion and metastasis formation. Gliomas are the most frequent primary malignant brain tumours in adults, and one of the most aggressive human cancers. nAChRs are expressed by astrocytes and those that contain α7, α9 and α10 subunits are detected in human glioblastoma (GBM) U87MG cells, where their activation by nicotine increases cell proliferation, which is blocked by subtype-specific nicotinic antagonists.

The triethylammoniumethyl ether of 4-stilbenol F1, a known antagonist of α7- and α9/α10-nAChRs, has an antiproliferative activity on U87MG cells. The structural analogy of F1 with the mitocan RDM-4'BTPI, a triphenylphosphoniumbutyl ether of pterostilbene which is more potent than F1 on reducing cell viability of U87MG cells, although it does not own nicotinic activity, suggested us that a molecular hybridisation might result in novel antitumor agents with higher potency and selectivity on the nAChRs that promote GBM cell growth. We found that the replacement of ethylene with butylene in the triethylammonium derivatives (see N4C compound) resulted in more potent and selective toxicity towards U87MG cells, which was paralleled by an increased α7- and α9/α10-nAChR antagonism and an improved ability in reducing mitochondrial ATP production. A further elongation of the alkylene linker (see N8C compound) enhanced the selectivity for α7- and α9/α10-nAChRs, as well as the ability to reduced GBM cell viability after a 72-hour treatment. Moreover, when we treated U87MG cells with the triethylammonium derivatives described above, we observed a decrease in basal cell proliferation, which was only partially antagonised by co-incubation with the α7/α9-antagonists aBungarotoxin (aBgtx) and methyllycaconitine (MLA). Therefore, in addition to the α7/α9-nAChR antagonism, we believe that F1, N4C and N8C may also have other non nAChR-mediated effects.

Our aim is to understand the whole mechanism of action of our newly-synthetised drugs, as well as F1, and to test them in primary GBM cell lines derived from patients and in xenografted NOD/SCID mice in order to determine whether nAChRs are new possible targets for glioma and GBM therapy.



THE HUMAN-RESTRICTED DUPLICATED FORM OF THE α7 NICOTINIC RECEPTOR, CHRFAM7A: EXPRESSION AND TRANSCRIPTIONAL REGULATION IN INFLAMMATORY CELLS

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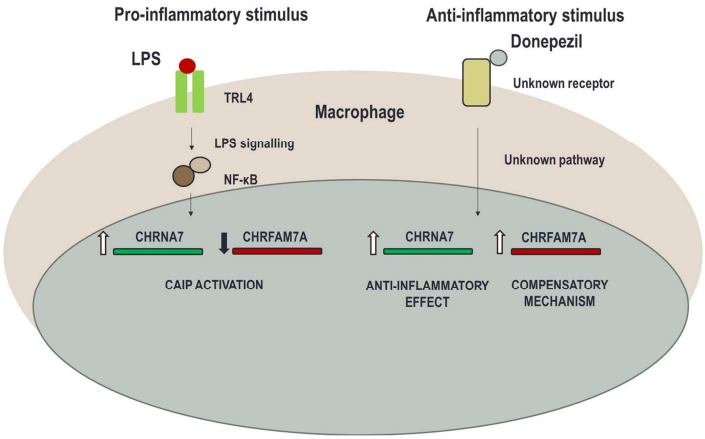

Introduction: The α7 nicotinic acetylcholine receptor (CHRNA7) plays a role in the modulation of the inflammatory response through the activation of the "cholinergic anti-inflammatory pathway". In humans, a recombination event involving the exon 5 to 10 of *CHRNA7* gene, fused to four novel exons A, B, C and D (*FAM7A*), gave rise to the *CHRFAM7A* gene. This hybrid gene, located on chromosome 15q13-q14, 1.6 Mb apart from *CHRNA7*, is highly expressed in inflammatory cells, where it can regulate the anti-inflammatory effects of α7 activation. Acute treatment of macrophages with LPS down-regulates *CHRFAM7A* by a mechanism driven by NF-κB, paralleled by *CHRNA7* up-regulation. As studies are emerging, which identify *CHRFAM7A* expression alteration in inflammatory or infective pathologies, the regulation of its expression may become a key step in the modulation of inflammation. However, the region driving the transcriptional regulation of *CHRFAM7A* gene in human immune tissues is largely unknown.
Materials and methods: human monocytic-derived macrophages and THP-1 cell line have been used to characterized the *CHRFAM7A* regulatory region.
Results and conclusions: we provide a detailed analysis of the *CHRFAM7A* gene regulatory region and its pro-inflammatory stimuli responsiveness. Furthermore, given the anti-inflammatory potential of the acetylcholinesterase inhibitor donepezil, we investigated the *CHRFAM7A* expression profile in macrophages treated with donepezil, showing an unexpected up-regulation of both *CHRFAM7A* and *CHRNA7* gene, thus highlighting a possible role for *CHRFAM7A* gene product in the control and modulation of the cholinergic anti-inflammatory pathway, and/or in the modulation of CHRNA7 function.

PREVALENCE OF ATRIAL FIBRILLATION IN THE ITALIAN ELDERLY POPULATION. FINAL RESULTS FROM THE PROGETTO FAI

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Objective. To evaluate the prevalence of atrial fibrillation (AF), a major risk factor for stroke and thromboembolism, in a representative sample of the Italian elderly population.
Methods. This survey is part of the National Research Program: Progetto FAI. La Fibrillazione Atriale in Italia, coordinated by the Regione Toscana, and funded by the Italian Ministry of Health-CCM. The Program included a cross-sectional examination, which started in 2016, of all subjects aged 65 years and over, from three general practices in Lombardia, Toscana and Calabria, and a follow-up survey of AF patients 6 months after the baseline examination. All participants were administered an *ad hoc* developed screening procedure, followed by a diagnostic evaluation with ECG confirmation. All ECGs were centrally evaluated by expert cardiologists.
Results. The study sample included 6,016 subjects. After exclusion of 235 non-eligible subjects, in the remaining 5,781 the overall participation rate was 78.3%, which left 4,528 participants (mean age 74.5±6.8 years, 47.2% men). A total of 331 AF prevalent cases were identified, including 183 men (55.3%) and 148 women (44.7%). AF patients were significantly older (mean age 78.5±6.9 vs.74.2±6.6 years), and more likely to have a lower education level (P=0.001) as compared with individuals without AF. A number of vascular risk factors and comorbid conditions were also significantly more frequent in AF patients: hypertension (P<0.001), myocardial infarction (P<0.001), heart failure (P<0.001), diabetes (P=0.009), renal disease (P<0.001), and previous stroke (P<0.001). The final prevalence of AF was 7.3% (95% CI, 6.6-8.1), 8.6% (95% CI, 7.5-9.8) in men and 6.2% (95% CI, 5.3-7.2) in women, and increased with advancing age. Rates ranged from 3.0% in patients aged 65-69 years to 16.1% in those aged 85 and over. In the same age groups, figures ranged from 3.4% to 19.0% in men, and from 2.5% to 13.8% in women. When rates were standardized to the 2016 Italian population, the final prevalence was 8.1% (95% CI, 5.9-11.1). Using data from the prevalence survey, the number of Italian elderly having AF was estimated at ~1.1 million (51.5% women).
Discussion and Conclusions. Subjects with AF have a 5-fold increased risk of stroke, and AF-associated stroke currently accounts for over one third of ischemic strokes managed by stroke units in Europe. Our results indicate a prevalence of AF higher than previously estimated. Considering the demographic transition, reliable epidemiological data on AF burden are essential both for clinicians and policy-makers.

SECRETED PHOSPHOLIPASES A2 GO INSIDE CELLS: POSSIBLE ROLE IN REGULATION OF EICOSANOID BIOSYNTHESIS

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Eicosanoids are signalling molecules made by the enzymatic or non-enzymatic oxidation of arachidonic acid or other polyunsaturated fatty acids (PUFAs), involved in numerous physiological and pathological processes. The majority of eicosanoid metabolism requires arachidonic acid that is primarily stored in an esterified form in membrane phospholipids. Phospholipase A2 (PLA2) enzymes are crucial for increasing the levels of free arachidonic acid for metabolism and eicosanoid biosynthesis under most physiological conditions, and in inflammatory cell activation. As free fatty acids and derivatives can rapidly diffuse out of the cell, or become reincorporated into membrane lipids by re-acylation, enzymes involved in their transformation are often physically associated.

In studying PLA2 toxins of snake venom and a recombinant form of human secreted PLA2, PLA2G2A, we noticed that these proteins are internalized in various types of cells and that, depending on the toxin and on the cell state, they are localized at mitochondria or para-nuclear/nuclear zone. Using special probes, we observed an increase in reactive oxygen species (ROS), in the subcellular areas where the secreted PLA2s are transported and ROS formation may be related to oxidation reactions of PUFAs released by PLA2. The secreted PLA2s object of our study are not specific for arachidonic acid but, various observations suggest that they stimulate the activity of cytosolic PLA2s specific for this PUFA. According to results of our studies and of other groups, secret PLA2s tend to form, in contact with the lipid membranes, amyloid-type aggregates, which, hypothetically, serve to control the lipid transformation activity of themselves and of other enzymes. Our goal is to help clarify whether and how secret phospholipases influence the activity of intracellular PLA2 and other enzymes involved in the biosynthesis of eicosanoids.

THE TUSCANY STROKE NETWORK:TEAM IS BRAIN

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Aims. In January 2015 a major system change for acute ischemic stroke (AIS) care was implemented across the entire Tuscany region: the Tuscany Stroke Network (TSN). AIS patients are first taken to the nearest spoke hospital for possible t-PA treatment, assessed for eligibility to endovascular intervention, and quickly transferred to the hub hospital, where appropriate. We investigated differences in quantity of treatments for acute ischemic stroke (AIS) patients before and after the TSN implementation, to explore and monitor its effectiveness.
Materials and methods. We included all patients with AIS consecutively treated in each of the 22 TSN stroke hospitals from January 1, 2014 to December 31, 2017. We estimated an expected number of 9000 AIS patients per year. We measured and monitored short-term TSN efficacy by estimating and comparing annual numbers and rates of AIS treatments, as well as health benefits in terms of Disability Adjusted Life Years (DALYs) avoided, based on 0,605 DALYs avoided for each treated patient, before (2014) and after (2015-2017) TSN implementation.
Results. The network spans across 23000 Km^2 with 3,8 million inhabitants, 26 hospitals with no stroke service, 3 hub hospitals and 19 spoke hospitals. Through 2014, 382 AIS patients were treated, mainly with t-PA. Number and rates of treatments increased up to 669 (7,4%) in 2015 and to 1078 (12%) in 2017, being 382 (4.2%) in 2014. The implementation of the TSN resulted in 1014 additional patients treated with t-PA from 2015 to 2017, yielding to an health benefit of 613,5 DALYs avoided. An increasing number of both secondary and Drip&Ship transfers have been activated, yielding to an increasing number of rescue and thrombectomies performed eventually by the hub hospitals throughout the observation period.
Conclusions and implications. The logistic interventions provided by the TSN resulted in more stroke patients receiving the benefits of t-PA and thrombectomy, that are highly cost-effective. Increasing the ratio between treated and eligible AIS patients could thus provide savings both in economic and in DALYs areas.

**TSN Working Group: Baldereschi M, Balzi D, Baruffi MC, Bellomo F, Bertini A, Bollani E, Bonuccelli U, Bracco S, Carneglia L, Caruso A, Centorrino S, Cesari V, Chiti A, Chiti I Colombai R, Conti A, Cosottini M, Cozza S, De Vito L, Del Dotto P, Di Fabrizio V, Donigaglia G, Fainardi E, Ferrini L, Fortini A, Frosini F, Galli R, Gambaccini G, Iannelli G, Inzitari D, Landini G, Laureano R, Lencioni MG, Linoli G, Luchini G, Mancuso M, Mandò M, Mangiafico S, Marconi R, Marrone A, Martelli F, Martini G, Masotti L, Mazzoni M, Menichetti C,Meucci G,Nencini P, Niccolini A, Nocentini S, Orlandi G, Orsitto E, Palumbo P, Panigada G, Pennati P, Pepe G, Pratesi M, Prosetti D, Ruggiano G, Scazzeri F, Spisni L, Spolveri S, Tassi R, Testa A, Tognarelli, Torri T, Vannini R, Vignali C, Volpi G.*

ROLE OF THE PRO-INFLAMMATORY CYTOKINE INTERLEUKIN 6 IN THE CONTROL OF THE GABA SWITCH IN HIPPOCAMPAL NEURONS

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The transition of GABA signalling from excitatory to inhibitory represents a critical process of brain development. The process is determined by a change in intracellular chloride concentration, through the activity of two chloride co-transporters, NKCC1 and KCC2. An altered GABA switch is associated with neurodevelopmental disorders, including autism, which are frequently related to inflammatory states. Hence, we investigated whether immune molecules produced during inflammation can impinge the GABA switch. Using a combination of calcium and chloride imaging techniques, we found that IL-6, a major pro-inflammatory cytokine, is able to accelerate the GABA switch in neuronal cultures by potentiating the GABAergic transmission. This effect involved STAT-3 activation, a transcription factor activated by IL-6 signalling, as the STAT3 inhibitor Stattic prevented the IL-6–mediated GABA switch potentiation. Moreover, qRT-PCR analysis revealed that KCC2, but not NKCC1, gene was up-regulated upon IL-6 treatment thus suggesting a possible effect of this cytokine specifically on KCC2 expression, and also on KCC2 protein trafficking and its surface expression. In fact, the same higher KCC2 surface expression was observed in young mice deriving from IL-6-injected pregnant dams. Taken together, these results show that IL6 may regulate the developmental GABAergic switch in early neuronal development by guiding transcriptional and cytoplasmic effects. Further experiments will allow to clarify the role of IL-6 as an important player in such a critical process.

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