# IN CNR Annual Retreat 2019

Area della Ricerca di Pisa
October 2–4, 2019

# Oral communications and posters: schedule and abstracts


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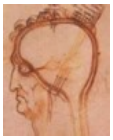

# IN CNR Annual Retreat 2019

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## INVITED LECTURE and ORAL COMMUNICATIONS: SCHEDULE AND ABSTRACTS

| N | When | Speaker | Title |
|---|---|---|---|

### Special lecture

**Chairman: Giorgio Carmignoto**

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|---|---|---|---|
| | Wednesday, October 2 16.30-17.00 | **Pozzan Tullio** DIRECTOR DSB-CNR | *CNR: A retrospect and a look forward* |

### Plenary lecture

**Chairman: Matteo Caleo**

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|---|---|---|---|
| | Wednesday, October 2 18-19 | **Montecucco Cesare** IN-PD | *Degeneration and regeneration in the peripheral nervous system* |

### Training course speakers and topics

| | | | |
|---|---|---|---|
| | Thursday, October 3 14.30-17.30 | **Maffei Lamberto** | *Ricordo di un esperimento di Lucia* |
| | Thursday, October 3 14.30-17.30 | **Wong Rachel** | *Mechanisms underlying synaptic wiring specificity in the retina* |
| | Thursday, October 3 14.30-17.30 | **Rizzo Stanislao** | *Artificial vision: dream or reality* |
| | Thursday, October 3 14.30-17.30 | **Falsini Benedetto** | *Cone dysfunction and degeneration in retinitis pigmentosa: diagnosis, natural history and therapeutic approaches* |

### Oral communications

| Session 1 | ***Breaking news by young IN researchers*** | | **Chairman: Michela Matteoli** |
|---|---|---|---|
| OC S1.1 | Wednesday, October 2 | **Silvia Landi** IN-MI | *Diurnal oscillation of intracellular Chloride: a new modulator of cortical* |

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|---|---|---|---|
| | 14.30-16.30 | | *excitability?* |
| OC S1.2 | Wednesday, October 2 14.30-16.30 | **Luca Murru** IN-MI | *Spotlight on Lateral habenula (LHb) function in tetraspanin7 (TSPAN7) knockout* |
| OC S1.3 | Wednesday, October 2 14.30-16.30 | **Eleonora Vannini** IN-PI | *Bacterial toxins as innovative tools for exploring brain disorders* |
| OC S1.4 | Wednesday, October 2 14.30-16.30 | **Antonella Borreca** IN-MI | *Translation efficiency is upregulated in hAPP mice before and immediately after the onset of cognitive impairments: insights for anticipating AIzheimerDisease diagnosis and treatment* |
| OC S1.5 | Wednesday, October 2 14.30-16.30 | **Diana Pendin** IN-PD | *A Synthetic Fluorescent Mitochondria-Targeted Sensor for Ratiometric Imaging of Calcium in Live Cells* |
| OC S1.6 | Wednesday, October 2 14.30-16.30 | **Fossati Matteo** IN-MI | *Selective control of inhibitory synapse development by the glutamate receptor delta-1 in cortical pyramidal neurons* |

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|---|---|---|---|
| Session 2 | ***Neuroplasticity and neuroimaging*** | | **Chairman**: **Alessandro Sale** (IN-PI) |
| OC S2.1 | Thursday, October 3 9:00-10:30 | **Debora Napoli** IN-PI | *MiR-29 coordinates age-dependent plasticity brakes in the adult visual cortex* |
| OC S2.2 | Thursday, October 3 9:00-10:30 | **Paola Tognini** IN-PI | *Nutrition and gut microbiota impact on cortical plasticity* |
| OC S2.3 | Thursday, October 3 9:00-10:30 | **Anna Letiza Allegra Mascaro** IN-PI | *Mesoscale imaging of neuronal activity coupled with light-evoked motor mapping reveal movement-specific spatiotemporal patterns of cortical activation in awake mice* |
| OC S2.4 | Thursday, October 3 9:00-10:30 | **Alessandro Benedetto** IN-PI | *Voluntary action modulates visually evoked cortical responses in primary visual cortex: an integrated ultra-high field fMRI and EEG* |
| OC S2.5 | Thursday, October 3 9:00-10:30 | **Ferdinando Sartucci** IN-PI | *Improvement of visual acuity in amblyopic patients following unilateral application of cathodal transcranial direct current stimulation (tDCS)* |
| OC S2.6 | Thursday, October 3 9:00-10:30 | **Paola Binda** IN-PI | *Pupillometry provides new insights on figure-ground segregation and its covariation with autistic traits.* |

| Session 3 | ***Brain cellular physiology and pathology*** | | **Chairman: Stefano Morara** (IN-MI) |
|---|---|---|---|
| OC S3.1 | Thursday, October 3 11:00-12:30 | **Maria Elena Castellini** IN-TN | *The interphotoreceptor matrix: investigating the role of IMPG2 in autosomal recessive retinitis pigmentosa* |
| OC S3.2 | W 11:00-12:30 | **Marco Mainardi** IN-PI | *Quantitative mapping of hippocampal synaptic memory engrams* |
| OC S3.3 | Thursday, October 3 11:00-12:30 | **Cristina Spalletti** IN-PI | *Combining Rehabilitation and Neuromodulation after stroke: novel approaches in a mouse model* |
| OC S3.4 | Thursday, October 3 11:00-12:30 | **Claudia Alia** IN-PI | *Novel cell-based strategies to promote brain repair and motor function after stroke in mice* |
| OC S3.5 | Thursday, October 3 11:00-12:30 | **Silvia Penati** IN-MI | *Molecular and cellular mechanisms underlying the relationship between metabolic alterations and cognitive decline* |
| OC S3.6 | Thursday, October 3 11:00-12:30 | **Maria Luisa Malosio** IN-MI | *Intracerebral Injection of Extracellular Vesicles from Mesenchymal Stem Cells Exerts Reduced Aβ Plaque Burden in Early Stages of a Preclinical Model of Alzheimer's Disease* |

| Session 4 | ***Neurodevelopmental and neurodegenerative disorders*** | | **Chairman: Claudia Lodovichi** (IN-PD) |
|---|---|---|---|
| OC S4.1 | Friday, October 4 9:00-10:30 | **Luigi Balasco** IN-TN | *Somatosensory hypo-reactivity to whisker stimulation in the Cntnap2 -/- mouse: a genetic mouse model of autism spectrum disorder* |
| OC S4.2 | Friday, October 4 9:00-10:30 | **Leonardo Lupori** IN-PI | *The visual system as a biomarker in a mouse model of CDKL5 deficiency disorder* |
| OC S4.3 | Friday, October 4 9:00-10:30 | **Vania Broccoli** IN-MI | *Whole brain delivery of an instability-prone Mecp2 transgene rescues behavioral and molecular pathological defects in mouse models of Rett syndrome* |
| OC S4.4 | Friday, October 4 9:00-10:30 | **Simone Bido** IN-MI | *Neurodegeneration in a mouse model with alpha-synuclein accumulation in the microglia* |
| OC S4.5 | F 0-10:30 9:00-10:30 | **Marcello Serra** IN-CA | *D2 receptors on indirect medium spiny neurons modulate L-DOPA-induced dyskinesia* |
| OC S4.6 | Friday, October 4 9:00-10:30 | **Giorgia Pallafacchina** IN-PD | *Characterization of the role of sigma-1 receptor mutation in the etiology of dHMN focusing on cell homeostasis and intracellular CA2+ signaling* |

| Session 5 | ***Neuromodulation and hormonal regulation of brain circuits*** | | **Chairman: Anna Lisa Muntoni** (IN-CA) |
|---|---|---|---|
| OC S5.1 | Friday, October 4 11:00-12:30 | **Valentina Gigliucci** IN-MI | *New light on oxytocin receptors* |
| OC S5.2 | Friday, October 4 11:00-12:30 | **Cristina Cadoni** IN-CA | *Role of genotype in the longlasting effects of nicotine exposure on mesolimbic dopamine transmission: a likely mechanism of nicotine gateway effect* |
| OC S5.3 | Friday, October 4 11:00-12:30 | **Patrizia Porcu** IN-CA | *The brain as a target of hormonal contraceptives: evidences from animal studies* |
| OC S5.4 | Friday, October 4 11:00-12:30 | **Roberto Bizzotto** IN-PD | *Glucose sensitivity, insulin sensitivity and their longitudinal changes are strong independent determinants of type 2 diabetes progression: an IMI DIRECT study* |
| OC S5.5 | Friday, October 4 11:00-12:30 | **Laura Baroncelli** IN-PI | *Creatine transporter deficiency: new insights on cell-specific vulnerability to metabolic failure* |

# DIURNAL OSCILLATION OF INTRACELLULAR CHLORIDE: A NEW MODULATOR OF CORTICAL EXCITABILITY?

S. Landi[1], O. Cozzolino[2], E.Pracucci[2], G.Nardi[2], V. Pillai[2], D.Lamers[2], F.Trovato[2], V. Beatini[2], L. Baroncelli[1], G.M. Ratto[2].

[1]*Istituto Neuroscienze CNR;* [2]*NEST, Istituto Nanoscienze CNR and Scuola Normale Superiore, Pisa.*

The interplay between excitation and inhibition continuously regulates brain output in physiological conditions. GABAergic activity dictates neuronal firing range and timing**,** synaptic plasticity, and the flow of information in neuronal networks. The principal target of GABA are the ionotropic $GABA_A$ receptors that are conductive mainly to Chloride. The present paradigm states that, in physiological conditions, the GABA-activated current has a reversal potential (EGABA) close to the neuronal resting potential, consequently, the effect of GABAergic signaling is inhibitory. However, the inhibitory action of GABA currents are very sensitive to changes in the $Cl^-$ electrochemical gradient, thus supporting the yet untested hypothesis that intracellular $Cl^-$ ($[Cl^-]_i$) could be an important regulator of neuronal activity. Unfortunately, until now, this hypothesis was untestable because of the lack of a sound methodology for the measurement of $[Cl^-]_i$ *in vivo*. Recently, we demonstrated that by means of two-photon spectroscopy, it is possible to measure $[Cl^-]_I$ *in vivo* employing ClopHensor-II, a genetically encoded sensor for $Cl^-$ and pH developed @NEST (Sato et al., 2017). By transducing ClopHensor in layer 2/3 pyramidal neurons (PNs) after *in utero* electroporation, we provided the first demonstration *in vivo* of the switch of GABA signaling during postnatal development, but we also observed a high degree of heterogeneity of $[Cl^-]_i$ in the adult cortex. Surprisingly, the search for factors justifying this heterogeneity, led us to discover that $[Cl^-]_i$ in PNs follows a diurnal cycle: $[Cl^-]_i$ is minimal during the day, at the time of mice resting period, and rises during the night, when mice are mostly active. Superfusion with bumetanide, an inhibitor of cation-chloride cotransporter NKCC1, reduces intracellular $Cl^-$ concentration during the night, but not during the day, thus suggesting that, contrary to the common understanding, NKCC1 plays an important role in $[Cl^-]_i$ regulation also during adulthood. We expect that diurnal oscillation of $[Cl^-]_i$ plays an important role in controlling PNs excitability which is higher during active behavior and lower during sleep, possibly exerting a protective role during slow-wave hyper-synchronisation (Petrucco et al, 2017). We are currently exploring changes in network excitability related to the diurnal oscillation of $[Cl^-]_I$, by recording visual induced oscillations in behaving head-restrained mice and by recording steady-state activity in freely behaving mice.

# SPOTLIGHT ON LATERAL HABENULA (LHb) FUNCTION IN TETRASPANIN7 (TSPAN7) KNOCK-OUT MICE

Luca Murru[1], Luisa Ponzoni[2], Anna Longatti[1], Mariaelvina Sala[1], Maria Passafaro[1]

*[1]CNR Institute of Neuroscience, Milan, Italy; [2]Fondazione Zardi Gori, Milan, Italy*

Mutations in many genes have been linked to increased risk of developing Intellectual disability (ID) and autism spectrum disorders (ASD) so far, including tm4sf2 that encodes for tetraspanin7 (TSPAN7) protein. Indeed, patients displaying mutated tm4sf2 gene has been diagnosed for ID and ASD. We previously demonstrated defects in hippocampal function and related behaviors in tm4sf2 knock-out ($Tm4sf2^{-/y}$) mice (Bassani S. et al., 2012; Murru L. et al., 2017).

Since hippocampal activity has been demonstrated to be coordinated with the lateral habenula (LHb) and LHb has emerged as master regulator of several brain areas involved in ID- and ASD-related behaviors, including the limbic system and monoaminergic nuclei, we decided to study LHb neuronal activity in $Tm4sf2^{-/y}$ mice.

Our electrophysiological data showed a strong reduction in action potential (AP) firing activity, an aberrant AP firing pattern together with altered sodium and potassium voltage-gated channel conductances in LHb of $Tm4sf2^{-/y}$ mice. Furthermore, we analyzed $Tm4sf2^{-/y}$ mice for ASD-like behaviors showing a minor sociability, an increased self-grooming, an altered marble burying behavior, a decreased sucrose preference and an increased depressive-like state.

With our data we demonstrated strong alterations in LHb neuronal activity together with an ASD-like behavior in $Tm4sf2^{-/y}$ mice, suggesting that an aberrant LHb activity could be causative for behavioral phenotypes common in ASD.

# EPILEPTIC ACTIVITY AFFECTS VESICULAR POSITIONING AT CORTICAL SYNAPSES: NEW THERAPEUTIC TARGETS

Eleonora Vannini[1,2,3], Yuri Nishimura[3], Marialaura Dilillo[4], Matteo Caleo[1,5], Liam McDonnell[4], Vincenzo Marra[3], Laura Restani[1]

*[1]CNR Neuroscience Institute Pisa, Italy; [2]Fondazione Umberto Veronesi Milan, Italy; [3]University of Leicester, United Kingdom; [4]Fondazione Pisana per la Scienza ONLUS; [5]University of Padua, Italy*

Networks' hyperexcitability is often caused by an unbalance between excitatory and inhibitory neurotransmission, manifested in patients as a propensity for epileptic seizures. While much is known about the causes of some forms of epilepsy, plastic rearrangements that maintain the epileptic focus and their effects are only partly understood. We focus on functional and structural changes in vesicular array at cortical synapses of mice injected with tetanus neurotoxin (TeNT) in the visual cortex. Previous studies have already showed that TeNT-injected mice show epileptic seizures together with altered visual transmission and impaired dendritic spines (Vannini et al., 2016 BSAF). Using an ultrastructural readout of *in vivo* activity we focused on synapses, investigating the positioning of synaptic vesicles released in response to visual stimulation at two different stages (10 and 45 days) after toxin injection. The nanoscale analyses, performed with a combination of photoconversion and electron microscopy, show that TeNT-injected mice: i) have an unchanged proportion of activated synaptic vesicles at excitatory synapses, but the positioning of such vesicles is no longer biased towards the active zone as happens in control animals; ii) at inhibitory synapses display a longer active zone. These changes might be due to homeostatic plastic rearrangements of the presynaptic terminals, consequent to hyperexcitability development. Proteomic analysis of synaptosomes revealed an up-regulation of specific presynaptic proteins (i.e. Carboxypeptidase E, Synaptotagmin V, Dickkopf 3 Protein 3 and Secretogranin I) involved in synaptic vesicles' availability and positioning in TeNT-injected mice. Remarkably, we found that the selective inhibition of Carboxypeptidase E (CPE) provokes a decrease in both ictal events and general hyperexcitability of TeNT-injected mice, highlighting the relevant importance of CPE as a potential target for anticonvulsivant therapies. Altogether, these data suggest that the presynaptic remodelling could represent the base of hyperexcitability maintenance.

# TRANSLATION EFFICIENCY IS UPREGULATED IN hAPP MICE BEFORE AND IMMEDIATELY AFTER THE ONSET OF COGNITIVE IMPAIRMENTS: INSIGHTS FOR ANTICIPATING ALZHEIMER DISEASE DIAGNOSIS AND TREATMENT

Antonella Borreca[1§], Francesco Valeri[2], Mariassunta De Luca[2], Lysianne Ernst[3], Arianna Russo[4], Alberto Cordella[2,5], Veronica Corsetti[6], Annalisa Nobili[2], Giusy Amadoro[7], Nicola Biagio Mercuri[2,8], Marcello D'Amelio[2,5], and Martine Ammassari-Teule [1,2§]

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Introduction

Overexpression of full-length Amyloid Precursor Protein (APP) is a common feature of familiar or sporadic Alzheimer Disease (AD) patients and mouse models of AD. Which dysfunction of translational efficiency mediates APP overexpression is, however, unknown.

Methods

A polysome gradient analysis was carried out in hippocampal extracts taken from Tg2576 mice at 1, 3 and 6 months of age using quantitative real time PCR Hippocampal levels of eukaryotic initial translation factors (p-eIF2$\alpha$/eIF2$\alpha$, p-eIF4E/eIF4E, and eIF4G), APP, A$\beta$. BACE-1 and caspase-3 levels were assessed by western blotting in 3-month old Tg2576 mice receiving intracerebral or peripheral injections of salubrinal, a blocker of eiF2$\alpha$ de-phosphorylation which inhibits overall translation. Synaptic plasticity, dendritic spines, memory, and memory-induced hippocampal *c-fos* immunoreactivity were investigated in the same 3-month old salubrinal injected Tg2576 mice.

Results

While upregulation of eIF2α phosphorylation in fully symptomatic patients and mouse models of AD is well documented, we found that elF2$\alpha$ phosphorylation is downregulated when Tg2576 mice are pre-symptomatic and early symptomatic. Decreasing translation efficiency by salubrinal injections in early symptomatic mice rescued AD-related molecular, neural, and behavioral alterations

Discussion

Our findings agree with the suggestion that p-eIF2$\alpha$ regulation is a promising therapeutic target for AD. The observation that p-eIF2$\alpha$ levels in AD mice are initially downregulated and then upregulated suggests that p-eIF2$\alpha$ should be regulated in directions which differ according to stage of the pathology.

# A SYNTHETIC FLUORESCENT MITOCHONDRIA-TARGETED SENSOR FOR RATIOMETRIC IMAGING OF CALCIUM IN LIVE CELLS

Pendin D[1,2], Norante R[2], De Nadai A[3], Gherardi G[2], Vajente N[2], Basso E[1,2], Kaludercic N[1,2], Mammucari C[2], Paradisi C[3], Pozzan T[1,2,4], Mattarei A[5]

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$Ca^{2+}$ handling by mitochondria is crucial for cell life. It is implicated in energy production, shaping of cytosolic $Ca^{2+}$ rises, determination of cell fate and still provides researchers with new and old challenges. It is therefore of crucial interest to directly measure mitochondrial $Ca^{2+}$ concentration in living cells. Genetically encoded $Ca^{2+}$ indicators (GECIs) have greatly facilitated this task, however they require demanding delivery procedures. Synthetic fluorescent $Ca^{2+}$ indicators provide a straightforward loading technique that allows reliable cytosolic $Ca^{2+}$ measurements in many cell types. Unfortunately, existing mitochondria-targeted synthetic $Ca^{2+}$ indicators are instead plagued by several drawbacks.
We have synthesized and characterized a new, highly selective, fluorescent $Ca^{2+}$ sensor named mt-fura-2, obtained by coupling two triphenylphosphonium cation-containing groups to the molecular backbone of the cytosolic ratiometric $Ca^{2+}$ indicator fura-2. Mt-fura-2 binds $Ca^{2+}$ with a dissociation constant of ~1.5 μM *in vitro*. When loaded in different cell types as acetoxymethyl ester, the probe shows proper mitochondrial localization and accurately measures matrix [$Ca^{2+}$] variations, proving its superiority over available non-ratiometric dyes. mt-fura-2 can be successfully applied to cell types where the delivery of GECIs is troublesome, paving the way for a new class of easily deliverable mitochondria-targeted $Ca^{2+}$ probes

# SELECTIVE CONTROL OF INHIBITORY SYNAPSE DEVELOPMENT BY THE GLUTAMATE RECEPTOR DELTA-1 IN CORTICAL PYRAMIDAL NEURONS

Matteo Fossati[1,2,3], Nora Assendorp[1], Olivier Gemin[1], Sabrina Colasse[1], Guillame Arras[4], Florent Dingli[4], Damarys Loew[4], Cécile Charrier[1]

*[1]Ecole Normale Supérieure, Institut de Biologie de l'ENS (IBENS), Inserm U1024-CNRS UMR 8197, Paris, France; [2] CNR - Institute of Neuroscience, Milano, Italy; [3]Humanitas Clinical and Research Center, Rozzano, Italy; [4]Mass Spectometry and Proteomic facility, Institut Curie, Paris*

Balanced development of excitatory and inhibitory synapses is required for normal brain function, and alterations may result in severe neurodevelopmental and psychiatric disorders. How this equilibrium is achieved is poorly understood. In the present study we report the role of the glutamate receptor delta-1 (GluD1), a member of the delta subfamily of ionotropic glutamate receptors, as a synaptic organizer of inhibitory synapses in pyramidal neurons of the neocortex. Human mutations affecting its gene, *GRID1*, have been implicated in schizophrenia, autism spectrum disorders and other cognitive diseases. However, its role remains poorly understood. Using *in utero* electroporation to manipulate the function of GluD1 in sparse layer 2/3 cortical pyramidal neurons *in vivo*, we found that GluD1 depletion decreases the density of inhibitory synapses along dendrites without affecting the number of excitatory synapses. Consistently, GluD1 regulates GABAergic synaptic transmission, but not the glutamatergic counterpart, and it localizes in the postsynaptic compartment of inhibitory synapses. Using an *in vivo* structure-function analysis, we demonstrated that GluD1 regulates the development of inhibitory synapses by interacting with Cerebellin, a protein secreted by presynaptic terminals, which bridges postsynaptic GluDs and presynaptic neurexins. In addition, GluD1 forms a complex with Cerebellin-4, which is specifically expressed by somatostatin-positive interneurons. GluD1 also requires binding to its agonist glycine or D-serine, but not ion flux through its pore. Using an unbiased proteomic approach, we dissected the signaling pathways activated by GluD1 in developing synapses. We identified two major partners of GluD1, the signaling molecule specific for the RhoA GTPase ARHGEF12 and the protein phosphatase 1 regulatory subunit 12A (PPP1R12A), as critical regulators of inhibitory synapse formation. Together, our results unravel a *trans*-synaptic signaling pathway required for the proper establishment of inhibitory connectivity in the neocortex by mediating the formation of inhibitory synapses between pyramidal neurons and $SST^+$-interneurons and shed a new light on the implication of GluD1 in neurodevelopmental disorders.

# MiR-29a COORDINATES AGE-DEPENDENT PLASTICITY BRAKES IN THE ADULT VISUAL CORTEX

*Napoli Debora[1,2], Leonardo Lupori[1,2], Raffaele Mazziotti[2,7], Giulia Sagona[7], Sara Bagnoli[1], Chen Siwei[5], Erika Kelmer Sacramento[4], Johanna Kirkpatrik[4], Cristopher Magnan[5], Alessandro Ori[4], Elena Putignano[2], Muntaha Samad[5], Eva Terzibasi Tozzini[1], Paola Tognini[1,2], Laura Baroncelli[2,6], Pierre Baldi[5], Jessica Kwok[3,8], Alessandro Cellerino[1,4], Tommaso Pizzorusso[1,2,7].*

*[1]BIO@SNS lab, Scuola Normale Superiore, Pisa, Italy; [2]Institute of Neuroscience, National Research Council, Pisa, Italy; [3]School of Biomedical Sciences, University of Leeds, Leeds, UK; [4]Leibniz Institute on Aging – Fritz Lipmann Institute (FLI), Jena, Germany;. [5]Institute for Genomics and Bioinformatics, School of Information and Computer Sciences, University of California, Irvine, CA, US;. [6]Department of Clinical and Experimental Medicine, University of Pisa; Department of Developmental Neuroscience, IRCCS Stella Maris Foundation, Pisa, Italy; [7]Department of Neuroscience, Psychology, Drug Research and Child Health NEUROFARBA University of Florence, Area San Salvi - Pad. 26, Florence, Italy; [8]Institute of Experimental Medicine, Czech Academy of Science, Prague, Czechia.*

Cortical activity-dependent plasticity is high during critical periods of postnatal development and then declines over time. The molecular bases of this decline are still poorly known although some structural and functional brakes have already been discovered. How these brakes are coordinated during the transition from development to adulthood remains an open question. MiRNAs are good candidates because they can control different pathways. In our work, we found a strong age-dependent increase of miR-29a in the visual cortex who can control two important gene categories for plasticity: epigenetic enzymes and extracellular matrix. By inhibition of miR-29a expression in adult mice using antagomirs, we have physiologically and molecularly demonstrated that it is possible to restore juvenile plasticity induced by monocular deprivation. Indeed, proteomic and transcriptomic analyses reveal changes of epigenetic enzymes and extracellular matrix as main gene ontology categories, then confirmed by real-time PCR and western blot. In particular, mir29a inhibition causes a significantly upregulation of Dnmt3a and Tet3 expression possibly unlocking experience-dependent plasticity gene expression. Moreover, miR29a inhibition had an effect on another plasticity brake such as the perineuronal nets (PNNs); PNNs intensity was significantly decreased and its chemical composition was made more permissive for plasticity as demonstrated by the strong decrease of chondroitin sulfate with 4-O-sulfation (C4S).
Conversely, we found that the upregulation of miR29a during critical period made the cortical circuits resilient to experience-dependent changes induced by monocular deprivation associated with a precocious increase of extracellular matrix deposition. Taken together, our results demonstrate that miR29a may be a central hub of experience-dependent plasticity acting as an intrinsic biological clock. This approach could be used to design miRNA-based targeted therapeutic treatments to enhance adult plasticity.

# NUTRITION AND GUT MICROBIOTA IMPACT ON CORTICAL PLASTICITY

Leonardo Lupori[1] , Sara Cornuti[1], Giulia Sagona[2], Raffaele Mazziotti[2,3], Tommaso Pizzorusso[2,3], Paola Tognini[2,3]

*[1]Laboratory of Biology, Scuola Normale Superiore, Pisa, Italy; [2]NEUROFARBA Department, University of Florence, Florence, Italy; [3]Institute of Neuroscience, National Research Council, Pisa, Italy.*

Vision is probably one of the best-understood processes in the human and rodent brain. As such, the mouse visual system is a classic model to study postnatal neurodevelopment, stimuli processing and neuronal plasticity. The visual system has been widely used to examine how external stimuli impinge on neuronal function with clear physiological and behavioral correlates, often distinctive in in young and adult subjects. Here, we investigated how specific and poorly examined exogenous and endogenous factors could impinge on brain plasticity in mouse models. We focused our attention on nutrition and the gut microbiota. Both of them have been shown to be key regulators of physiology and molecular processes in peripheral tissues, however how metabolism and the gut microbiota modulate neuronal function in sensory system is totally unexplored. We found that fasting is able to enhance ocular dominance plasticity in adult mice, and to shift the preference of binocular cortical neurons toward specific spatial frequency in critical period mice. These functional effects were accompanied by dramatic changes in the transcriptional and epigenetic landscape of the visual cortex. Fasting promotes ketosis and the subsequent increase in beta-hydroxyl-butyrate (BHB) plasma concentration. Indeed, we observed significant differences in Lysine beta-hydroxyl-butyrylation on histone H3, suggesting that metabolite-driven changes in epigenetic marks may be a mechanism underlying fasting-driven cortical plasticity. Furthermore, gut microbiota manipulations through antibiotic treatment or fecal transplantation were able to respectively hinder or enhance visual cortical plasticity in adult rodents. Gene expression analysis shows modulation of plasticity genes. Our data indicate that signals coming from the gut could affect plastic processes in the central nervous system through still unknown mechanisms. Our work will pave the way to a new concept in neuroscience: the metabolic status through metabolite/microbiome signals-dependent changes in the brain epigenome, could influence sensory system stimuli processing and plasticity, and, therefore, our perception and behaviour in determined situations.

# MESOSCALE IMAGING OF NEURONAL ACTIVITY COUPLED WITH LIGHT-EVOKED MOTOR MAPPING REVEAL MOVEMENT-SPECIFIC SPATIOTEMPORAL PATTERNS OF CORTICAL ACTIVATION IN AWAKE MICE

Anna Letizia Allegra Mascaro[1,2], Francesco Resta[2,3], Elena Montagni[2], Giuseppe De Vito[4], Alessandro Scaglione[2,3], Francesco Saverio Pavone[2,3,5]

*[1] Neuroscience Institute, National Research Council, Pisa, Italy; [2]European Laboratory for Non-Linear Spectroscopy (LENS), Sesto Fiorentino, Italy;*

A fundamental goal of neuroscience is to understand how the spatiotemporal patterns of neuronal activity drive behaviour. To this aim, the use of light to monitor and manipulate the activity of neuronal networks has several advantages, such as the low invasiveness and the possibility to target with high precision specific population of cells. However, the combination of these tools is still arduous, mainly due to the spectral overlap between actuators and indicators. Here we developed an all-optical system that couples large-scale cortical imaging with chronic light-based motor mapping in awake mice. By adeno-associated virus-mediated cortical transfection we induced the co-expression of the red-shifted genetically encoded calcium indicator RCaMP1a and light-sensitive actuator ChR2 over both the rostral and caudal forelimb areas, which was stable over several months. No evidence of cross talk was detected by cortical LFP recordings during illumination of ChR2+ neurons with the light source used for RCaMP1a excitation. By performing single pulse irradiation, we showed the rise of optogenetically-evoked calcium peaks with increasing laser power, which reached a plateau around 10 mW. Light-based motor mapping coupled with wide-field imaging of neuronal activation in awake mice showed non-overlapping cortical representations for grasping and tapping. Interestingly, our results revealed spatiotemporal patterns of cortical activation specific for each movement category. Finally, preliminary results on pharmacological and optical interference indicate that successful block of movement generation is correlated with reduced spread of cortical activation. We anticipate that the combination of tools shown here will play a key role in the study of optogenetic-guided rehabilitation after stroke.

# VOLUNTARY ACTION MODULATES VISUALLY EVOKED CORTICAL RESPONSES IN PRIMARY VISUAL CORTEX: AN INTEGRATED ULTRA-HIGH FIELD fMRI AND EEG STUDY

Alessandro Benedetto[1], Paola Binda[2], Mauro Costagli[2], Michela Tosetti[2], Maria Concetta Morrone[1,2]

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In previous studies we have shown that voluntary actions can induce long-lasting theta behavioral oscillations. These oscillations have been observed for several visual functions, including temporal order judgments, orientation and contrast discrimination. To study how behavioral oscillations are related to endogenous neural oscillations, we investigated the spatiotemporal characteristics of the visual response around the time of a voluntary action in an ultra-high-field (7T) fMRI experiment. Participants (N= 18) discriminated the spatial frequency of two very brief gratings, presented randomly in the upper or lower visual field after a free self-paced button-press. The stimulus was displayed randomly with either 70ms or 150ms delay from button-press, corresponding to the first minimum\maximum of the sensitivity oscillation. Stimuli presented at 150ms evoked a stronger V1 BOLD response than the stimulus presented at 70ms (i.e., the predicted peak\through of the excitability cycle, respectively). These results suggest an early visuo-motor interaction, at the level of V1. The rhythmic modulation points to a synchronization between vision and action, shaping vision by alternatively suppressing and enhancing visual processing.

# IMPROVEMENT OF VISUAL ACUITY IN AMBLYOPIC PATIENTS FOLLOWING UNILATERAL APPLICATION OF CATHODAL TRANSCRANIAL DIRECT CURRENT STIMULATION (tDCS).

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Objective: Amblyopia is a neurodevelopmental disorder characterized by visual acuity and contrast sensitivity loss, refractory to pharmacological and optical treatments in adulthood. We investigated the response of the visual cortex to transcranial Direct Current Stimulation (tDCS) applied over the primary visual area (V1) contralateral to the “lazy eye”.
Methods: Visual acuity (logMAR) was assessed before (T0), immediately after (T1) and 60’ following the application of cathodal tDCS (protocol: 2.0 mA, 20’). At each time point, Visual Evoked Potentials (VEPs) triggered by grating stimuli of different contrasts (K90% and K20%) were recorded in both hemispheres and compared to those obtained at baseline and in healthy volunteers.
Results: Cathodal tDCS improved visual acuity (Holm–Sidak, $p < 0.0001$), whereas sham polarization had no significant effect. tDCS produced an inhibitory effect on VEPs amplitudes in the targeted side and a concurrent facilitation of responses in the hemisphere ipsilateral to the amblyopic eye; the facilitation persisted at T2 for high contrasts (K90%; $p < 0.001$), while the stimulated hemisphere recovered more quickly from inhibition ($p < 0.001$).
Conclusions: tDCS represents a promising treatment for amblyopia in adults. The recovery of excitability and the persistent transcallosal disinhibition following tDCS support the role of interhemispheric pathways in the pathophysiology of amblyopia.

# PUPILLOMETRY PROVIDES NEW INSIGHTS ON FIGURE-GROUND SEGREGATION AND ITS COVARIATION WITH AUTISTIC TRAITS

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We used pupillometry to investigate the processes of figure-ground segregation and its inter-individual variability while minimizing interference with the observer's perceptual task. Twenty-five observers classified silhouettes as depicting meaningful real-world or meaningless novel objects. The borders of half the novel objects suggested portions of meaningful objects on the ground side. Participants were directed to focus their attention on the central object for the full duration of the stimuli (2s). Pupil constriction/dilation responses to the central figure (respectively, brighter/darker than its ground) were stronger when its borders suggested meaningful objects on the ground side. This is inconsistent with attention shifting away from the meaningless central figure towards the meaningful ground objects (which would have predicted the opposite pupillometry pattern), but may suggest active suppression of the meaningful figure on the ground. We investigated the inter-individual variability of the responses and found a surprisingly strong correlation between the strength of pupillary light responses and autistic traits, estimated with the Autistic Quotient. Participants with stronger AQ have reduced pupil constrictions, particularly in response to silhouettes with meaningful objects on the ground side. This could be an index of autistic traits influencing figure-ground processing, which is revealed by the sensitive and objective pupillometric technique.

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# THE INTERPHOTORECEPTOR MATRIX: INVESTIGATING THE ROLE OF IMPG2 IN AUTOSOMAL RECESSIVE RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is one of the most commonly inherited retinal dystrophies, with a prevalence of approximately 1:4000. It leads to progressive degeneration of rod and cone photoreceptors and of RPE (retinal pigmented epithelium). The genetic background of RP is heterogeneous, as are inheritance modes. Among these, autosomal recessive RP (arRP) was found to be associated with mutations in 42 different genes (van Huet et al., *Invest Ophthalmol Vis Sci.,* 2014). Recent studies have reported that mutations in the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene, responsible for the introduction of premature stop codons and the production of a truncated protein, are associated with arRP in humans (Bandah-Rozenfeld D. et al., *Am J Hum Genet.,* 2010). This gene encodes the proteoglycan IMPG2, expressed in the interphotoreceptor matrix (IPM). IPM is the extracellular matrix, mainly composed of proteoglycans and glycosaminoglycans, that surrounds retinal photoreceptor outer segments and ellipsoids. IMPG2 is synthesized by both rods and cones and it is secreted in the IPM. We chose zebrafish to investigate IMPG2 function and expression, since its visual system is very comparable to that in humans. In zebrafish, IMPG2 is present in two isoforms, IMPG2a and IMPG2b, which have diverged during evolution. Their expression as well as their role and possible differences are not yet known.

RT-qPCR experiments performed on zebrafish embryos at different developmental stages revealed that IMPG2a and IMPG2b are transcribed from 3 days post fertilization (dpf) in whole fish. In adults, both isoforms have an eye-specific expression. Western blot analyses showed a similar expression pattern for the proteins. Furthermore, immunohistochemistry experiments performed on eye sections showed that IMPG2 is specifically found in the outer segment of photoreceptors starting from 5 dpf. Microinjection of antisense morpholinos oligonucleotides (MOs), specific for each of the two isoforms, provided preliminary evidence that IMPG2 is involved in eye and head development and RPE pigmentation in zebrafish. Moreover, morphant embryos show an increase in cell proliferation in the ciliary marginal zone (CMZ), the region at the periphery of the retina composed of retinal stem and progenitor cells. To better characterize the CMZ we plan to microinject MOs in zebrafish transgenic lines to be used as reporter for signalling pathways such as Shh. Currently, we are generating a zebrafish line carrying the human IMPG2 protein truncations, by using CRISPR/Cas9 technology. Our aims are to phenotypically characterize mutant fish during development and in adulthood and to perform large-scale testing of therapeutic compounds to discover a possible treatment for this type of retinopathy. Finally, next generation sequencing on this new animal model would help us to better analyse the cellular and molecular mechanisms underlying IMPG2-related retinopathies, since little is known about IPM components and their involvement in retinal disorders.

# QUANTITATIVE MAPPING OF HIPPOCAMPAL SYNAPTIC MEMORY ENGRAMS

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The brain tailors its responses to the variation in external stimuli via the acquisition of new information and its subsequent recall i.e., "learning and memory". The search for a physical substrate of these processes has been guided by the concept of "engram", which indicates a structure whose activity is both necessary and sufficient for the recall of a given memory. Our current knowledge on this topic is based on seminal discoveries based on the expression of fluorescent reporters and optogenetic probes driven by the activity-dependent promoter from the *cFos* gene (Tonegawa et al., 2018, *Nat Rev Neurosci* 13:485-98). Data using this approach have permitted the identification and manipulation of the cellular engrams of the hippocampus. However, structural and functional modification serving learning and memory occur via plasticity phenomena which do not involve all the synapses of a given neuron at once, but are thought to recruit specific subsets of a given neuron synaptic array (Sossin, 2018, *Front Syn Neurosci* 10:5).

To investigate this point, we took advantage of the "SynActive" (SA) toolbox (Gobbo et al., 2017, *Nat Comm*, 8:1629), which allows expression of a given protein of interest specifically at synapses undergoing activity-dependent potentiation. The SA gene construct is based on regulatory sequences borrowed from the *Arc* immediate-early gene mRNA, plus a synthetic synaptic retention aminoacyl sequence. We placed a green fluorescent protein-based reporter under SA control, and restricted the temporal window for its expression using a "TetON", doxycylin-dependent system. This resulted in a pair of AAVs, namely (i) synapsin::rtTA-IRES-tdTomato, and (ii) TREp::SA-Venus, injected into the hippocampus of mice, which were subsequently exposed to contextual fear conditioning. Using confocal imaging, we show here that this approach results in tagging of potentiated synapses supporting an associative episodic memory. Our approach can be used to obtain the precise topography of synaptic memory engrams, providing quantitative data to be integrated into computational models.

# NOVEL CELL-BASED STRATEGIES TO PROMOTE BRAIN REPAIR AND MOTOR FUNCTION AFTER STROKE IN MICE

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Brain injuries causing chronic sensory or motor deficit, such as stroke, are among the leading causes of disability worldwide, according to the World Health Organization; furthermore, they carry heavy social and economic burdens due to decreased quality of life and need of assistance. Cell-based approaches have emerged as an intriguing and promising strategy to promote brain repair, in particular, pluripotent stem cells (PSCs) hold great promises for their clinical applications, such as cellular replacement of damaged neural tissues with autologous neurons. Notwithstanding these potential applications, PSC-derived neurons have to match the precise sub-type, positional and functional identity of the lesioned neural tissue, both if they were obtained *in vitro* or directly *in vivo*.
Recently, we demonstrated that manipulating Wnt and BMP signaling, it is possible to steer the differentiation of mouse embryonic stem cells (ESCs) toward a cortical or hippocampal fate. These two types of cells showed different degrees of axonal outgrowth and targeted different regions when co-transplanted *in vivo*. In hippocampus, only precursor cells with hippocampal molecular identity were able to extend projections, contacting CA3 neurons. Conversely, cortical-like cells were capable of extending long-range axonal projections only when transplanted in motor cortex, sending fibers toward both intra- and extra-cortical targets. An ischemic photothrombotic damage greatly enhanced the capability of cortical-like cells to extend far-reaching projections. Our results indicate that neural precursors generated by ESCs carry intrinsic signals specifying axonal extension in different environments.
A second approach to promote neural repair after a brain injury exploits the possibility to direct reprogram endogenous perilesional reactive astrocytes into neurons with the major advantages of obtaining neurons with the correct positional identity and immunogenic profile. This would be possible forcing the expression of pro-neural transcription factors through flexed AAVs injection in GFAP-Cre transgenic mice. 60 days after stroke induction, we observed a successful reprogramming in about 35% of transfected astrocytes.
Currently, Gridwalk and Schallert Cylinder tests are used to evaluate the therapeutic effect of this innovative cell-therapy approach in promoting motor function after stroke, alone and in combination with motor rehabilitation by means of a robotic-platform developed for mice (M-Platform). Moreover, electrophysiological experiments are currently used to shed light on the effective integration of newborn cells in the host damaged circuitry, assessing their role in achieving post-stroke motor recovery.
Our results are important to move the field forward and to bridge the gap between pre-clinical studies and clinical developing of new combined therapeutic strategies for stroke patients.

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

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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 the effect of ischemic stroke in mice forelimb Primary Motor Cortex (Caudal Forelimb Area, CFA) and the effectiveness of different therapeutic strategies.
Results: We 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 recording 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., 2017). 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 improved the rehabilitative treatment on the robotic platform with a real-time control of friction and isometric measure of forces (Pasquini et al., 2018). We coupled 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 and in WT animals with transcranial Alternated Current Stimulation.

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

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Increasing evidence suggests an association between metabolic disorders, notably insulin-resistance and type 2 diabetes, with cognitive decline and Alzheimer's Disease. Recent studies have shown that diet-induced changes in peripheral insulin sensitivity contribute to alterations in brain insulin signaling and cognitive functions. Deranged glucose metabolism in the brain accompanied by elevated levels of fatty acids and chronic low-grade inflammation has been postulated as the pathogenic mechanism associating type 2 diabetes with Alzheimer's Disease.
We set up a preclinical animal model of diet induced-glucose intolerance: mice were fed with 45% and 60% high fat diets for several weeks, the effects on body weight gain, glucose-, pyruvate- and insulin-tolerance were tested. To assess the presence of cognitive impairments behavioral tests were done. We investigated the effect of the high fat diet on neurotransmission, myelination and endoplasmic reticulum stress through western blot analyses in hippocampus and prefrontal cortex of mice. To investigate more in depth the molecular mechanism underlyingglucose-intolerance in the brain, palmitic acid treatments were used *in vitro* on primary neuronal cell cultures to mimic the metabolic condition determined by high fat diet.
Starting from 2 weeks of diet a significantly higher body weight was observed in the groups of animals fed with highfat diets compared to controls. Moreover, metabolic tests on the same animals showed glucose impairment after 3 weeks compared to controls, whereas insulin intolerance was observed starting after 5 weeks of diet. Open Field test showed significant alterations in stereotyped activity, rearing activity and anxiety-like behaviors, with no changes in locomotor activity. Hippocampal tissues of animals exposed to high fat diet showedaltered levels of p-AKT, reduced levels of excitatory subunits receptors and elevated level of BIP, a marker of endoplasmic reticulum (ER) stress. Moreover myelin protein PLP was significantly reduced in both Hippocampus and Prefrontal cortex of high fat diet fed mice. Hippocampal neurons treated *in vitro* with palmitate showed the same pattern of ER stress activation showed in ex vivo hippocampal tissues.
Our results suggest that high fat diet, even for a short period of exposure, can alter relevant brain functions, including neurotransmitter receptors and myelination. The precise molecular mechanismsunderlying these effects are still under investigation.
By this work we want to identify novel pathways affected by high fat diet and to eventually translate this knowledge into the clinic for halting cognitive decline in at risk subjects.

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# INTRACEREBRAL INJECTION OF EXTRACELLULAR VESICLES FROM MESENCHYMAL STEM CELLS EXERTS REDUCED Aβ PLAQUE BURDEN IN EARLY STAGES OF A PRECLINICAL MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), the leading cause of dementia, has recently been attracting a lot of attention from the scientific community, since millions of people are affected by this incurable pathology; furthermore, the number of patients is destined to increase in the coming decades. The limited knowledge of the etiology of AD has rendered in vain numerous attempts hitherto pursued to find a resolutive treatment that is not simply limited to the alleviation of symptoms. Therefore, a worldwide effort is underway to discover the mechanisms responsible for the disease onset and progression and to find an efficacious therapy, developing either novel treatments or preventive strategies.
Cell therapy is becoming a new reality for many diseases. Due to the plasticity and multifaceted features of stem cells, recent studies have also focused on their possible exploitation in AD. Bone marrow Mesenchymal Stem Cells (BM-MSCs), due to their strong protective and anti-inflammatory abilities, have been widely investigated in the context of several diseases for their possible therapeutic role, based on the release of a highly proactive secretome composed of soluble factors and Extracellular Vesicles (EVs). BM-MSC-EVs, in particular, convey many of the beneficial features of parental cells, including direct and indirect β-amyloid degrading-activities, immunoregulatory and neurotrophic abilities.
We examined the therapeutic potential of BM-MSC-EVs injected intracerebrally into the neocortex of APPswe/PS1dE9 AD mice at 3 and 5 months of age, a time window in which the cognitive behavioral phenotype is not yet detectable or has just started to appear. We demonstrate that BM-MSC-EVs are effective at reducing the Aβ plaque burden and the amount of dystrophic neurites in both the cortex and hippocampus. The presence of Neprilysin on BM-MSC-EVs - a neutral endopeptidase, which is the dominant Aβ peptide-degrading enzyme in the brain - opens the possibility of a direct β-amyloid degrading action of EVs. Our results suggest a potential therapeutic role for BM-MSC-EVs already in the early stages of AD, suggesting the possibility of intervening before overt clinical manifestations.

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# SOMATOSENSORY HYPO-REACTIVITY TO WHISKER STIMULATION IN THE CNTNAP2 -/- MOUSE: A GENETIC MOUSE MODEL OF AUTISM SPECTRUM DISORDER

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Autism spectrum disorders (ASD) form a heterogeneous group of neurodevelopmental syndromes characterized by deficits in social interactions, repetitive behaviors, and language impairments. Recently, the American Psychiatric Association also included hyper/hypo-reactivity to sensory stimuli as diagnostic criteria for ASD in the last edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). Abnormal reactivity and defensive behaviors in response to tactile stimuli are indeed common symptoms in ASD, with 90% of individuals facing atypical sensory experiences. Similarly, both somatosensory hyper-sensitivity and hypo-sensitivity have been described in different mice lacking ASD-associated genes.
Our recent work showed that, when tested in the whisker nuisance (WN) test, En2-/- but not wild-type (WT) mice were over-reactive to repeated whisker stimulation. Moreover, En2-/- mice undergoing WN exhibited decreased c-Fos-positive neurons in layer IV of the primary somatosensory cortex and increased immunoreactive cells in the basolateral amygdala, as compared to WT.
Stemming from these results, we explored somatosensory impairments following whisker stimulation in Cntnap2 -/- mice. Our hypothesis is that somatosensory impairments seen in En2-/- mice could be a common feature shared by other ASD mouse models. Preliminary results show that Cntnap2-/- mice are under-reactive to repeated whisker stimulation. This finding is in line with the literature of sensory defects in ASD mouse models demonstrating how sensory deficits are a common feature yet reporting divergent outcomes. This impaired sensory response could be linked to neurological defects at the cortical level and could represent the neurological substrate of these aberrant behaviors.

# THE VISUAL SYSTEM AS A BIOMARKER IN A MOUSE MODEL OF CDKL5 DEFICIENCY DISORDER

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CDKL5 deficiency disorder (CDD) is a neurodevelopmental disorder characterized by a severe global developmental delay, early-onset seizures and, notably, visual abnormalities often clinically diagnosed as Cortical Visual Impairment. Murine models of CDD have been recently generated, raising the possibility of preclinical testing of treatments. However, unbiased, quantitative biomarkers of high translational value to monitor brain function are still missing. Moreover, the analysis of treatment is hindered by the challenge of repeatedly and non-invasively testing neuronal function.

To addressed these issues, we analysed the development of visual responses in a mouse model of CDKL5 disorder to introduce visually evoked responses as a quantitative method to assess cortical circuit function. Cortical visual responses were assessed in CDKL5 null male mice, heterozygous females, and their respective control wild-type littermates by repeated transcranial optical imaging. Starting from P27-P28, defective responses appeared both in heterozygous and homozygous CDKL5 mutant mice. These results were confirmed by visually evoked potentials (VEPs) in adult animals, revealing a persistent reduction of response amplitude, reduced visual acuity and defective contrast function in mutants. These results suggest that monitoring visual responses represents a promising biomarker for preclinical and clinical studies on CDKL5 disorder.

To assess which is the relative contribution of cortical and subcortical circuits to the described visual deficits, we proceeded in two ways: first, we performed an in-depth morphological analysis of the visual pathway, from the retina to the primary visual cortex (V1), of CDKL5 null mice. We found that CDKL5 lack produced no alteration in the organization of retinal circuits, but, conversely, it reduced density and altered morphology of spines and decreased excitatory synapse marker PSD95 in the dorsal Lateral Geniculate Nucleus and in V1. Second, using a conditional KO model, we showed that selective cortical deletion of CDKL5 from excitatory cells is sufficient to produce abnormalities of visual cortical responses, demonstrating that the normal function of cortical circuits is dependent on CDKL5.

# WHOLE BRAIN DELIVERY OF AN INSTABILITY-PRONE *MECP2* TRANSGENE RESCUES BEHAVIORAL AND MOLECULAR PATHOLOGICAL DEFECTS IN MOUSE MODELS OF RETT SYNDROME

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Rett syndrome (RTT) is an incurable neurodevelopmental disorder caused by mutations in the gene methyl-CpG binding-protein 2 (*MECP2*). Gene therapy for this disease presents inherent hurdles since MeCP2 is expressed throughout the brain and its duplication also leads to severe neurological conditions. However, the recent introduction of PHP.eB, an engineered capsid with an unprecedented efficiency in crossing the blood-brain barrier upon intravenous injection, has provided an invaluable vehicle for gene transfer in the mouse nervous system. Herein, we use PHP.eB to deliver a novel instability-prone *Mecp2* (*iMecp2*) transgene cassette which prevents supraphysiological MeCP2 protein levels in transduced neural tissues by increasing RNA destabilization and inefficient protein translation of the viral Mecp2 transgene. Intravenous injections of the PHP.eB-*iMecp2* virus in symptomatic male and female *Mecp2* mutant mice resulted in complete protection from disease progression with improved locomotor activity, coordination, lifespan and normalization of altered gene expression, epigenetic deficits and mTOR signaling. Remarkably, PHP.eB-*iMecp2* administration was safe in female *Mecp2* mutant and wild-type animals at all viral doses with only a marginally increase in MeCP2 protein levels throughout the brain. In contrast, we observed a strong immune response to the transgene in treated male *iMecp2* mutant mice that was partially overcome by immunosuppression. Overall, PHP.eB-mediated delivery of the *iMecp2* cassette provided widespread and efficient gene transfer and achieved physiological levels of MeCP2 total protein in the brain. This combination defines a novel viral system with strong therapeutic efficacy and increased levels of safety holding important clinical implications for RTT.

# NEURODEGENERATION IN A MOUSE MODEL WITH ALPHA-SYNUCLEIN ACCUMULATION IN THE MICROGLIA

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The role of immune response in the development of Parkinson's disease (PD) has had an increasing interest in the last decade. It has become evident that in parallel with alpha-synuclein neuronal toxicity, the inflammatory process also occurs in PD patients. One of the most important actors in mediating inflammation in the brain is microglia, with its ability to patrol and monitoring the micro-environment. Microglia behaviour can be influenced by a wide range of different molecular mediators (cytokines, LPS, antibodies, neurotransmitters, metabolites) making the microglia able to mediate a number of responses ranging from the release of cytotoxic molecules to the overt phagocytic activity. Microgliosis has been shown in post-mortem tissue of PD patients. Although the neuronal loss is able to trigger the inflammatory response, some studies suggested that microglia activation could be not related only to cell death but rather to alpha-synuclein deposition. As matter of fact in transgenic lines expressing mutated alpha-synuclein under the TH promoter, microgliosis precedes the cell death. However, the available PD murine models does not allow to dissect the primary role of microglia either as a trigger or only as an enhancer of the PD pathology. In this work we took advantage from a unique animal model where the expression of alpha-synuclein is limited to CX3CR1-expressing microglia. We achieved such of high selective expression by injecting Cre mouse lineage with lentiviral vector expressing the flexed human form of mutated alpha-synuclein (hA53Tfl). Therefore, we analysed the possible neurodegeneration elicited by hA53Tfl-expressing microglia and compared with other models, in particular with animals overexpressing hA53T specifically in the dopaminergic neurons. Unexpectedly hA53Tfl-expressing microglia results to be more toxic toward the dopaminergic neurons than the accumulation of alpha-synuclein in the dopaminergic neurons themselves. This effect is mediated by the shift of microglial cells to a M1 status with the consequent release of pro-inflammatory and cytotoxic cytokines. Moreover, the toxic response mediated by the microglia is followed by peripheral immune-cell infiltration. Taken together these results strongly suggest a predominant non-cell autonomous toxicity mediated by the microglia over the cell-autonomous dysfunction. The two processes are probably entangled together and culminate with the dopaminergic cell loss. Apparently, alpha-synuclein accumulation in the microglia comes first with changes in the microenvironment which, in turn, results detrimental for the dopaminergic neurons. This scenario opens to different therapeutic strategies aimed to counteract microglia misbehaviour, therefore, delaying or slowing down the neurodegenerative process.

# D2 RECEPTORS ON INDIRECT MEDIUM SPINY NEURONS MODULATE L-DOPA-INDUCED DYSKINESIA

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Within the Caudate-Putamen nucleus (CPu), dopamine (DA) regulates basal ganglia functions balancing striatal neuronal population activity.

L-DOPA-induced dyskinesias (LIDs) consist of disabling involuntary movements which can affect either discrete body parts or the entire body and are observed in parkinsonian patients receiving dopamine replacement agents. Previous studies have demonstrated that LIDs are associated with dramatic changes of the striatal network connectivity and are promoted by the imbalanced activity of the direct and indirect projecting medium spiny neurons (MSNs). In the CPu, the DA D2 receptor (D2R) is broadly expressed and modulates striatal motor functions by inhibiting the release of several neurotransmitters such as dopamine, acetylcholine, glutamate and GABA. The purpose of the present project is to determine how the absence of the D2R either on the indirect MSNs (iMSNs) or in the striatal cholinergic interneurons (ChIs) modulates the expression of LIDs. To achieve our goal, we used 6-OHDA unilaterally lesioned mice carrying a cell-specific deletion of the D2R either on iMSNs (iMSN-D2RKO mice) or on ChIs (ChIs-D2RKO mice). Three weeks after the lesion, mice were treated with: i) Saline; ii) 15 mg/kg of L-DOPA (once a day for 11 consecutive days) or iii) an ascending-dose regimen of L-DOPA (1.5, 3, 6 mg/kg, once a day for 9 consecutive days), and abnormal involuntary movements (AIMs), as index of dyskinesia, were evaluated. Afterward, electrophysiological and immunohistochemical experiments were performed on striatal brain samples to further investigate mechanisms involved in LIDs development and expression.

Our results suggest that, when nigrostriatal terminals are almost entirely degenerated, the stimulation of the D2R located on iMSNs, but not on ChIs, is implicated in the modulation of the dyskinetic behaviour induced by repeated L-DOPA administration. Indeed, under the same experimental conditions, iMSN-D2RKO mice expressed more severe LIDs than WT and ChIs-D2RKO mice. These behavioural results were supported by our immunohistochemical data where striatal sections from dyskinetic iMNS-D2RKO mice showed the highest immunoreactivity toward established protein markers associated with an abnormal dopaminergic stimulation and LIDs. Finally, local field potentials recordings acquired after the delivery of stimuli promoting long-term depression, revealed an abnormal synaptic plasticity in the CPu of iMSN-D2RKO and ChI-D2RKO mice. The present study sheds light on how the D2R signalling is implicated in the modulation of LIDs and opens new perspectives for future therapeutic strategies.

# CHARACTERIZATION OF THE ROLE OF SIGMA-1 RECEPTOR MUTATION IN THE ETIOLOGY OF dHMN FOCUSING ON CELL HOMEOSTASIS AND INTRACELLULAR $CA^{2+}$ SIGNALING

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Our and other groups have recently identified *SIGMAR1* as the causative gene of a series of Hereditary Motor Neuropathies (dHMNs), a family of clinically and genetically heterogeneous neurological disorders whose hallmark is the degeneration of peripheral motor neurons. In our previous work, we characterized two *SIGMAR1* mutations mapping at position E138Q and E150K in the sigma-1 receptor (sigma-1R) protein, which were found in two Italian families affected by dHMN. Sigma-1R is a highly conserved 28 kDa chaperone protein of the ER with no homology to any known mammalian protein. We demonstrated that sigma-1R variants have a "loss-of-function" behavior in neuroblastoma cell lines, impinging on cell viability and altering $Ca^{2+}$ homeostasis due to the impairment of ER-mitochondria tethering.

In the present study we focus on the functional effects of sigma-1R variants in a cellular model consisting of patient primary skin fibroblasts homozygous for the E150K mutation. Our comprehensive analysis of sigma-1R distribution, intracellular $Ca^{2+}$ signaling and ER-mitochondria contact sites in these cells clearly show a significant mislocalization of the mutated protein, which is present in cytosolic aggregates, an altered global $Ca^{2+}$ handling and a remarkable disorganization of the ER-mitochondria tethers compared to controls. Importantly, patient fibroblasts display a reduced amount of the sigma-1R protein, which is probably due to the increased clearance of the mutated protein. In addition, cells expressing sigma-1R E150K variant display a substantial upregulation of basal autophagy and alterations of mitochondrial metabolism. Moreover, transmission electron microscopy data reveal the presence of impaired mitochondria architectures and abnormal intracellular structures in mutant fibroblast compared to controls.

Concluding, our data support the involvement of sigma-1R in the maintenance of cell and protein homeostasis and highlight the crucial role of this protein in the establishment of 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.

# NEW LIGHT ON OXYTOCIN RECEPTORS

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The neurohormone oxytocin (OXT) plays a role in various functions including endocrine and immune functions but also parent-infant bonding and social interactions. OXT triggers social behaviors by binding to oxytocin receptors (OXTRs) and both distribution and number of OXTRs in the brain affect the type and degree of behavioral responses. Receptor autoradiography has been used for over 30 years to study the presence of OXTRs in the brain and other organs, and still represents the only possibility to visualize the OXTR physical distribution in tissues. However, this technique presents different limits: reduced anatomical resolution, impossibility to perform double staining, concerns related to the use of radioactive materials and high costs. As specific OXTR antibodies are difficult to produce and are not available, especially for murine OXTR, we have generated two different fluorescent OXT analogues. These analogues maintain all the pharmacological properties of OXT in term of affinity and selectivity for OXTR. When we used them in cells, we were able to visualize receptor binding and to follow receptor trafficking in response to OXT stimulation. Moreover, we obtained a beautiful staining for OXTR on brain slices. In particular, not only we confirmed the same OXTR distribution and expression levels observed with brain autoradiography but we obtained anatomically high-resolution images. We were also able to compare the distribution of OXTR and dopamine D2R receptor using our OXT-fluorescent analog and an antibody for D2R on the same brain slice. Our newly developed ligands make possible to precisely determine the level and distribution of OXTRs within the brain and can be applied to study whether the actions of OXT are altered in response to a genetic condition, phenotypic abnormality, disease state or drug treatment. This knowledge can then be used to the development of therapies to treat neurodevelopment and neuropsychiatric disorders associated with dysregulation of the OXT system, such as autism and schizophrenia.

# ROLE OF GENOTYPE IN THE LONGLASTING EFFECTS OF NICOTINE EXPOSURE ON MESOLIMBIC DOPAMINE TRANSMISSION: A LIKELY MECHANISM OF NICOTINE GATEWAY EFFECT

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Epidemiological evidence suggests that individual who begin experimenting with drugs of abuse during adolescence are more likely to develop substance use disorders. Smoking during adolescence is considered a risk factor to develop nicotine dependence, but also to co-abuse other drugs such as cocaine [1-4]. On the basis of epidemiological observation, it has been hypothesized that nicotine might be a gateway toward use and abuse of other illicit drugs [1,5,6]. However, the progression of the individual to subsequent drug (co)abuse might be due to genetic background. Indeed heritability estimates for nicotine initiation and nicotine dependence range from 40 to 70 % [4,7,8].

Despite great efforts to understand underlying neurobiological mechanisms of this progression, less attention has been paid to the role of genetic factors. Here, we investigated the influence of both genetic background and age at first nicotine exposure in the long-lasting effects on mesolimbic dopamine transmission and on cocaine rewarding effect.

Mid-adolescent (6 weeks of age) and adult rats (10-12 weeks of age) of inbred strains Lewis (addiction prone) and Fischer 344 (addiction resistant) were administered nicotine (0.4 mg/kg) or vehicle once daily for 5 days. Changes in dopamine transmission were investigated by in vivo microdialysis [9] and electrophysiology [10] after 30 days of withdrawal, whereas changes in cocaine rewarding effect were assessed via conditioned place preference paradigm [9]. Nicotine pre-exposure differentially changed mesolimbic dopamine transmission depending on strain and age of pre-exposure. A potentiation of dopamine response to nicotine was observed in nucleus accumbens (NAc) core of both strains and age groups, whereas dopamine response in NAc shell was enhanced exclusively in Lewis rats exposed to nicotine during adolescence. A similar response was observed following cocaine challenge at adulthood. Changes in VTA dopamine cell population and activity were observed only in adolescent nicotine-pretreated Lewis rats, which also showed an increased cocaine rewarding effect at adulthood. These results highlight the influence of genetic background in the long-lasting effects of nicotine exposure and suggest that exposure during adolescence might increase nicotine and cocaine rewarding properties in genetically vulnerable individuals, thereby facilitating progression toward dependence.

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# THE BRAIN AS A TARGET OF HORMONAL CONTRACEPTIVES: EVIDENCES FROM ANIMAL STUDIES

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Endogenous steroid hormones regulate brain development as well as neuronal plasticity and excitability, thus influencing mood, affective behavior and cognition. By contrast, effects of exogenous synthetic steroids, such as those used in hormonal contraceptives (HC) formulations, on brain function and synaptic plasticity are poorly investigated. HC are the most prescribed drugs among young, healthy women; however, their use is sometimes associated with emotional liability and episodes of affective disorders such as depression or mood changes, suggesting effects of these drugs on the central nervous system. HC inhibit ovulation, thereby decreasing the levels of the endogenous hormones estradiol and progesterone. In agreement, we showed that long-term treatment with the combination of ethinylestradiol (EE) and levonorgestrel (LNG), two compounds frequently used in HC formulations, decreased brain levels of progesterone and its neuroactive metabolite allopregnanolone in female rats. Likewise, HC prevent the increase in allopregnanolone concentrations during the luteal phase of the menstrual cycle in women. These changes may contribute to some of the emotional and affective disorders sometimes observed in HC users. Allopregnanolone and estradiol are also involved in regulation of neuronal plasticity and cognition. We thus examined whether long-term treatment with EE-LNG was associated with altered brain plasticity and learning and memory in female rats.

Adult female rats were orally treated with a combination of EE (0.020 mg) and LNG (0.060 mg) once a day for 3 weeks and were tested 24 hours after the last administration. Long-term EE-LNG treatment reduced the abundance of brain-derived neurotrophic factor (BDNF) and the extent of long-term potentiation (LTP) in the hippocampus, compared to vehicle-treated rats tested in the proestrus phase of the estrus cycle. However, these effects were not accompanied by changes in spatial learning and memory and cognitive flexibility in the Morris water maze task.

Given that allopregnanolone also regulates the stress response and affective behavior, we evaluated the effects of EE-LNG treatment on such parameters. Long-term EE-LNG treatment induced an anxiety-like behavior in the elevated plus maze test, reduced immobility behavior in the forced swim test, but failed to affect sucrose preference, a measure of anhedonia. EE-LNG treatment also increased basal plasma corticosterone levels, and blunted the corticosterone and allopregnanolone responses to acute restraint stress.

These results might be relevant to explain some of the effects sometimes exhibited by women taking HC. Understanding the neurobiological effects of HC may improve women's health and may help women making informed choices on the advantages and disadvantages of hormonal contraception.

This work was supported by Fondazione di Sardegna (Grant 2019.1440).

# GLUCOSE SENSITIVITY, INSULIN SENSITIVITY AND THEIR LONGITUDINAL CHANGES ARE STRONG INDEPENDENT DETERMINANTS OF TYPE 2 DIABETES PROGRESSION: AN IMI DIRECT STUDY

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**Background and aims:** Many studies have described clinical parameters associated with type 2 diabetes (T2D) onset or "time-to-failure" markers, while predictors and mechanisms of progression in established and newly-diagnosed T2D are unclear. Here, we have evaluated predictors and determinants of $HbA_{1c}$ progression among metabolic parameters from the prospective DIRECT study.

**Materials and methods:** We studied 621 T2D patients recruited to the DIRECT study (baseline treatment as diet only or metformin, $HbA_{1c}$<60mmol/mol, diabetes duration <24 months). $HbA_{1c}$ concentrations were collected at months 0, 9, 18, 27 and 36 after start of the study. Mixed-meal tests were performed at 0, 18 and 36 months, for assessment of insulin sensitivity (as OGIS index) and β-cell glucose sensitivity (GS). $HbA_{1c}$ data were described as linear functions of time, with slope (σ) describing underlying progression, adjusted for changes in BMI and in standardized dosage of antidiabetic treatments. Changes of various clinical parameters during follow-up were computed as uncorrected slopes (π). Multivariate linear and logistic regression of σ was performed considering the baseline parameters and the π slopes as potential independent variables. In the logistic regression, subjects were divided into fast and average progressors based on a σ threshold of 2.7 mmol $mol^{-1}$ $y^{-1}$ (chosen based on the presence of a right tail in the σ distribution). Fast progressors were 26.

**Results:** The most relevant independent determinants of progression from linear regression were lower baseline GS and OGIS, and greater decrease in GS and OGIS (Table; adjusted $R^2$ = 0.23). The AUC ROC from the logistic regression using the same determinants was 0.90 ([0.82, 0.95] 95% CI). Fast and average progressors were clearly different in terms of $\pi_{GS}$ (-19.2±3.3 (mean±se) *vs* -1.3±1.3 pmol $min^{-1}$ $m^{-2}$ $mM^{-1}$, $p$ = $10^{-6}$) and $\pi_{OGIS}$ (-38.7±8.2 *vs* -12.5±1.7 ml $min^{-1}$ $m^{-2}$, $p$ = $10^{-4}$).

**Conclusion:** 1) Greater $HbA_{1c}$ progression is independently associated with baseline characteristics (fattier liver, and lower fasting glucose, age, GS and OGIS) as well as increments with time in triglycerides, ALT liver enzyme, β-cell glucose insensitivity and insulin resistance. 2) A much greater OGIS and GS deterioration was observed in fast progressors. 3) The strongest progression determinants are GS, OGIS and their longitudinal changes.

| Parameter | Multivariate linear regression | | | Multivariate logistic regression | |
|---|---|---|---|---|---|
| | Standardized β | Partial ρ | p-value | Standardized β | p-value |
| GS | -0.26 | -0.19 | $10^{-6}$ | -1.78 | 0.0005 |
| OGIS | -0.40 | -0.22 | $10^{-7}$ | -1.55 | 0.0002 |
| age | -0.12 | -0.10 | 0.01 | -0.47 | 0.03 |
| FPG | -0.15 | -0.09 | 0.03 | -0.79 | 0.02 |
| FLI | 0.18 | 0.08 | 0.0003 | 0.22 | 0.19 |
| $\pi_{GS}$ | -0.30 | -0.24 | $10^{-9}$ | -1.89 | 0.0001 |
| $\pi_{OGIS}$ | -0.33 | -0.25 | $10^{-9}$ | -0.75 | 0.02 |
| $\pi_{TG}$ | 0.12 | 0.11 | 0.01 | 0.30 | 0.06 |
| $\pi_{ALT}$ | 0.14 | 0.12 | 0.003 | 0.28 | 0.14 |

GS: β-cell glucose sensitivity; OGIS: oral glucose insulin sensitivity; FPG: fasting plasma glucose; FLI; fatty liver index; $\pi_{GS}$: uncorrected slope for GS; $\pi_{OGIS}$: uncorrected slope for OGIS; $\pi_{TG}$: uncorrected slope for triglycerides; $\pi_{ALT}$: uncorrected slope for ALT liver enzyme.

# CREATINE TRANSPORTER DEFICIENCY: NEW INSIGHTS ON CELL-SPECIFIC VULNERABILITY TO METABOLIC FAILURE

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Creatine Transporter Deficiency (CTD) is an X-linked inherited metabolic disorder presenting with cerebral creatine (Cr) deficiency, early intellectual disability, epilepsy and autistic-like behaviour. Although rare, CTD represents a major issue in health care, leading to a significant decrease of life expectancy and causing chronic illnesses with a large impact on patient quality of life and health-care system. There is no cure for this devastating disorder. Despite much knowledge about the natural history of CTD and the role of Cr in energy metabolism, little is known about the brain alterations underlying the impairment of multiple functional domains in CTD. To provide a characterization of brain morphological and neurofunctional alterations associated to CTD, we used an integrated approach combining neuroanatomical, electrophysiological and behavioural techniques. These analyses were performed in ubiquitary CrT ko mice and conditional transgenic mice carrying CrT deletion only in parvalbuminergic interneurons. We report that anomalies in GABAergic neurotransmission, particularly depending on the failure of parvalbuminergic interneurons, constitute a pathological hallmark of CTD. Consistently, CTD mice show a very specific EEG signature and a severe epileptic phenotype, as assessed through behavioural observation and video-EEG recordings in awake animals. We demonstrated that aberrant development and function of a selective neuronal population contribute to the etiology of CTD disorder. These findings will allow us to identify new potential targets for pharmacological treatment. Moreover, EEG spectral signature could be used as classifying non-invasive biomarker for evaluation of brain function in CTD and treatment assessment. Importantly, EEG is routinely included in the follow-up of patients, increasing the translational value of this biomarker.

## POSTER SESSION 1: SCHEDULE AND ABSTRACTS

| N | When | Speaker | Title |
|---|---|---|---|
| **Session 1** | | | |
| P1 | Wednesday, October 2 | Martins Manuella | *MicroRNA-mediated control over callosal projection neurons in the developing cerebral cortex* |
| P3 | Wednesday, October 2 | Trovato Francesco | *A Cre-amplifier to generate and detect genetic mosaics in vivo.* |
| P5 | Wednesday, October 2 | Cornuti Sara | *Metabolic changes influence brain plasticity in mice* |
| P7 | Wednesday, October 2 | Animali Silvia | *Indeexing arousal with pupillometry and EEG: implication for normal vs. pathological aging* |
| P9 | Wednesday, October 2 | Cicchini Guido Marco | *Specialized visual module for numerosity* |
| P11 | Wednesday, October 2 | Angelini Monica | *Short-term immobilization reduces the extent of the self-perceived peripersonal space: an immersive virtual reality study* |
| P13 | Wednesday, October 2 | Talani Giuseppe | *Early life stress induces changes on specific brain areas for cognitive and motivational functions: insight the multimodal effect of maternal separation in C57BL6J mice* |
| P15 | Wednesday, October 2 | Baldereschi Marzia | *Successful performance of the tuscany stroke network: a before-and-after study. Tuscany Stroke Network* |
| P17 | Wednesday, October 2 | Massa Verediana | *Distinct activity of pyramidal and fast-spiking neurons during a motor act in mice* |
| P19 | Wednesday, October 2 | Giordano Nadia | *Neurons derived from mescs extend projections into lesioned brain: a strategy for stroke recover* |
| P21 | Wednesday, October 2 | Montecucco Cesare | *Bacteria in the Brain: the case of Eubacterium tarantellae* |
| P23 | Wednesday, October 2 | Canzi Alice | *Lack of IL-1R8 affects interneurons development and generation* |
| P25 | Wednesday, October 2 | D'Arrigo Giulia | *Astrocytes-derived Extracellular Vesicles in motion at the neuron surface* |
| P27 | Wednesday, October 2 | Pucci Susanna | *New stilbene-ammonium nicotinic ligands as anti-glioblastoma agents* |
| P29 | Wednesday, October 2 | Redolfi Nelly | *Development and characterization of a new transgenic mice line expressing a mitochondrial Cameleon probe for real-time calcium imaging* |
| P31 | Wednesday, October 2 | Vajente Nicola | *Microtubules stabilization by mutant spastin affects ER morphology and* |

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| | | | *Ca2+ handlingmajor neuropathological hallmarks of the human pathology* |
| P33 | Wednesday, October 2 | Furlan Sandra | *Comparative analysis of Ca2+ handling proteins across vertebrate species* |
| P35 | Wednesday, October 2 | Francia Simona | *Mechanism of activation and function of the axonal odorant receptor* |
| P37 | Wednesday, October 2 | De Marco Doriana | *My kinematics as a template to decode your actions: the role of motor resonance in intention prediction* |
| P39 | Wednesday, October 2 | Scalona Emilia | *Virtual reality in virtual patients: efficacy of VR action observation treatment in speeding up the recovery of the shoulder joint* |
| P41 | Wednesday, October 2 | Vecchiato Giovanni | *NuARCH: the interplay between architecture and the brain as revealed by eeg and virtual reality* |
| P43 | Wednesday, October 2 | Mazziotti Raffaele | *Creatine transporter disorder: new insights into epileptic phenotype and diagnostic biomarkers* |
| P45 | Wednesday, October 2 | Viglione Aurelia | *Pupil fluctuations as a biomarker for cdkl5 disorder* |
| P47 | Wednesday, October 2 | Giona Federica | *Neuronal dysfunctions underlying Phelan−McDermid syndrome and theirs rescue by acute and chronic modulation of mGlu5 signaling* |
| P49 | Wednesday, October 2 | Maset Andrea | *Altered migration of inhibitory interneurons in a mouse model of intellectual disability* |
| P51 | Wednesday, October 2 | Santini Francesca | *Alterations of oxytocin receptor expression in the brain of Magel2-KO mice, a model of Prader Willi-like syndrome* |
| P53 | Wednesday, October 2 | Piano Ilaria | *Pharmacological strategies to slow down cone death and vision loss in animal models of Retinitis Pigmentosa* |
| P55 | Wednesday, October 2 | Falcicchia Chiara | *Microglia-derived extracellular vesicles carrying aβ impair cortico-hippocampal network* |
| P57 | Wednesday, October 2 | Dazzo Emanuela | *Epilepsy-causing Reelin mutations result in intracellular degradation and impaired secretion of mutant proteins* |
| P59 | Wednesday, October 2 | Gomiero Chiara | *Calcium signalling and mitochondrial function in presenilin 2–knock-out mice: is there any loss-of-function phenotype related to alzheimer's disease?* |
| P61 | Wednesday, October 2 | Elia Chiara | *Deregulated microglial production of exosomes in a mice model of Alzheimer's disease* |
| P63 | Wednesday, October 2 | Cozzolino Olga | *Evolution of epileptiform activity in zebrafish by statistical-based integration of electrophysiology and 2-photon Ca2+ imaging* |
| P65 | Wednesday, October 2 | Di Carlo Antonio | *Prevalence of atrial fibrillation subtypes in the Italian elderly. Progetto FAI* |
| P67 | Wednesday, October 2 | Ponzoni Luisa | *Increased sensitivy to the rewarding effects of Δ9-tetrahydrocannabinol and MDMA after exposure to nicotin in mice and zebrafish* |
| P69 | Wednesday, October 2 | Lobina Carla | *Can chronic Red Bull treatment during adolescence affect the mesolimbic* |

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| | | | *dopamine transmission and the cardiovascular system in adult rats?* |
| P71 | Wednesday, October 2 | Maccioni Paola | *Wide spectrum of efficacy of saikosaponins (active ingredients of bupleurum falcatum) on alcohol self-administration in rats* |
| P73 | Wednesday, October 2 | Santoni Michele | *Effect of n-acylethanolamine acid amidase inhibition on locus coeruleus noradrenergic neuronal responses to morphine* |

## POSTER SESSION 2: SCHEDULE AND ABSTRACTS

| N | When | Speaker | Title |
|---|---|---|---|
| **Session 2** | | | |
| P2 | Thursday, October 3 | Tozzi Francesca | *Associative learning and synaptic plasticity in the lateral entorhinal cortex* |
| P4 | Thursday, October 3 | Torelli Claudia | *Active training promotes recovery of visual functions in adult amblyopic rats* |
| P6 | Thursday, October 3 | Steinwurzel Cecilia | *Inter-individual variability of short-term ocular dominance plasticity in human adults* |
| P8 | Thursday, October 3 | Castaldi Elisa | *Residual visual responses in patients with retinitis pigmentosa revealed by functional magnetic resonance* |
| P10 | Thursday, October 3 | Nuara Arturo | *Catching the imposter in the brain: a clinical, neuroimaging and neurophysiological single case-study on Capgras delusion* |
| P12 | Thursday, October 3 | Righi Marco | *Quantitative analysis of OCT-A retina scans from healthy and AMD vascular plexa according to signal amount and dispersion of caliber-classified vessels* |
| P14 | Thursday, October 3 | Baldereschi Marzia | *A summary of stroke concepts from late antiquity to current days* |
| P16 | Thursday, October 3 | Baldereschi Marzia | *Prospective evaluation of post stroke rehabilitation in Florence, Italy* |
| P18 | Thursday, October 3 | Salluzzo Marco | *Direct reprogramming of reactive astrocytes in neurons in mouse motor cortex after stroke* |
| P20 | Thursday, October 3 | Panzi Chiara | *Interneuronal transfer of clostridial neurotoxins: cell specificity and long-range action in the central nervous system* |
| P22 | Thursday, October 3 | Desiato Genni | *A systematic molecular study of neuroimmune mechanisms in aging* |
| P24 | Thursday, October 3 | Corradini Irene | *Maternal Immune Activation induces synaptic alterations in the offspring* |
| P26 | Thursday, October 3 | Pillai Vinoshene | *Intravital two-photon imaging of glioblastoma mouse models* |
| P28 | Thursday, October 3 | Colombo Sara | *Role of rare missense variants of the human β4 subunit in the expression and surface exposure of α3β4 nicotinic receptors* |
| P30 | Thursday, October 3 | Tonello Fiorella | *Human secreted phospholipase A2 GIIA is tranported to the cell nucleus* |
| P32 | Thursday, October 3 | Di Benedetto Giulietta | *Defining the mitochondrial camp signalling: regulation and possible role in metabolic flexibility* |

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| P34 | Thursday, October 3 | Gomez-Gonzalo Marta | *Astrocyte control of glutamatergic transmission in ventral tegmental area* |
| P36 | Thursday, October 3 | Mostallino Maria Cristina | *Chronic treatment with bifidobacterium (longum, breve, infantis) modulates GABAA receptor gene expression, neuronal function and structure in the rat* |
| P38 | Thursday, October 3 | Del Vecchio Maria | *More than just somatosensory: intracortical responses of SII to action observation* |
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# MICRORNA-MEDIATED CONTROL OVER CALLOSAL PROJECTION NEURONS IN THE DEVELOPING CEREBRAL CORTEX

Manuella Martins[1,2], Silvia Galfrè[1,3],Marco Terrigno[1,2], Luca Pandolfini[1], Edoardo Sozzi[1], Keagan Dunville[1,2],Andrea Marranci[4], Milena Rizzo[4], Alberto Mercatanti[4], Irene Appolloni[5], Paolo Malatesta[5], Laura Poliseno[4],Robert Vignali[6], Federico Cremisi[1,2]

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Mouse embryonic cortical layering is controlled by the activity of few genes coding for transcription factors that identify radial glia (RG; Sox1, Sox2), basal progenitor cells (BP; Neurogenin2, Eomesodermin), and the mature neurons they originate, namely: deep projection neurons (DPN; Tbr1, Bcl11b, Fezf2) and superficial projection (callosal) neurons (SPN; Cux1, Satb2). The analysis of a scRNA-seq dataset (Yuzwa et al., Cell Reports 2017) highlighted early and not clustered Satb2 mRNA expression, which does not correspond to the reported protein expression pattern. We investigated the mechanisms of Satb2 expression during corticogenesis.

We developed a new method of scRNA-seq analysis to assay the co-expression of two mRNA species in single cell by Contingency Table Analysis (COTAN). COTAN analysis revealed that Satb2 mRNA has the lowest levels of mRNA co-expression and anti-co-expression with other mRNAs at single cell level among cortical transcription factor genes, suggesting a deregulated transcription. Satb2 RNA stability increases during corticogenesis. Moreover, Satb2 3'UTR confers translational bias both in cultured early progenitor cells and in vivo, where it preferentially supports GFP translation in Ctip2-negative cells. miRNAomes of corticalized mES cells cluster with embryonic miRNAomes of corresponding developmental times, indicating that these cells are an in vitro reliable model of corticogenesis. miRcatch reveals a number of miRNAs which bind to Satb2 RNA 3'UTR of in vitro corticalized mES cells. miRNAomes of Sox1:GFP sorted cells and AraC treated cells report miRNA expression profiles of progenitor cells and post-mitotic cells, respectively. In progenitor cells, only miR-92a/b and miR-541 show general high expression levels and marked decrease near at the onset of Satb2 protein expression, between DIV12 and DIV17. LNA-oligonucleotides blocking miR-92a/b and miR-541 increase Satb2 translation. miR-541 inhibition acts through predicted sites in the 3'UTR.

The translation of Satb2, the transcription factor controlling the development of callosal neurons, is under miRNA control. One main miRNA, miR-541, is contained in the evolutionarily new lncRNA Mirg1 of Eutherians, which evolved the corpus callosum, and is absent in acallosal mammals.

# ASSOCIATIVE LEARNING AND SYNAPTIC PLASTICITY IN THE LATERAL ENTORHINAL CORTEX

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The entorhinal cortex (EC) represents a major hub between the hippocampal formation and polymodal associative areas. Based on cytoarchitecture, connectivity and function of the EC can be subdivided into two main subregions: the lateral entorhinal cortex (LEC) and the medial entorhinal cortex (MEC). From the functional point of view, the activity of MEC neurons appears to be spatially modulated, whereas LEC neuronal firing correlates with object position. However, the synaptic changes induced by object-place-context associative learning in the intrinsic circuitry of the LEC have not been investigated. Our aim is to study the plasticity phenomena occurring in the LEC after the novel object-place-context recognition test (OPCRT). This test is based on a sequential presentation of objects in two different contexts. Mice are tested for their ability to discriminate novel object-place-context associations with respect to familiar ones. Changes in synaptic plasticity were studied recording field excitatory post-synaptic potentials (fEPSP) in LEC superficial layer II using acute brain slices obtained from C57BL6/J mice. We found that the main forms of synaptic plasticity, namely LTP and LTD, can be elicited by high frequency stimulation (HFS, three trains of 100 pulses at 100 Hz, 10 s interval) and low-frequency stimulation (LFS, 900 paired pulses at 1 Hz, 30 ms interpulse interval), respectively. We first demonstrated that bilateral LEC lesion impairs the associative learning in the OPCRT but not the non-associative task in the novel object recognition test (ORT). We then characterized the time course of the associative memory performing the OPCRT test trial at different time points (1h, 6h, 12h and 24h) and looked at the effects on LTP and LTD in EC slices following the execution of the task. Our results confirm the involvement of the LEC superficial layer plasticity in the OPCRT paradigm and encourage us to further investigate the specific role of LEC neurons subtypes in the formation and retrieval of associative memories.

# A CRE-AMPLIFIER TO GENERATE AND DETECT GENETIC MOSAICS IN VIVO

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Genetic mosaicism refers to the presence of genetically distinct cellular populations within the same individual. Mosaic modelling is a major theme in life science, as mosaicism is associated to many pathological conditions: several Mendelian disorders, chromosomal aberrations, neurological dysfunctions as focal dysplasia or autism spectrum disorders, and cancer have been directly or indirectly connected to certain degrees of mosaicism. Moreover, sparse mosaic labelling is a powerful tool for the study of single-cell functions and cell-autonomous effects of selective overexpression/knockout. Currently, many diffused approaches for the generation of mosaic models make use of the Cre-Lox technology. In these systems, Cre activated reporters are used to tell recombinant and non-recombinant cells apart. Our goal is to define a Cre-based strategy to differentially label both wild type (WT) and knockout cells (KO) for a gene of interest, with a fine control of the mosaicism level.

The general idea is to co-transfect two different plasmids. The first plasmid, based on the Cre-switch design, is a Cre reporter relying on the FLEx system that switches expression between two fluorophores (RFP/GFP) depending on Cre activity. The second plasmid, carries Cre recombinase and it acts as a trigger for the recombination. The concentration of the trigger plasmid determines the mosaicism entity. However, the necessity to have a bi-univocal correspondence between the genomic floxed allele status and the Cre-reporter readout is difficult to fulfill with the low levels of recombinase activity required to induce a sparse recombination. Indeed, at low Cre concentration, recombination has
been described to occur in the reporter, but not in the gene of interest or vice versa, thus leading to an incorrect reporting of the recombination state of the genomic floxed sites.

We designed Beatrix, a general-purpose tool specifically devised to amplify weak Cre recombinase activity, and we used it to develop a powerful approach for the in vivo generation and detection of sparse mosaics of mutant and wild type (WT) cells. In this way we can generate genetic mosaic of arbitrary density where each cell reports its genomic state by the expression of a specific fluorescent protein: RFP expressing cells are wild type, while GFP cells are mutated. By means of this tool we have generated three different mosaic system: 1) in primary cultures of neurons from a model of Rett syndrome (MeCP2 flox/flox ) we generated a cultured mosaic of WT and MeCP2 -/- neurons; 2) by applying post-natal electroporation in a PTEN flox/flox mouse we generated a mosaic of WT and PTEN -/- interneurons in the olfactory bulb; 3) by in utero electroporation we generated a mosaic of WT and PTEN -/- pyramidal neurons in the visual cortex. In these models, we could rapidly detect a morphological and functional phenotype associate to the mutation thus opening the way to the study of the physiology of individual mutated cell within a genetic mosaic.

# ACTIVE TRAINING PROMOTES RECOVERY OF VISUAL FUNCTIONS IN ADULT AMBLYOPIC RATS

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

# METABOLIC CHANGES INFLUENCE BRAIN PLASTICITY IN MICE

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It is becoming clear that diet and lifestyle can affect brain physiology. In spite of the well-known effects of specific diets' on the outcome of several neurological diseases, little is known about how metabolism modulates neural function. For instance, fasting and ketogenic diet (KD) control seizures in epileptic kids, although how fasting and ketone bodies' affect brain physiology, and the molecular mechanisms involved, are still enigmatic. Here, we investigate how a specific metabolic challenge: i.e. fasting, could influence neural physiology and plasticity in mouse models. Since the visual system is probably the deepest understood system of the human brain and a classic model to study experience-dependent plasticity in rodents, we focused on the visual system to assess 48 hours (h) fasting impact on neural function and plasticity in mice, and to analyze the molecular/epigenetic adaptation to this metabolic challenges.

Using intrinsic optical signal imaging, we found that ocular dominance(OD) plasticity was enhanced in adult mice after 48h fasting simultaneously to 2 days of monocular deprivation. In critical period (CP) mice undergoing the same protocol, OD plasticity was not affected by 48h fasting. However, following a deeper analysis of visual responses to different spatial frequencies, we observed specific changes correlating with alterations in blood glucose concentration.

To further investigate the effect of 48h fasting on mouse general activity along the 12h light:12h dark cycle, locomotor activity was assessed both in juvenile and in adult mice. As expected, we observed increase in general activity in both young and adult mice during the fasting period.

To look inside the molecular mechanisms underlying fasting-driven plasticity we performed a RNA-seq on the visual cortex of CP mice. Strikingly, a large set of genes was differentially expressed in the fasting group compared to ad libitum fed control animals, Moreover, significant alterations in gene expression was also detected in adult mice subjected to fasting. In particular, plasticity related genes, like *Npas4, Bdnf, Arc,* were increased after fasting.

Finally, since fasting is able to increase beta-hydroxyl-butyrate (BHB) plasma concentration, and BHB is a new epigenetic factor, we analysed the new post-translational modification K9-beta-hydroxyl-butyrylation (bhb) on histone H3. ChIP-seq revealed a significant enrichment of H3K9-bhb in promoter and enhancer regions of genes upregulated in the fasting group compared to control animals.

In summary, our data suggest that fasting is able to affect brain physiology and, particularly, to modify plasticity level in the visual cortex through different mechanisms still under-investigation and probably involving BHB-driven molecular changes.

# INTER-INDIVIDUAL VARIABILITY OF SHORT-TERM OCULAR DOMINANCE PLASTICITY IN HUMAN ADULTS

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

# INDEEXING AROUSAL WITH PUPILLOMETRY AND EEG: IMPLICATION FOR NORMAL VS. PATHOLOGICAL AGING

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

# RESIDUAL VISUAL RESPONSES IN PATIENTS WITH RETINITIS PIGMENTOSA REVEALED BY FUNCTIONAL MAGNETIC

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Purpose: We evaluated the potential of magnetic resonance imaging in identifying signs of cortical visual processing with greater sensitivity than standard ophthalmological measures in RP patients at advanced stage.
Methods: Eight patients affected with retinitis pigmentosa with only bare light perception and weak or absent visual evoked potential (VEP) or electroretinogram (ERG) responses to flashes of light were tested. Visual impairment was evaluated by means of psychophysical testing, where patient were asked to discriminate the drifting direction of a contrast modulated grating. Patients underwent MRI scanning and the behavioral performance was correlated with both Blood-Oxygenation-Level-Dependent (BOLD) signal elicited by flashes of lights and cortical thickness measured in primary visual area.
Results: Contrast sensitivity to drifting gratings of very low spatial and temporal frequency was greatly impaired yet measurable in all patients. Weak luminance flashes elicited significant BOLD responses in striate and extra-striate cortex, despite the stimuli were not perceived during scanning. Importantly, patients with less severe impairment of contrast sensitivity showed stronger V1 BOLD responses. Striate cortical thickness did not correlate with visual sensitivity.
Conclusions: BOLD responses provide a sensitive and reliable index of visual sparing more than VEPs or ERGs, often absent in RP patients. The minimal residual vision can be assessed by optimal visual stimulation in two alternative forced choice discrimination tasks and by BOLD responses. Imaging techniques can provide useful information to monitor progressive vision loss.

# SPECIALIZED VISUAL MODULE FOR NUMEROSITY

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There is strong evidence that humans can make rough estimates of the numerosity of a set of items, almost from birth. However, as numerosity covaries with many non-numerical variables, the idea of a direct number sense has been challenged. Here we summarize recent research which test which variable (size, density, numerosity or ink) is the one that subjects consider most when they are asked implicit tasks (i.e. find the odd-stimulus or reproduce the pattern).
Our research shows that numerosity is the dominating dimension and that changes in density and area do matter only if combined with each other to yield a numerosity estimate.
This demonstrates that numerosity is a primary feature with high biological relevance that subjects develop with high reliability. In addition we demonstrate that these properties appear even before schooling begins and are preserved in dyscalculic subjects. Overall our research reveals that numerosity, whilst requiring a complex classification of visual images, it is learned in a formally correct way even early in development and it is preserved throughout lifetime

# CATCHING THE IMPOSTER IN THE BRAIN: A CLINICAL, NEUROIMAGING AND NEUROPHYSIOLOGICAL SINGLE CASE-STUDY ON CAPGRAS DELUSION

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Objectives: Capgras delusion (CD) is a rare condition characterized by the belief that some people –mostly familiars– have been replaced by imposters[1]. Despite the preserved ability to overtly recognize familiar faces, CD is often associated with an impaired selectivity of autonomic responses between familiar vs unfamiliar faces[2]. Two main abnormalities are thought to lead to CD: the failure to attach emotional value to familiar faces, and an impaired consistency-checking mechanism favoring delusional belief[3].
Materials and Methods: Here we describe a case of I.F., an 87-year-old male without previous psychiatric or neurological history, except the incidental MRI finding of a 6-cm-diameter right-anterior temporal arachnoid cyst. Three months before our observation, he developed the delusional belief that his son was substituted by an imposter. Such belief – not directed toward his daughter – was selective for visual modality. A FDG-PET performed after delusional onset demonstrated fronto-parietal hypometabolism. Neurological examination was normal, excepting mild episodic memory disturbances (Mini-Mental-Status-Examination 28/30, Clock-Drawing-Test 5/6).
We collected nasal-tip temperature (NT) by means of cutaneous Thermal Infra-Red (FT-IR) imaging simultaneously to autonomic skin-conductance-responses (SCR) to visual presentation of three different face categories to visual presentation of three different face categories: familiar delusion-related (Face-S, i.e. son's face), familiar not-delusion-related (Face-D, i.e. daughter's face), not-familiar (Face-NF). A similar protocol evaluating autonomic responses throughout auditory modality was applied using pre-recorded voices of the son (Voice-S), daughter (Voice-D) and not-familiars (Voice-NF). FT-IR mean temperatures, as well as SCR peak-to-peak amplitudes were converted to Z-scores. The ability to overtly recognize emotion expressions, as well as familiar faces and Benton Facial-recognition Test (BFT) were also assessed.
Results: NT decreased during Face-D presentation (-0.21) in comparison to both Face-S and Face-NF (respectively 0.05 and 0.13). NT diminished during Voice-D and Voice-S presentation (respectively -0.12 and -0.15) in comparison to Voice-NF (0.04). SCR resulted greater for Face-D (0.18) in comparison to both Face-S and Face-NF (respectively -0.07 and -0.09). SCR to voices was higher for both Voice-D and Voice-S (respectively 0.30 and 0.21) in comparison to Voice-NF (-0.43). Taken together, these data indicated an autonomic modality-dependent dissociation coherent with delusional behavior. Of note, I.F. showed normal performance in emotion recognition task (10/10), face-familiarity (18/20) and BFT (17/18).
Discussion: Our case support the "two-hit-hypothesis"[3] about CD etiopathogenesis: the first condition is here represented by the right-temporal compressive lesion involving face-recognition key areas and limbic structures such as amygdala, resulting in an impaired autonomic and affective coupling to familiar faces. The second one relies on the prodromal fronto-parietal neurodegeneration favoring delusional behavior. Without any "emotional glow" surrounding familiar-face representation, I.F. creates separate memory traces of the same person (i.e. the son). Interestingly, such duplicative-paramnestic phenomenon presents a double selectivity for both modality (i.e. visual) and person (i.e. son), suggesting the involvement of modality-specific stages in the retrieval of affective familiarity-related properties during person-recognition.

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# SHORT-TERM IMMOBILIZATION REDUCES THE EXTENT OF THE SELF-PERCEIVED PERIPERSONAL SPACE: AN IMMERSIVE VIRTUAL REALITY STUDY

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Introduction

Short-term arm immobilization and nonuse induce not only a reduction of motor cortex excitability and a deterioration of motor performances [1], but also deficits in motor-related perceptual processing, such as space encoding and categorization, which are fundamental for context-dependent action planning and, more in general, motor control. For instance, the extent of the peripersonal space (PPS), i.e. the body-centered region immediately surrounding our body and in which objects can be grasped and manipulated, is reduced by brief (10-24 hrs) arm immobilization periods in healthy people [2, 3]. To date, however, whether the PPS reduction derives mainly from an immediate effect of the arm restriction, or rather it accumulates over the immobilization period remains an open issue. The present study addressed this question, administering a Reachability Judgment Task (RJT) before and during a 1-hour immobilization time-window and taking advantage of immersive virtual reality (VR) technique.

Methods

Sixteen healthy, right-handed volunteers (9 females, age 25.8±4.1 years) were recruited. They sat on a chair wearing a HTC Vive Pro head-mounted display (HMD), which presented experimental stimuli in an immersive VR environment. Participants performed a Reachability Judgment Task (RJT) [3], by assessing the extension of individual PPS. RJT stimuli consisted in a ball appearing in different positions (from 30 to 170 cm, with steps of 5 cm) on a 2x4 m virtual table. Participants had to quickly report via button push, whether the ball was reachable or not. RJT was performed at three time points: 1) at baseline (T0); 2) immediately after the application of a soft arm bandage to participants' arm and forearm, preventing any arm movement (T1); 3) after 1 hour of immobilization, before the removal of the bandage (T2). During the 1-hour immobilization, participants observed 3D virtual video clips showing landscape devoid of any biological motor content. The individual boundary of the reachable space was determined by fitting data with a logistic regression and calculating the point of subjective equality (PSE). Statistical analyses on PSE values were carried out through a repeated-measures ANOVA ($p$<.05) with Time (3 levels: T0, T1, T2) as within-subject factor and Tukey's post-hoc tests.

Results

A significant TIME effect was found on the PSE values (mean±SD T0=98.11±16.1 cm, T1=88.7±19.5 cm, T2=90.04±20.7 cm; $F(2,30)=10.637$, Greenhouse and Geisser-corrected $p=0.001$). Post-hoc comparisons revealed that the PPS extent was significantly larger at T1 and T2 relative to T0 ($p=0.006$, $p=0.003$, respectively), while no significance was found between T1 and T2 ($p=0.8$).

Our preliminary data show that a shrinkage of the PPS occurs immediately after arm immobilization and that this effect remains stable after 1 hour of immobilization. The perception of the action space plays a key role in the pre-movement phases of action control, providing body- and environment-related constraints, which ultimately may lead to the decision about to act. Hence, in clinical conditions leading to arm non-use (such as in neurological patients with damages to the motor cortex or orthopaedics patients with imposed limb immobilization), the baseline and follow-up evaluation of motor space perception could represent a useful neurophysiological index for the monitoring of motor system recovery during rehabilitative interventions. These findings present practical implications, suggesting that: 1) the cognitive functions proper of the cortical motor system (here, PPS encoding) may provide clinical indicators in rehabilitative settings, even when real movements are impeded, and 2) starting rehabilitative interventions as early as possible could promote a wider and more complete recovery of motor and cognitive functions after cerebral or musculoskeletal damages, leading to arm nonuse.

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# QUANTITATIVE ANALYSIS OF OCT-A RETINA SCANS FROM HEALTHY AND AMD VASCULAR PLEXA ACCORDING TO SIGNAL AMOUNT AND DISPERSION OF CALIBER-CLASSIFIED VESSELS

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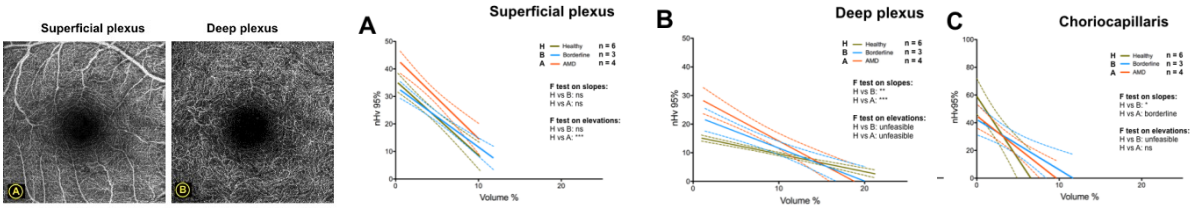
Description of vascular angioarchitectures observed in different plexa of physio-pathological retinas is a difficult task. In this respect, a new analytical approach based on amount and dispersion of caliber-classified vessels was recently validated in tumors. There, vascular angioarchitectures from xenotransplanted experimental tumors could be summarized as a near-linear relationship on the basis of the amount, dispersion and caliber of the observed vascular trees [1]. Furthermore, the slope of the resulting lines correlates with the angiogenic potential of the tumors. The same approach, applied to cerebral vessels of the *twitcher* mouse (a model of Krabbe disease), confirmed evidence of reduced angiogenesis in the developing frontal cortex [2].

Here we applied this image-analysis approach to retinal tissues developing a semi-automatic quantitative protocol for descriptions of microvascular angioarchitectures on the basis of a small set of interdependent parameters. Briefly, OCT-A images were obtained with a TOPCON scanner and elaborated by ImageJ custom scripts. Efforts resulted in a rationale for automatic isolation of binary representation of retinal vascular plexa. The resulting signals were then classified according to the minimal vascular cross-section observed on the XY Cartesian planes and OCT-A artefacts removed assigning to vessels a depth not greater of their planar caliber. The final analysis was carried out on partially reconstituted vascular trees, obtained by serially combining the vessels with different calibers belonging to the same plexus. Analyses of automatically segmented retinal tissues from OCT-A scans were carried out on physiological eyes and on a minimal cohort of patients suffering from macular degeneration (AMD). In some of these patients, we quantified a vascular reduction in the deep plexus of pathological eyes together with an increase in the microvascularization of corresponding choroidal




tissues. In addition to contributing to the identification of vascular alterations, this approach candidates itself as an useful tool to monitor disease progression and the effectiveness of anti-AMD therapies. Such an approach can be extended to the many neurodegenerative pathologies affecting retinal vasculature. Coupling low invasiveness with high sensitivity, OCT-A can turn out very useful in monitoring retinal alterations in their very first phases.

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# EARLY LIFE STRESS INDUCES CHANGES ON SPECIFIC BRAIN AREAS FOR COGNITIVE AND MOTIVATIONAL FUNCTIONS: INSIGHT THE MULTIMODAL EFFECT OF MATERNAL SEPARATION IN C57BL6J MICE

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Stress occurring early in life may be predictive for the development of neuropsychiatric disorders as well as increased vulnerability to drug use disorders in adulthood. Repeated maternal separation (RMS) in rodents is a powerful model to investigate the consequences of neonatal stress on brain plasticity and vulnerability to ethanol (EtOH) abuse. Here we extended our recent findings about the potential mechanisms involved in the long-term effects of RMS in C57BL/6J adult mice by evaluating the changes in neuronal plasticity at both GABAergic and glutamatergic synapses in the hippocampus (hip) and nucleus accumbens (NAcc), areas involved in learning and memory function and drug reward, respectively. Patch-clamp experiments performed in the hip revealed that RMS causes a significant enhancement in the tonic component of the GABAergic inhibition in dentate gyrus granule cells from male, but not females, mice. RMS is also accompanied in males by a marked increase in the frequency of GABAergic IPSCs recorded in the same neurons. Interestingly, all these changes induced by RMS were paralleled by an impairment in LTD formation in the CA1 subregion in male but not in female mice, an effect that may involve an increased function of the endocannabinoid system and which was accompanied by an impaired cognitive performance in the Barnes maze test. RMS is also associated in males with a marked increase of EtOH intake and preference in the two-bottle free choice paradigm, while in females this result is not statistically relevant. We observed a significant RMS-induced changes in synaptic plasticity with a reduction of LTD formation in the NAcc MSNs, an effect that is accompanied with an impairment of the AMPA/NMDA ratio. Interestingly, these functional and behavioral changes were no longer appreciable in RMS male mice when treated with a single injection of beta-ethinylestradiol at PND3, suggesting that alteration in the hormonal asset may strongly influences the neuronal and behavioral impairments induced by RMS. Taken together, these findings demonstrate that RMS is associated with long-lasting effects on synaptic plasticity in both hip and NAcc of C57BL/6J male mice, alteration of learning and memory as well as goal directed behavior. In line with previous findings, our data may support a gender-dependent effect of RMS. Supported by CNR-DISVA-Sardegna Ricerche

# A SUMMARY OF STROKE CONCEPTS FROM LATE ANTIQUITY TO CURRENT DAYS

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BACKGROUND. The long-standing concept of apoplexy (or the latin equivalents of morbus attonitus and sideratio) can be followed from antiquity, passing through the Middle Ages and Renaissance, and reaching the modern era and the present day, with the new designation of stroke.
METHODS. Relevant primary sources and secondary material as well as selected illustrations were identified and interpreted in their historical context.
RESULTS. Ancient descriptions and concepts of apoplexy were not guided by observations , were unconcerned with the pathogenesis, and concentrated on symptomatology and treatment. Apoplexy definition can be divided, by the history of post-mortem examination, into a period predating this practice, which spanned from antiquity until Renaissance, and an autopsy period of the Modern era. In the first period apoplexy was believed to be a result of the imbalance of the four humors (yellow bile, black bile, phlegma, and blood). When autopsies became relatively common in the 16th century the humoral theory began to be questioned. During the 16th and 17th centuries scholastic approaches were merging with an observational approach to medicine and Galen's speculation that apoplexy was due to an accumulation of phlegma or black bile in the cerebral ventricles was seriously challenged. Vesalius, Willis, Wepfer, and Morgagni greatly contributed in forming the notion that apoplexy was the result of a disturbance of cerebral vasculature. Nevertheless humoral physiology was still employed as a foundation for apoplexy treatment. It was R. Virkow (1812-1902) who would expand the vascular theories of occlusive stroke with the introduction of the terms thrombosis and emboli into the medical literature, focusing attention on the physiological mechanisms of ischemic stroke. Pathologists at this time believed that ischemic stroke was of inflammatory origin and that thrombosis occurred as a a consequence of inflammation. Following the mid 20th century work by C. Miller Fisher, with the recognition of the importance and the therapeutic implication of the carotid artery in stroke, the specialty of stroke medicine came into being. Therapeutic nihilism was replaced by an increasing armamentarium of therapeutic interventions. During 1970s and 1980s the advent of CT and later MRI scanning made it possible to visualize the brain parenchyma and paved the way to the evidence-based current treatments.
CONCLUSIONS. To sum up, the history of stroke is characterized by great cognitive successes but by a slow and late therapeutic progress.

# SUCCESSFUL PERFORMANCE OF THE TUSCANY STROKE NETWORK: A BEFORE-AND-AFTER STUDY

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AIMS. Faced with the burden of stroke, a regional strategy was implemented in Tuscany at the beginning of 2015. A hub-and-spoke model was established throughout tuscanian stroke hospitals adopting a common protocol to standardize acute ischemic stroke (AIS) care, leading to the implementation of 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 nearest hub hospital, where appropriate. We investigated differences in quantity of revascularization treatments for AIS patients before and after the TSN implementation, to explore and monitor its effectiveness.
MATERIALS AND METHODS. This interventional study was conducted from January 1, 2014 to December 31, 2018 and included all patients with AIS consecutively treated in each of the 22 TSN hospitals. Before and after analysis was conducted using data covering the entire region. We estimated an expected number of 9000 AIS patients per year. We measured 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 (2015-2018) 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 1312 (14,6%) in 2018. The implementation of the TSN resulted in 1549 additional patients treated with t-PA from 2015 to 2018, yielding to an health benefit of 937,1 DALYs avoided. An increasing number of both secondary transfers have been activated, yielding to an increasing number of endovascular treatments performed eventually by the hub hospitals throughout the observation period.
DISCUSSION. The logistic interventions provided by the TSN resulted in more than 1500 stroke patients receiving the benefits of revascularization treatments, that are highly cost-effective. Our data provide a sound feedback that AIS care delivery can be improved through organization and logistics.
CONCLUSIONS. Increasing the ratio between treated and eligible AIS patients could thus provide savings both in economic and in DALYs areas. Our data suggest both the need and the benefit of organized stroke systems.

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# PROSPECTIVE EVALUATION OF POST STROKE REHABILITATION IN FLORENCE, ITALY

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AIMS. Recovery and return to a full and meaningful life following stroke are the main goals for stroke survivors, their families and health professionals. Stroke rehabilitation requires coordination and integration of in- and out-patient health services to be effective. This survey aimed at auditing access, quality and effectiveness of the transition from in-hospital to out-patient rehabilitation of all the adult stroke survivors in the Florence area.
METHODS. All adult (18 years of age and over) acute stroke patients discharged from all the three in-patient rehabilitation facilities of the Florence area from October 1, 2016 to September 30, 2017 were enrolled in the study. A standardized telephone questionnaire was prepared and then administered by two trained physiotherapists at 6 and 12 months after discharge from each in-patient rehab facility. Questionnaire was designed to estimate self-perceived rehab accessibility, duration and effectiveness. Moreover, functional outcomes were estimated by the means of modified Rankin Scale (Van Swieten et al., 1988). The study protocol was approved by the Institutional Review Board of the Tuscany Regional Health System. Informed consent was obtained from each subject before enrollment.
RESULTS. After excluding patients who died or received palliative care, 120 stroke patients were enrolled in the study. Their median age was 74 years (IQR, 64-83 years) and 51,7% were men. Out of the 80 (67%) stroke patients who were prescribed with continuing rehabilitation, the 31,3% could not take advantage of public rehab services, mainly for logistic reasons. Public rehab services were available but time schedule and transportation issues precluded their utilization. The 36% of stroke patients contacted and used private rehab services.
Most stroke patients and caregivers perceived rehabilitation as effective, but did complain a too short duration. All the 45 patients (56,3%) who managed to continue rehab beyond the first 6 months acknowledged further effectiveness, and the Modified Rankin Scale score was <3 in 36% of those patients.
DISCUSSION. Our data suggest that logistic improvements are needed to make public out-patient rehab really accessible, and highlight the unmet needs of extending rehabilitation duration beyond the current availability (two 1-month rounds).
CONCLUSIONS. Our study could monitor, in the real world, the sequential transition from in-patient to out-patient post-stroke rehabilitation, that is commonly reported as a bottleneck in the entire stroke care pathway (Allen D and Rixson L, 2008). This study also serves as an exploratory research to develop and model effective post-stroke rehabilitation interventions in Italy.

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# PHYSIOLOGICAL BIOMARKERS TO PREDICT MOTOR RECOVERY AFTER STROKE IN MICE

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Stroke is the second leading cause of death and the third leading cause of disability worldwide. Although stroke damage can be devastating, many patients survive the initial event and display a spontaneous recovery, which can be further increased by rehabilitation therapy. Recovery is possible due to a reorganization of spared areas and connections, but the extent of the functional outcome is highly variable. Currently, there are no ways to determine either the extent or time-course of recovery in individual subjects. Great is therefore the need for reliable biomarkers predictive of spontaneous recovery and responsive to rehabilitation to allow a better patient stratification in clinical trials and to personalize therapies, maximizing the final outcome. In our study we took advantage of a mouse model of middle cerebral artery occlusion (MCAO), which is the main cause of ischemic insult in humans, to investigate novel prognostic and therapeutic tools in preclinical models. In order to determine the amount of spontaneous recovery after MCAO, we conducted, at different time point, a battery of behavioral tests: gridwalk test, skilled reaching test, and a retraction task in the M-platform, a robotic device that permits to quantitatively evaluate several kinetic and kinematic parameters related to forelimb movement. Moreover, to mimic clinical scales used in stroke patients, we implemented a novel “Motor Score” comprehensive of the motor performance assessed using single motor tests. The Motor Score was able to detect a motor deficit after MCAO, showing a certain degree of variability in terms of spontaneous recovery, considering late time points (30 days post-stroke). Mice were also implanted with chronic electrodes in the caudal forelimb area (CFA) to record local field potentials (LFPs) from both hemispheres during the retraction task in the M-platform and in freely moving condition. Novel quantitative methods were also implemented to evaluate lesion size, location and shrinkage in histological brain sections obtained 30 days after injury. Particularly, evaluating the exact lesion location for each subject allows to measure the percentage of intact CFA, possibly an important index underling post-stroke recovery.

Finally, electrophysiological and anatomical changes will be then correlated with the extent of motor outcome to highlight physiological predictive biomarkers of spontaneous recovery.

The results deriving from this study could shed light on the mechanisms used by the central nervous system to reorganize and rewire the spared areas following a stroke, and could finally find reliable biomarkers with high translational potential to predict functional recovery in stroke patients.

# DIRECT REPROGRAMMING OF REACTIVE ASTROCYTES IN NEURONS IN MOUSE MOTOR CORTEX AFTER STROKE

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

# DISTINCT ACTIVITY OF PYRAMIDAL AND FAST-SPIKING NEURONS DURING A MOTOR ACT IN MICE

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The premotor cortex is necessary for motor planning. In mice, a putative premotor area controlling voluntary licking has been identified and physiologically mapped in the anterior-lateral motor cortex (ALM). However, the role of distinct physiologically identified ALM neuronal classes in voluntary movement planning and execution is still unknown.

To address this issue, we used head-restrained mice trained to lick a reward delivered at random intervals. Mice spontaneously performed either single isolated licks or a burst of consecutive licking events (6-8 Hz), which we categorized, a posteriori, into two classes: single (=1 lick) and multiple licks (≥3 consecutive licks). During the task, we extracellularly recorded single unit activity from the ALM using a 16-channels single shank silicon probe. We identified putative pyramidal (PNs) and fast-spiking neurons (FSNs) based on well-established physiological features of their spike waveforms, and then we investigated their functional properties during the licking task.

We found that most of the neurons' activity anticipated the licking onset by 100-200 ms. This is consistent with an involvement of the ALM in lick planning. Most of the neurons (about 90%) increased their firing frequency in correspondence with the movement, but suppressive modulations were also observed in a subset of units. For both PNs and FSNs, we found significantly greater discharge during multiple than single licks. Notably, FSNs modulated their activity about 100 ms earlier than the PNs.

During multiple vs single licking events, the peak discharge was significantly delayed for both PNs and FSNs. Furthermore, almost all FSNs showed a peak in their response before the beginning of the sequence of licks.

The differential timing of activation of PNs and FSNs suggests that inhibitory activity may be relevant for voluntary movement initiation, and FSNs appears to be more directly related to the planning of an entire motor sequence.

# INTERNEURONAL TRANSFER OF CLOSTRIDIAL NEUROTOXINS: CELL SPECIFICITY AND LONG-RANGE ACTION IN THE CENTRAL NERVOUS SYSTEM

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Tetanus neurotoxin (TeNT) and botulinum neurotoxins (BoNTs) are clostridial neurotoxins, which show high specificity for the nervous system. They impair neurotransmitter release, causing spastic and flaccid paralysis, respectively. For TeNT, although is believed that this toxin enters GABAergic terminals following transport, no clear evidence has been reported in literature to date. Since the routes of transport and spreading exploited by the neurotoxins could be also hijacked by pathological protein aggregates, as it has been shown for tau in the brain, it has become increasingly important to study and understand such mechanisms. The aim of this work is to study the molecular mechanisms of TeNT spreading and cell specificity, by using both *in vitro* and *in vivo* approaches. In the *in vivo* experiments, we analyzed the spreading of TeNT in the CNS after injection of toxin in peripheral muscles. In particular, we injected TeNT in peripheral muscles, such as the naso-labial or hindlimb musculature, of wild type mice and analysed the presence of its cleaved substrate (cleaved VAMP/synaptobrevin) in the CNS. We have determined which synaptic terminals in the brain are preferentially targeted by the neurotoxin after retrograde axonal transport from the periphery. Our data in the facial nucleus (FN) demonstrate that TeNT, following transport, preferentially targets inhibitory terminals in the CNS. The toxin also presents a significant affinity for VAChT positive terminals in the FN, probably corresponding to C-boutons afferents. In a second part of the *in* vivo experiments, we have tried to selectively target specific mechanisms controlling exocytosis and endocytosis, such as clathrin-dependent endocytosis, using drugs (DYNGO-4a and/or Pitstop 2) administered via intracerebroventricular injection and comparing the amount of cleaved VAMP in the CNS. For *in vitro* part, we are currently collaborating with Schiavo's laboratory at the UCL to set up a model to study the molecular mechanisms used by the toxins and the cellular populations that are preferentially targeted. To approach the problem, we plan to use microfluidic chambers (MCFs). This study will allow us to dissect the molecular mechanism of trans-synaptic transfer of these neurotoxins, and insights on the routes exploited by pathogenic protein aggregates to spread across neuronal circuits. This strategy is likely to reveal potential novel targets for therapeutic intervention for nervous system disorders.

# BACTERIA IN THE BRAIN: THE CASE OF *EUBACTERIUM TARANTELLAE*

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Introduction: The brain is generally considered an organ free of bacteria, that can be infected only after the alteration of anatomical barriers by inflammation of the meninge caused by bacteria that have established a first infection in anoher part of the body (spirochetes, meningococci etc.). However, in Udey et al. (J. Fish. Res. Board Can. (1977) 34:402-409) described the first report of a bacterium isolated and cultivated from the brain of apparently normal fish, as well as from fish that were affected by twirling and whirling movements, both collected in Byscaine Bay, Florida, USA. Accordingly, this bacterium was named *Eubacterium tarantellae*. The bacterium was found to be anaerobic and non spore forming. No other papers followed this first report apart from a publication describing the isolation of *E. tarantellus* from fish larval gut flora (2010) and from one human case of joint septic arthritis (2019).
Aim: To characterize *Eubacterium tarantellae* and to identify the bacterial molecule(s) responsible for inducing the characteristic twirling / twisting movement in fish.
Methods: The bacterium was cultivated either in fortified cooked meat medium or in synthetic medium thioglycollate broth under anaerobiosis. It was subjected to SEM after fixation/dehydration and gold-shadowing. DNA was sequenced with the Illumina technology with a 60x coverage. DNA alignment and analysis was performed with the SPAdes software 3.10.1. The bacterium or its culture supernatant were injected in zebra fish or in mice using a stereotactic apparatus. Movement analysis was performed by videorecording.
Results: 1) The supernatant was collected by centrifugation of a synthetic liquid culture medium and injected in the brain of fish and mice. Mice did not show any alteration of movement with any of the bacterial preparations no matter in which part of the brain they were injected. 2) Scanning Transmission Microscope analysis of *E. tarantellae:* bacterial cultures showed very elongated bacteria, with 0.8-1 µm diameter and 20-40 µm length 3) Genome sequencing of *E. tarantellae*: The *E. tarantellae* show the highest homology with *Clostridium perfringens* (40% of genes encoding for known proteins and for predicted preteins are similar) and then a lower similarity with *Clostridium botulinum* and with other species of the genus *Clostridium.* This genome includes several toxins present in other Clostridia.
Conclusion: *Eubacterium tarantellae* was identified and named by Udey (1977) following the observation of the typical twirling / twisting movements that it induced in several fish species including striped mullet, bluefish, seatrout, menhaden and snook. The bacterium was always isolated from the brain with only 7 % isolation from the intestine and 5% from liver and was found to be anaerobe and non spore forming. The ensemble of these properties led us to decide to investigate more in detail this bacterium with the final goal of identifying the molecule(s) responsible for the singular phenotype induced. We have defined a biological read out in a convenient laboratory fish model species, i.e. the zebra fish, and have defined that the putative toxic molecule ( a bacterial toxin ?) is a protein. We have determined the genome whose analysis is ongoing, but already allow us to support the proposed inclusion of *E. tarantellae* within the genus *Clostridium* with the novel species name: *Clostridum tarantellae*.

# A SYSTEMATIC MOLECULAR STUDY OF NEUROIMMUNE MECHANISMS IN AGING

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Age-related intellectual disabilities represent one of the crucial public health problems of this century making it crucial to define strategies to dampen the effects of aging, preventing or delating the occurrence of neurodegenerative pathologies, such as Alzheimer's Disease (AD).
Recent evidence suggests that these disorders are associated with enhanced neuroinflammation which, in turn, directly or indirectly affects neuronal function and survival, giving rise to a vicious cycle possibly causing or accelerating the neurodegeneration. Aim of this work is to understand the molecular mechanisms through which neuroinflammation leads to age-related pathologies in the CNS and to provide a proof-of-concept for therapeutic treatment. We performed a deep immune-profiling of an Alzheimer's disease (AD) murine model (APPswe/PSEN1dE9) at distinct pathological phases of the disease (before, during and after the onset of the disease), in order investigate the precise timing of the occurrence of neuroinflammation. We found an early elevation of several pro-inflammatory cytokines and chemokines (Th1 response, featuring IL-6 and CXCL10) in hippocampus and cortex, two brain regions particularly affected by this disease. A similar analysis is being performed a cohort of elderly patients with or without cognitive impairments, recruited within the project Train-The-Brain, who will be examined for the levels of a wide panel of inflammation-derived mediators in the attempt to provide clear associations between inflammation and cognitive impairment. To explore the molecular underpinnings of inflammation-mediated neuronal impairments we used an in vitro approach to assess the role of single cytokines in cultured neurons. In particular, we found that IL-6 application enhances the synaptic transmission of mature hippocampal neurons, suggesting that inflammation might lead to a detrimental hyperexcitability, thereby mimicking the early neuronal impairment occurring during the first phases of the disease.

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# LACK OF IL-1R8 AFFECTS INTERNEURONS DEVELOPMENT AND GENERATION

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There is a general consensus that immune system abnormalities and inflammation can modify the risk and/or the severity of a variety of brain diseases, from neurodevelopmental to neurological disorders. Recently, our group demonstrated that the lack of IL-1R8 in neurons and the hyperactivation of the IL-1Receptor pathway lead to morphological and functional impairment of excitatory synapses, as a result of the activation of mTOR pathway and the increase of MeCP2 protein levels. MeCP2 is an epigenetic regulator that is fundamental also for the development of GABAergic inhibitory circuits. However, whether IL-1R pathway hyperactivation affects this process is still an unexplored issue.
This study is aimed at characterizing the generation of inhibitory GABAergic interneurons and inhibitory synapses in IL-1R8KO mice, as a model for IL-1Receptor pathway hyperactivation.
Similarly to what we have found in excitatory neurons, Parvalbumin-expressing GABAergic interneurons of IL-1R8 KO mice display a significantly increased level of MeCP2.
We then evaluated the density of inhibitory synapses in the hippocampal region and we found that IL-1R8 KO mice show a reduction of the number of vGAT-positive puncta with respect to age-matched wt mice. On the other hand the number of GABAergic inhibitory interneurons is significantly increased in the cortex of IL-1R8 KO mice with respect to wt, in particular this increase is due to the parvalbumin-positive and calretinin-positive interneuron subpopulations whereas the somatostatin interneurons do not change. To investigate the molecular mechanisms underlying these alterations in the number of GABAergic interneurons we performed RNAseq analyses on Medial Ganglionic Eminence (region which contains inhibitory neuron precursors) isolated at embryonic day 13.5 from IL-1R8 KO and WT mice. Of note this analysis revealed that a pattern of genes specifically controlled by Nkx2.1 is altered in IL-1R8 KO MGE with respect to wt. Finally a preliminary analysis show that IL-1R8 KO mice display changes of EEG basal activity with an alteration in the power of the principal brain rhythms.
Alterations in the molecular pathway underlying the formation of the inhibitory network during brain development may be at the basis of the learning and memory defects observed in IL-1R8 KO mice.

# MATERNAL IMMUNE ACTIVATION INDUCES SYNAPTIC ALTERATIONS IN THE OFFSPRING

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In the last fifteen years, groundbreaking genetic progress has underlined a convergence onto coherent synaptic pathways for most psychiatric and neurodevelopmental disorders, which are now collectively called “synaptopathies”. However, the modest size of inheritance detected so far indicate a multifactorial etiology for these disorders, underlining the key contribution of environmental effects to them. Inflammation is known to influence the risk and/or severity of a variety of synaptopathies. In particular, pro-inflammatory cytokines, produced and released in the brain by activated astrocytes and microglia, appear to play a pivotal role in these pathologies. Inflammation during pregnancy is known to increase the risk for the development of neuropsychiatric disorders in the offspring. In particular, alterations in the maternal immune system leading to heightened inflammation during pregnancy, even in the absence of clear maternal or neonatal signs and symptoms, are consistently related to an increased likelihood of long-term multiple psychiatric disorders in affected offspring, including autism, schizophrenia, attention-deficit hyperactivity disorder and mood disorders. We have recently demonstrated that the injection of the viral mimicking molecule poly I:C, a doublestranded, synthetic RNA that binds to toll-like receptor 3 like viral nucleic acid, when delivered to pregnant mice at early stages of embryo development (E9) results in delayed GABA switch in the offspring, resulting from a lower expression of KCC2 as compared to controls. The alterations in nKCC1/KCC2 ratio resulted in GABA being excitatory and offspring more susceptible to seizures in the adult stage (Corradini et al., 2018). No alterations in the density of excitatory or inhibitory synapses were detected in mice prenatally exposed to poly I:C at E9. We have now evidence that the same treatment performed at E15, a period which corresponds to the last trimester of pregnancy in humans, results instead in altered density of excitatory synaptic contacts in the offspring brain. Interestingly, the synapse changes were specific for hippocampus versus cortex and displayed a clear gender-specificity. Besides underlining the importance of the developmental window for the consequences of maternal immune challenge, these data unveil important differences in the impact of prenatal inflammation in males versus females and prompt to the need of adopting gender-specific approaches when investigating the cellular and molecular basis of neurodevelopmental disorders consequent to environmental stimuli.

# ASTROCYTES-DERIVED EXTRACELLULAR VESICLES IN MOTION AT THE NEURON SURFACE

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

# Intravital Two-photon Imaging of Glioblastoma Mouse Models

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Glioma is a brain tumor that derives from glial cells and represents about 40% of all diagnosed central nervous system tumors. The most malignant form of these tumors is the glioblastoma multiforme (GBM), that has extensive cellular and genetic heterogeneity, with a median life expectancy of only 14 months[1]. The major obstacle to a cure is the ability of the tumor to invade and migrate into different areas of the brain. A general emerging concept relating to the aggressive invasiveness of GBM, is that the tumor cells migrate along extracellular routes, often exploiting brain vasculature and collagen fibers, which leads to the formation of tumor cell streams and distant satellite tumors[2]. The motility of tumor cells,within the confined spaces of the brain, is a physical stimulus that is able to initiate cellular signaling and crosstalk that is important in controlling cellular processes, including cell proliferation, cell division and programmed cell death[3]. Calcium signaling has been proposed to be directly involved in cancer proliferation and invasion, but there are no demonstrations *in vivo* of the correlation between GBM cell motility and calcium signaling. In light of this idea, we have produced a strain of mouse glioma cells (GL261) expressing LifeAct-mDsRed2, a red fluorescent protein that stains the actin cytoskeleton and a green fluorescent protein, GCaMP6s, a genetically encoded calcium sensor. Upon transplant in a host mouse, these cells produce a fluorescent tumor and, by two-photon microscopy, we have performed intravital imaging to study tumor morphology, infiltration and intracellular calcium activity. Our data demonstrate that most of the tumor volume is occupied by tightly packed spherical cells characterized by little or no motility and rare Ca activity. This core, is surrounded by sparse cells displaying a very polarized morphology and migrating in a coordinated way. These cell streams are characterized by very active Ca signaling, with Ca waves propagating within cells and between distinct ensembles of cells based on their common activation. We postulate that these regions represent the infiltrating component of the tumor. Finally, we generated an on demand glioma model by exploiting a construct that allows the knock in of a constitutively active mutant of Ras (HRasV12)[4.] flanked by the consensus sequence for the *piggyBac* transposase. By *in utero* electroporation we transfected a small population of glia precursors with HRas, GCaMP6 and a red reporter. Two photon imaging demonstrated that, within 10 days from the electroporation, the brain is gradually invaded by hypertrophic cells characterized by elevated motility and infiltrative potential. Similarly, to the GL261 model, the infiltration is organized in cell streams endowed by very elevated Ca activity.

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# NEW STILBENE-AMMONIUM NICOTINIC LIGANDS AS ANTI-GLIOBLASTOMA AGENTS

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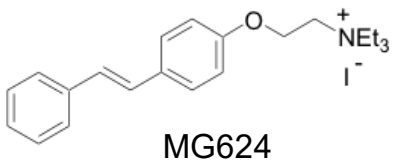
Human glioblastoma U87MG cells and primary cell lines derived from patients express mRNAs for α7- and α9-coding genes and nicotine activation of receptors containing these subunits increases cell proliferation, which is selectively blocked by α7,α9 receptor antagonists. The triethylammoniumethyl ether of 4-stilbenol MG624 (figure), which acts on α7,α9 neuronal nicotinic acetylcholine receptors, has antiproliferative activity on human glioblastoma cells. We found that the replacement of MG624 ethylene with butylene (see StN-4) resulted in a more potent and selective glioblastoma toxicity, which was paralleled by a decrease in mitochondrial ATP production after one hour of treatment, not paralleled by an increased production of reactive oxygen species within mitochondria. Further elongation of the alkylene linker (see StN-8) enhanced glioblastoma cell viability reduction, without changing neuronal nicotinic acetylcholine receptor selectivity. Moreover, after three days of exposure, MG624 and its elongated derivatives decreased U87MG cell proliferation, which was only partially restored by co-incubation with selective α7,α9 receptor antagonists, indicating that these compounds may also have other non nicotinic-mediated effect(s).

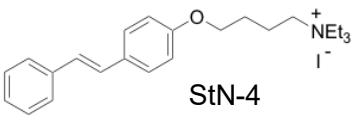
Docking data and functional studies of these compounds tested alone, in presence of acetylcholine or in presence of a positive allosteric modulator of α7 receptors have shown that MG624, StN-4 and StN-8 have slightly different nicotinic pharmacological profiles: partial agonist, silent agonist and full nicotinic antagonist, respectively.

The aim of this work is to identify the anti-tumour mechanism(s) of action of the three compounds on glioblastoma cells and for this we studied whether these compounds have apoptotic activity or inhibitory activity on cell cycle. FACS analysis of U87MG cells exposed for three days to $IC_{50}$ concentrations of these compounds show that only MG624 and StN-4, but not StN-8, increase the apoptotic rate and block U87MG cells at $G_1/G_0$ phase of the cell cycle. The effects of the compounds on the two canonical cancer-related signalling pathways ERK and Akt are also under study.

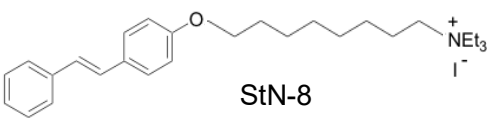
We are now generating, through CRISPR/Cas9 genome editing technology, U87MG cell lines knockout for α7 and α9 gene, as well as double knockout cells, in order to better characterize the effects of MG624 and its elongated derivatives. The next step of the project will be to test *in vivo* the α7,α9 antitumor agents in NOD/SCID mice xenografted with wild-type or knockout human glioblastoma cells.




MG624




StN-4




StN-8

# ROLE OF RARE MISSENSE VARIANTS OF THE HUMAN β4 SUBUNIT IN THE EXPRESSION AND SURFACE EXPOSURE OF α3β4

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Neuronal nicotinic acetylcholine receptors (nAChRs) are a family of cationic channels consisting of nine α (α2-α10) and three β subunits (β2-β4) which assemble in pentamers with different subunit composition. Two ligand binding sites are present at the interface between α and β pairs while the subunit in fifth position, that doesn't participate in the ligand binding, is called "accessory subunit". This subunit could be α or β leading to the formation of pentamers with two alternative stoichiometries: 2α/3β and 3α/2β that have similar agonist sensitivity but different antagonist sensitivity, and markedly different single-channel conductance.
To investigate the role of the subunit present in fifth position in the α3β4 nAChRs we set up a system to express single population of pentameric receptors with fixed stoichiometry. We found that 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 human 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 and their co-expression with the α3 subunit in hippocampal neurons, significantly altered the amplitude of nicotine-evoked currents. Taking advantage of the system that we have developed, we investigated the role of the β4 variants present in fifth position in the expression and exposure to the surface of α3β4 nAChRs.

# DEVELOPMENT AND CHARACTERIZATION OF A NEW TRANSGENIC MICE LINE EXPRESSING A MITOCHONDRIAL CAMELEON PROBE FOR REAL-TIME CALCIUM IMAGING

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Spatial and temporal organization of intracellular calcium ($Ca^{2+}$) signals is crucial for the correct function of all tissues (Streck E. et al., 2014). Among other functions, mitochondria play a relevant role in calcium homeostasis and their dysfunction has been correlated to several pathological conditions (i.e. from neurodegenerative diseases to malignant transformation). In the past years, several Genetically Encoded $Ca^{2+}$ Indicators (GECIs) have been developed to study $Ca^{2+}$ dynamics inside mitochondria. In particular, ratiometric, FRET-based GECIs such as Cameleons are largely used in cell cultures. However, the study of $Ca^{2+}$ dynamics in organelles *in vivo* or in *ex vivo* preparations, demands invasive techniques, such as viral injection.

The aim of this project is to develop and characterize a transgenic mice line expressing a mitochondria-targeted Cameleon probe. To achieve this goal, we engineered the Rosa26 mouse genomic locus by inserting the sequence of the mitochondria-targeted Cameleon 4mt-D3cpv (Palmer A.E. et al., 2006) preceded by a LoxP-STOP-LoxP sequence. The probe can be easily expressed in a tissue-specific manner upon Cre recombinase-mediated excision, obtainable by a single cross.

We exploited a CAG–Cre mice line (Nyabi O. et al., 2009) to drive the expression of the Cameleon probe in all tissues, included the brain. Preliminary data demonstrates the expression of the probe in the brain at the different mice age analyzed. Moreover, two-photon $Ca^{2+}$ imaging experiments performed in *ex vivo* brain slices, confirmed the functionality of the probe in live tissues.

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

# HUMAN SECRETED PHOSPHOLIPASE A2 GIIA IS TRANPORTED TO THE CELL NUCLEUS

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The phospholipases A2 (PLA2s) are enzymes that cleave glycerophospholipid sn-2 ester bond.
PLA2 superfamily is composed by at least 15 groups comprising secreted, cytosolic, and calciumindependent PLA2s. PLA2s of the second group are secreted compact proteins of about 120 amino acids, and seven disulphide bonds. PLA2G2A (group IIA)is expressed by many cell types both constitutively, and induced by pro-inflammatory cytokines. Its concentration in the plasma can increase a thousand times because of trauma or infection, rheumatoid arthritis or cardiovascular disease. It is also found at higher concentrations in the cerebrospinal fluid of Alzheimer's patients.
In Human Protein Atlas (https://www.proteinatlas.org/) PLA2G2A is reported to have a nuclear localization in HepG2, in U2OS cell cultures, and in many human tissues, based on staining with one validated antibody. This unexpected localization(as the protein is secreted), agrees with our data on the internalization and the nuclear localization of snake venom PLA2, homologous to PLA2G2A, so we decided to validate the nuclear localization of PLA2G2A with a second antibody and to produce the human recombinant protein to monitor is possible cell internalization pathway.
Our immunofluorescence experiments confirmed the nuclear localization of PLA2G2A in HepG2 and we found the same localization in A549 cells. By immunoblot of cell lysates we observed that PLA2G2A from these cells has an apparent molecular weight higher than expected,suggesting that the protein, in the nucleus or to be addressed to the nucleus, undergoes post-translational changes. Moreover, we found that he recombinant protein, labelled with a fluorescent tag, is endocytosed and transported, in few minutes, to paranuclear zone or to the nucleus depending on cell culture conditions.
We think that this unexpected localization of a secreted PLA2 will reveal new functions of this protein that plays an important role in lipid metabolism and inflammation.

# MICROTUBULES STABILIZATION BY MUTANT SPASTIN AFFECTS ER MORPHOLOGY AND $Ca^{2+}$ HANDLING

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The endoplasmic reticulum (ER) extends as a network of interconnected tubules and sheet-like structures in eukaryotic cells. ER tubules dynamically change their morphology and position within the cells in response to physiological stimuli and these network rearrangements depend on the microtubule (MT) cytoskeleton. Store-operated calcium entry (SOCE) relies on the repositioning of ER tubules to form specific ER-plasma membrane junctions. Indeed, the tips of polymerizing MTs are supposed to provide the anchor for ER tubules to move toward the plasma membrane, however the precise role of the cytoskeleton during SOCE has not been conclusively clarified. We used an in vivo approach involving the manipulation of MT dynamics in Drosophila melanogaster by neuronal expression of a dominant negative variant of the MT-severing protein spastin to induce MT hyper-stabilization. In this model, we observed the effect of MT stabilization on ER morphology and calcium handling, particularly SOCE.

# DEFINING THE MITOCHONDRIAL cAMP SIGNALLING: REGULATION AND POSSIBLE ROLE IN METABOLIC FLEXIBILITY

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Exploiting a FRET-based biosensor for cAMP in the mitochondrial matrix, we showed that the synthesis of cAMP inside mitochondria (mt-cAMP) is stimulated by raises in matrix [$Ca^{2+}$], and that increases in mt-cAMP result in increased efficiency of ATP synthesis. Thus, the regulation of oxidative phosphorylation (OXPHOS), which modulates the generation of ATP in response to nutrient availability and cellular demand, is at least partially achieved by mt-cAMP. These findings opened a number of questions regarding the identity of the effectors, the targets and the regulatory proteins of the cAMP signalling within the matrix, and the existence of additional functions regulated by mt-cAMP. Moreover, the structure, domain function and regulation of the mitochondria-targeted isoform(s) of the soluble adenyl cyclase (sAC), the matrix cAMP-generating enzyme, are still poorly known.

To unveil the localization, and its possible dynamic regulation, of sAC and other key molecules participating in the mt-cAMP signalling pathway, we recently set up a split-GFP-based tool. In addition, we employed the CRISPR/Cas9 technology and generated rodent and human sAC-deficient lines, currently under characterization, to study the effect of re-introducing mitochondrial versus cytosolic sAC isoforms.

Our working hypothesis is that the production of cAMP in the mitochondrial matrix could impinge on the regulation of the metabolic flexibility (MF). Mitochondria are emerging determinant in MF regulation, which involves the control of mitochondrial biogenesis and activity, resulting in the fine-tuning of oxidative metabolism. Interestingly, cAMP is involved in both bacteria and eukaryotes in the regulation of the response to fasting; additionally, the transcriptional networks governing mitochondrial biogenesis, the anabolism/catabolism shift and proteins involved in MF are highly conserved. We are thus currently testing the hypothesis of the existence of a feedback loop where nutrient availability could regulate mt-cAMP level, which, in turn, would participate in the processes underpinning MF through its action on mitochondrial metabolism.

Our preliminary data indicate that nutrients differentially modulate cAMP in the mitochondrial matrix.

# COMPARATIVE ANALYSIS OF Ca2+ HANDLING PROTEINS ACROSS VERTEBRATE SPECIES

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Calsequestrin is considered the major Ca2+-storage protein within the sarcoplasmic reticulum of both cardiac and skeletal muscle; it is also one of the high capacity Ca2+ buffers in the endoplasmic reticulum (ER), together with calreticulin, in other excitable cells. Little is known on Calsequestrin expression in the brain. The aim of this study was to analyze Calsequestrin expression in adult mammal brain at RNA and protein level and explore its cellular localization in relation to other Ca2+ store markers. At RNA level, by qPCR, we found significant expression of one transcript in mouse, *Casq2,* at difference with zebrafish that expresses two isoforms (*casq1* and *casq2*). At protein level, we confirmed the presence of Casq2 in total extracts from cerebrum and cerebellum by western blot. To investigate Casq2 localization in cells and subcellular compartments we compared cerebellar sections of mouse and zebrafish by immunofluorescence and confocal analysis. To identify Casq-specific Ca2+ stores co-localization analysis with other functional and structural markers were also performed. Our data show that in both zebrafish and mouse Purkinje cells express Casq2 in cell body, dendritic shafts and axons. Casq1 is expressed in granular cells of cerebellum and torus longitudinalis of zebrafish only. Double immunofluorescence experiments show that Casq2 rich compartments display an immunofluorescence pattern similar to Inositol Trisphosphate receptor 1 (Itpr1), according to chicken cerebellum, and to Ryanodine Receptor (Ryr) in both mouse and zebrafish. The co-localization of Itpr1 and Casq2 in the same compartments was confirmed for mouse cerebellum by co immunoprecipitation of the two proteins. In conclusion, our studies identify Casq2 in mouse, Casq1 and Casq2 in zebrafish, as useful markers for neuronal Ca2+ stores in adult cerebellum and extends the possibility of exploring structural and functional heterogeneity of neuronal ER Ca stores.

# ASTROCYTE CONTROL OF GLUTAMATERGIC TRANSMISSION IN VENTRAL TEGMENTAL AREA

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The mesocorticolimbic dopaminergic (DA) system originating from the Ventral Tegmental Area (VTA) plays a prominent role in the cognitive processing of aversion, motivation, pleasure and reward, including the development of addiction. A central aspect for the function of the brain reward system is the switch in the action potential firing pattern of VTA DA neurons from a tonic, low firing frequency to a phasic, high frequency bursts which cause an increase in phasic DA release at the VTA projecting structures. Glutamatergic afferent inputs to the VTA plays a central role in this transition from tonic to phasic-burst firing pattern and long-term changes of this signaling pathway can profoundly alter the output of DA neurons. Over the last years, astrocytes emerged as important regulatory elements of synaptic transmission in different brain circuits. They respond to neurotransmitters with $Ca^{2+}$ elevations through a mechanism that involves the production of inositol-1,4,5-trisphosphate (IP3) and the release of $Ca^{2+}$ from IP3-sensitive intracellular $Ca^{2+}$ stores. In turn, these $Ca^{2+}$ elevations in astrocytes evoke the release of gliotransmitters that modulates synaptic transmission. Whether a similar mechanism operates in VTA networks remains unexplored. By combining patch-clamp recording techniques and $Ca^{2+}$ imaging experiments in horizontal VTA slices of juvenile (P14-17) mice, we investigated whether astrocytes contribute to the modulation of excitatory synaptic transmission in the VTA circuitry. Our results demonstrate that astrocyte signaling contributes to long-term plastic changes of glutamatergic transmission in VTA circuitry. Our study opens a new perspective for the understanding of the cellular and molecular mechanisms that control the brain reward system.

# MECHANISM OF ACTIVATION AND FUNCTION OF THE AXONAL ODORANT RECEPTOR

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The 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 (OB), 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. This hypothesis could explain the role of the odorant receptor in the formation of the sensory map. The sensory map has a critical role in encoding odors, that are represented by a spatial pattern of activated glomeruli.
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. Among the active pool of molecule expressed in the OB, we identified a ligand that applied at the axon terminal, was able to induce $Ca^{2+}$ rise locally and modulate OSN axon turning behaviour. We confirmed that $Ca^{2+}$ rise observed in OSN was due to OR activation, by expressing OR in HEK293T cells, loaded with the calcium indicator fura 2. The identified ligand was able to activate the OR in HEK293T cells expressing distinctive OR. In addition we characterized the expression of the ligand in the OB and we found that is mostly expressed on the periglomerular cells, a suitable location for a guidance molecule acting on the incoming OSN axons.
If the odorant receptor ligand is involved in the formation of the sensory map, mouse carrying a null mutation of the ligand should exhibit an altered sensory map. To investigate the role of a putative ligand of the axonal receptors in sensory map formation, we analyzed the convergence of olfactory sensory neurons to form glomeruli in specific location of the olfactory bulb. We studied the organization of the sensory map in a mouse model carrying a null mutation for a odorant receptor ligand crossed with mice co-expressing GFP with distinct odorant receptors.
We found that the sensory map was disrupted by the presence of additional heterogeneous glomeruli and by a shifted position of the glomeruli in mutant in respect to control mice, indicating the instructive role of the identified ligand in the sensory map.
Consistent with a disrupted topographic map, we found that mice carrying a null mutation for the odorant receptor ligand, exhibited altered odor discrimination behaviour.

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.

# CHRONIC TREATMENT WITH BIFIDOBACTERIUM (LONGUM, BREVE, INFANTIS) MODULATES GABAA RECEPTOR GENE EXPRESSION, NEURONAL FUNCTION AND STRUCTURE IN THE RAT HIPPOCAMPUS

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Background: Changes in microbiota alter the modulation of the hypothalamic-pituitary-adrenal (HPA) axis sensitivity, to the effect of stress, an effect that may involve the inhibitory neurotransmitter GABA, one of the neurotransmitters known to modulate of emotional states. In our laboratory we studied in adult rats the effect of long-lasting effect of a 1-2 months chronic treatment with a preparation of three different Bifidobacterium (Longum, Breve, Infantis) on GABAA receptor gene expression and GABAergic function and structure in the hippocampus as well as the HPA axis sensitivity to acute foot- shock stress.
Methods: Were used adult male rats of Sprague Dawley strain. Rats were treated for 1 or 2 month per os, once a day, with a mixture of different three different Bifidobacterium: longum (BB536 strain, final concentration of 3*109 CFU), brevis (M- 16 strain, final concentration of 1*109 CFU) and infantis (M- 63 strain, final concentration of 1*109 CFU). In these rats were carried out the following studies in hippocampus: Western blot to assess the changes in α1, α4, δ and γ 2, GABAA receptor subunit and BDNF protein expression. We used a specific antibody against α1, α4, δ and γ2 (1:250; Phosphosolution) and BDNF (1:500; Santa Cruz Biotecnology). A specific antibody against GAPDH (1:1000; Millipore, USA) was used as standard; immunoistochemistry to assess the changes in α1, α4, δ and γ 2 GABAA receptor subunit in specific subareas of hippocampus. We used the same antibodies used for western blot experiments; electrophysiology, to assess the GABAergic tonic current and spontaneous GABAA receptor–mediated ISPCs (sIPSCs) current; Golgi impregnation, to assess the dendritic spines density; ELISA to assess the amount of corticosterone on plasma and allopregnanolone in the brain
Results: Plasma corticosterone (CTS) levels were measured in basal condition and after foot-shock stress in animals previously treated with bifidobacterium. This treatment failed to change the basal content as well as the increase of CTS levels elicited by acute footshock stress when compared to vehicle treated group. In contrast, western blot and immunohistochemistry analysis showed that two months of bifidobacterium treatment reduced α1, α4, and δ GABAAR subunits expression while increasing γ2 subunit. Moreover, this treat- ment significantly reduced plasma content of allopregnanolone, an agonist ligand for GABAA receptor containing α4 and δ subunits. Patch-clamp experiments performed in dentate gyrus granule cells showed no changes in synaptic currents while the tonic component of GABAergic inhibition was significantly decreased. The latter data are consistent with an observed increase of neuronal excitability measured in the same neurons of the dentate gyrus as well as with the parallel reduction of δ subunit and AP plasma content. Moreover, more recently we found that the same chronic treatment with bifidobacterium increased the number of dendritic spines in CA1 pyramidal neurons and in the granule cells of dentate gyrus.
Conclusion: Altogether our data show that this mixture of three (Longum, Brevis and Infantis) bifidobacterium given chronically to rats is able to modify the gene expression of specific GABAA receptor subunits in the rat hippocampus, an affect associate to functional and morphological changes of specific neuronal populations of dentate gyrus and CA1 and further support the crucial role of specific gut bacteria in the modulation of accordingly brain function. Accordingly, the concept of psychobiotics as new tools to be used in mental health has been recently suggested

# MY KINEMATICS AS A TEMPLATE TO DECODE YOUR ACTIONS: THE ROLE OF MOTOR RESONANCE IN INTENTION PREDICTION

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Background: Understanding the intention of others is an essential component of social behavior. The neural bases underlying this process have been identified in the motor system, specifically in the mirror mechanism. Recent studies have demonstrated that the kinematics of an action is modulated by the underlying motor intention, and that difference in observed kinematic patterns explains the performance in the recognition of others' intents. However, an open question is whether decoding others' intentions based on their kinematics depends solely on how much this varies across different actions, or rather it is also influenced by the similarly with the observer motor repertoire.

Methods: We performed a kinematic study including two tasks on the same group of 21 volunteers. In the first, participants were asked to perform a series of reach-to-grasp and place actions, which differed for the final motor intention regarding the size of the target (i.e., put an object in a big or a small container) or the presence of a social content (i.e., put an object in a box or give it to another individual).

In the second task, participants were asked to observe and predict the motor intention of an actor executing one of the actions previously performed. During the observation, the placing phase was visually-occluded requiring the participant to predict the intention from the observation of the sole reach-to-grasp phase. The reaching and grasping kinematics of the participants and of the actor were recorded with a 3D-optoelectronic system. Wrist and fingers markers position was monitored time-wise to calculate reach trajectories, reach velocity, grasp aperture, and grasp velocity. Moreover, the vertical projection of the distance between the wrist and the elbow markers was calculated ($WE_y$). This variable may be considered as an indirect estimation of the wrist rotation during the reaching movement.

Repeated measures ANOVAs were carried out on kinematic variables including SIZE and CONTENT as within-subjects' factors. The Linear Fit Method (LFM), complemented with the Root Mean Square Error (RMSE), was selected to assess the waveform similarity between each participant and the actor. The correlation between $R^2$/RMSE values and the intention recognition accuracy was evaluated.

Results: Reach and grasp kinematics was modulated by the different motor intentions. Reach elevation was higher and fingers aperture resulted wider and faster when participants had to place the object in a big target compared to a small target. When the task required a social action, the reaching was slower and $WE_y$ resulted smaller. Participants evidenced above chance accuracy in predicting the final intention of the action in the observation task, with higher performance for SIZE condition (85%) with respect to CONTENT condition (73%). Grasp Aperture $R^2$ and RMSE values significantly correlated with SIZE recognition accuracy ($r = 0.64$; $r = -0.54$). Instead, the recognition accuracy in CONTENT condition showed a significant correlation only for one parameter, i.e. $WE_y$ ($r = -0.53$), partially in line with the higher variability between the participants and the actor in terms of kinematic pattern.

Conclusion: The present study reveals that during action observation, low-level motor features provide cues for decoding the intentions of others and, more importantly, that their effect depends upon how similar they are to the observer motor repertoire. This provides further support to the view of action intention recognition as a visuo-motor process mediated by the motor mirror mechanism.

# MORE THAN JUST SOMATOSENSORY: INTRACORTICAL RESPONSES OF SII TO ACTION OBSERVATION

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# VIRTUAL REALITY IN VIRTUAL PATIENTS: EFFICACY OF VR ACTION OBSERVATION TREATMENT IN SPEEDING UP THE RECOVERY OF THE SHOULDER JOINT

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Introduction

The Action Observation Treatment (AOT) exploits the well-known mirror mechanism [1] for rehabilitation by stimulating the motor system of a patient with motor impairments through the observation of an action, which facilitates his/her functional recovery [2]. The efficacy of AOT was largely demonstrated in patients with neurological diseases [3] and, recently, also in non-neurological patients such as orthopedic and trauma patients [2,4-5].

Virtual reality, largely used in innovative rehabilitative procedures, provides an immersive experience which could favor an enhancement of AOT induced effects. To test this hypothesis, we temporarily immobilized 22 healthy subjects, administering half of them with VR-AOT during the immobilization period. The kinematics of the upper limb was recorded during a standardized set of movements before and immediately after the immobilization, in order to evaluate a possibly faster functional recovery induced by VR-AOT.

Methods

22 healthy adults (21.6 ± 2.7 years old) were enrolled in this study. Upper limb kinematics were simultaneously recorded with 3D-optoelectronic SMART system (BTS Bioengineering, Milano, Italy) and the Microsoft Kinect v2, embedded in the KHARE platform (INAIL). Four movements were requested to participants: (i) 90° shoulder flexion (low flexion); (ii) 90° shoulder abduction; (iii) 110° shoulder flexion (high flexion); (iv) dragging a target on the transversal plane. Each movement was repeated 10 times, for a total of 40 movements, both before (T0) and after (T1) the immobilization period (16 hours). During the immobilization period, aimed at creating a temporary impairment and thus a model of virtual patient, subjects performed 3 VR sessions with a 3D viewer (HTC Vive PRO). The VR-AOT group observed an avatar performing the same 4 movements from an egocentric perspective, while VR-CTRL group observed 4 landscape stimuli without any motor content. The Range of Motion (ROM) of the shoulder for each movement was calculated at both T0 and T1. In order to assess the rate of shoulder impairment/recovery, data at T1 were z-scored on T0. A quantitative comparison between the opto-electronic system and Kinect was conducted to evaluate the reliability of a low-cost and markerless solution for tracking the shoulder kinematic recovery.

Results

The results relative to the opto-electronic system indicated a marked decrease of the shoulder ROM at T1, compared to T0 for both experimental groups. Interestingly, while no difference emerged at trial 10, at trial 1 VR-AOT participants exhibited a more preserved shoulder kinematics with a z-score difference of about 1.5. Despite the greater variability, the KHARE platform results show a similar trend, suggesting the feasibility of tele-rehabilitative approaches for monitoring the progress of treatments conducted at home.

Discussion

VR-AOT proved to be a promising tool for preserving the cortical excitability of the motor system even when movements are impeded, ultimately speeding up the functional recovery.

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# TIME-FREQUENCY MODULATION OF EEG RHYTHMS ANTICIPATE BRAKING AND STEERING ACTIONS IN SIMULATED CAR DRIVING

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Car accidents are the leading cause of death for children and young adults all around the world. In order to improve car safety with devices adaptable to the driver's mental processes, it is fundamental to exploit the complexity of brain processes underlying the corresponding behavior. In order to obtain a time-frequency varying description of cerebral processes underlying braking and steering actions in a semi-ecological scenario, we performed 24 electroencephalographic (EEG) recordings during a driving simulation. After familiarizing with the whole setup, participants were asked to perform a single lap on the selected track, maintaining the right lane with no additional constraints. Telemetry and behavioral data from the driving simulator were recorded using the Lab Streaming Layer (LSL), which also served to synchronize these data with the EEG signals. Pedals position was used to identify and later segment the neural activity around the foot action onset, such as braking and accelerations. Instead, to extract left and right steering events we identified turns on the track as the periods when the curvature of the road trajectory was outside 5-95 percentile range. We performed the Adaptive Mixture Independent Component Analysis on the EEG signals followed by a cluster analysis to group significant and reliable activations. Next, EEG data were segmented into [-1500, 1500] ms epochs around the onset of braking, acceleration and steering actions. Neurophysiological brain activities were identified and then clustered to group EEG components according to their scalp topographies. For each cluster component, we computed the time-frequency decomposition by means of complex Morlet. Braking and acceleration, as well as left and right steering time-frequency patterns were statistically compared. The cluster analysis of these ICA topographies revealed 5 centroids corresponding to independent dipolar brain components which describe the whole driving session. Overall, these topographies refer to brain activity related to fronto-parietal networks, sensorimotor areas and premotor regions. Among these clusters: i) one is associated to significant modulation of theta activity in mesial premotor areas 800ms before braking actions (Cl5); ii) two are associated to sensorimotor areas (Cl3 and Cl6) and presented a significant desynchronization of the alpha and beta rhythms, which anticipates the left (Cl3) and right (Cl6) steering actions onset, 800ms and 600ms respectively. Overall, these findings show the activation of sensorimotor and 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, 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. These results show that it is possible to semi-ecologically discriminate braking and steering actions by means of modulation of theta, alpha and beta rhythms appearing more than half second before the event onset. Such findings are promising because they could provide a sufficient time interval to implement on-line braking and steering classification during driving, which could be further exploited to design driving assistance technology in the next future.

# NuARCH: THE INTERPLAY BETWEEN ARCHITECTURE AND THE BRAIN AS REVEALED BY EEG AND VIRTUAL REALITY

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Living space profoundly impacts on our behavior and mental states. Neuroscientific discoveries demonstrated that cerebral circuits underlying human cognition and bodily reactions are affected by the surroundings through a continuous sensorimotor interaction between the organism and its environment. The brain transforms sensory representations of other's behavior into one's own motor representations concerning that behavior and, depending on the location, can fulfill a range of cognitive functions, including action and emotion understanding. In particular, recent studies show that the recognition of emotions expressed by body postures and actions depends on the context in which they are perceived. This evidence set a promising direction of using neuroscience for architecture to investigate the relation between brain and built environment, thus using the body as the link between the two entities.

Objective of the present project is to investigate the active and the dynamic relationship between the organism and its living environment, by investigating the impact that architecture has on human perception. The study of the brain activity will be performed by collecting the electroencephalographic (EEG) signals of healthy people while perceiving expressive bodily actions within architectural environments represented in a virtual reality setting. The interaction between the simultaneous perception of bodily features and architecture will affect behavioral responses according to their mutual congruency. The analysis of reaction times and error rates in recognition tasks will return explicit behavioral variables of such congruency, whereas the implicit neural variables of this phenomenon will be retrieved by means of time-frequency EEG signatures. To this aim, the architecture will be designed with the purpose to convey different possibilities of movement. Similarly, expressive virtual avatars will be created in order to represent different degrees of movement. The experimental hypothesis is that congruent scenarios (architecture + avatar) will lead to faster and more accurate recognition performance, as well as an increase of cerebral activity of those cortical areas belonging and connected to the motor system. The EEG data analysis will have the objective to track the time-varying dynamic underlying perception of architecture. For this purpose, data driven oriented analysis of the EEG are suitable to study cortical processes and their dynamics in terms of large-scale functional networks. The whole experimental framework consisting in several tools coming from knowledge in neuropsychology, electrophysiology and virtual reality will allow the acquisition of neurophysiological variables in a highly ecological experimental setup.

NuArch investigates the architectural experience with the intention to understand the impact that built environment has on human behavior ad cognition. The resulting findings will help in understanding how architecture is experienced as meaningful based on the corporeal and automatic perception. These discoveries will help to design architecture as tuned to our internal states to match the man's changing need, and finally improving the overall well-being.

# CREATINE TRANSPORTER DISORDER: A PHARMACOLOGICAL APPROACH

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Creatine (Cr) transporter (CrT) deficiency is an orphan disorder (CTD, OMIM #300352) characterized by intellectual disability, epilepsy and autistic-like behavior. Mutation in the CrT gene causes one of the three syndromes associated with creatine deficiency, the other two being caused by mutations in the guanidinoacetate methyltransferase (GAMT) and L-arginine:glycine amidinotransferase (AGAT) genes, both coding for enzymes involved in the biosynthesis of creatine. In case of mutation occurring in GAMT and AGAT genes, in which creatine biosynthesis is impaired, the simple supplementation of creation by dietary mean is sufficient to revert the symptomatology. Although CTD shares much similarities with the other two syndromes, dietary creatine supplementation is ineffective in treating the disorder because due to its highly polar structure cannot cross cellular membrane without its transporter.
Here we used a longitudinal 6-months protocol to test cyclocreatine, Ccr, a creatine analog which thanks to its more hydrophobic structure is able to freely cross the cellular membrane. We found that cyclocreatine is able to prevent age related memory and cognitive deficits together with ameliorating autistic-like behaviours.

# CREATINE TRANSPORTER DISORDER: NEW INSIGHTS INTO EPILEPTIC PHENOTYPE AND DIAGNOSTIC BIOMARKERS

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Creatine (Cr) transporter (CrT) deficiency is an orphan disorder (CTD, OMIM #300352) characterized by intellectual disability, epilepsy and autistic-like behaviour. Epilepsy is one of the symptoms with the greatest impact on everyday life of patients and families. Animal models are crucial tools to dissect disease mechanisms and to develop new therapeutic strategies. Four murine models of CTD are available. However, they have been studied only at behavioural, neurochemical and anatomical level. To expand our knowledge about the face validity of the murine model, we monitored brain excitability and seizure susceptibility in the CrT knockout mouse using video-EE recordings in freely-moving animals. In addition, we studied the evoked activity of neuronal networks monitoring visual responses in the mouse model throughout the disorder progression using longitudinal transcranial intrinsic optical signal (IOS) imaging. Our data show that CrT loss-of- function results in spontaneous seizure occurrence and higher susceptibility to kainic acid-induced seizures, as assessed both at behavioural and electrophysiological level. Accordingly, we detected a prominent reduction of inhibitory parvalbuminergic synapses in the cortex. Moreover, we found marked abnormalities in theta, alpha, beta and gamma frequency oscillatory activity in the EEG of CrT ko mice both during wake and sleep. A specific increase of IOS responses was detected in CrT ko mice. Our results fill a substantial gap in the current literature providing a more comprehensive normative data for the evaluation of potential therapeutic strategies. Moreover, EEG signature and visual assessment could be used as classifying biomarker for CTD diagnosis and treatment assessment with high reliability. Importantly, EEG and IOS imaging can be readily applied to humans, increasing the translational value of these biomarkers.

# A FULLY 3D PRINTED AUTOMATED AND COST-EFFECTIVE SYSTEM FOR APPETITIVE CONDITIONING FOR BEHAVIORAL PHENOTYPING OF MOUSE MODELS OF NEURODEVELOPMENTAL DISORDERS

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Conditioned learning is a procedure widely used for evaluating learning curves in mice. Here we present a custom and automatic touch button chamber, based on appetitive conditioning principles and using visuomotor tasks. The procedure has been applied in a mouse model of CDKL5 syndrome, a neurodevelopmental disorder characterized by frequent drug-resistant seizures, lack of language, mental retardation, visual impairment, and poor motor control.

The conditioning chamber is created by using cost-effective 3D printed components and custom electronics, paired with open-source software. The behavior starts at 60 postnatal days and is preceded by an Intrinsic Optical Signal Imaging session to quantify visual amplitude. Using the chamber, we tested mice on a battery of different tasks, based on appetitive conditioning principles, designed to assess different aspects of learning and cognition. Through tracking analysis, it was possible to evaluate how they move inside the chamber and approach to the tasks.

This conditioning chamber is aimed at evaluating learning and other aspects of cognitive functions. From the first day, the technique highlights differences between genotypes, clustering different cognitive profiles. Indeed, ko and het mice show differences in learning curves and tracking parameters compared to wt. The use of this behavioral technique combined with the physiological investigation allows a multicomponent analysis to categorize different genotype.

In conclusion, we create a cheap and automatic chamber, suitable for the study of cognitive functions and able to discriminate between genotypes. Furthermore, this conditioning chamber seems to be capable of investigating other learning tasks such as visual discrimination or attention.

# PUPIL FLUCTUATIONS AS A BIOMARKER FOR CDKL5 DISORDER

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Mutations in CDKL5 causes in humans a devastating syndrome (CDD) characterized by seizures that begin in infancy and followed by significant delays in many aspects of development. During the last years mouse models have become available, open the way to mechanistic and preclinical investigations on possible therapies. These studies have produced a plethora of preclinical data, however the clinical efficacy assessment of these potential treatments depends on the availability of noninvasive biomarkers with high sensitivity that unfortunately are still missing. The pupillary light reflex regulates the amount of light that reaches the retina; its pathway involves the retinal ganglion cells, the Pretectal nucleus, the Edinger–Westphal nucleus, and the Ciliary ganglia. Recent studies have shown that pupil size could reflect brain processes in addition to merely sense the external illumination. Moreover, pupil function abnormalities have been reported for a wide range of disorders, including alcoholism, mental health disorders such as seasonal affective disorders, schizophrenia and generalized anxiety disorder, Alzheimer's and Parkinson's diseases. The aim of the present study was establishing a first background for the use of pupillometry as a biomarker for CDD. Thus, the central hypothesis of our project was that pupil size could reliably report the altered cortical processing present in CDKL5 disorder models. We performed pupillometry in awake head fixed fully symptomatic (P60-P80) CDKL5 null male mice and their wild type (WT) littermates. A head post was fixed to the skull 1 week before experimental training. During pupillometry the animal was head fixed for 30 minutes. We analyzed average pupil diameter and its oscillation (hippus) in different behavioral states and with different visual stimuli: a uniform gray background for 15 minutes followed by a virtual reality presented in two conditions, coherent and incoherent for 15 minutes each, 30 seconds of dark or white screen were randomly interleaved to measure pupil dynamic range. Movement sensor was used to record locomotor activity. We obtained preliminary data showing a significant difference in locomotion between mutants and controls. In particular, mutants show an higher locomotor activity during the task compared with their WT littermates. Interestingly, we found a significant lower pupil diameter in CDKL5 null mice, suggesting that pupillometry can be used for noninvasive assessment in mouse models of CDD.

# CORTICAL EXCITABILITY IN A CONDITIONAL MODEL OF PCDH19 EPILEPSY

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PCDH19 Epilepsy is characterized by epileptic seizure onset in early infancy and is frequently associated with intellectual disability and autism spectrum disorder[1,2,3]. The disease has been attributed to mutations in the X chromosomal Protocadherin 19 (PCDH19) gene[1], encoding a $Ca^{2+}$-dependent cell-cell adhesion protein. Interestingly, only heterozygous females and males with somatic mutations are affected[4], leading to the hypothesis that the disease is caused by a mosaic PCDH19 expression in the brain[5]. However, the mechanism by which PCDH19 mosaicism causes epilepsy and cognitive impairment is unknown.

Here, we employed a novel mouse model of PCDH19 Epilepsy and performed in vivo electrophysiological and imaging studies. We obtained a focal mosaic loss of PCDH19 by the injection of an AAV vector carrying CRE-EGFP in the visual cortex. The opposite hemisphere served as internal control in the electrophysiological experiments. Local field potential recordings in anesthetized animals demonstrate that mosaic PCDH19 patches in the brain have disrupted slow wave activity and show transient episodes of hyperexcitability and hypersynchronous activity. Intriguingly, in-depth analysis of the slope of slow wave activity and single unit statistics, suggest that the local network has an overall reduced firing rate. These findings are consistent with the attenuation of slow wave activity but are at odds with the onset of transient episodes of hyperexcitability.

To further explore the neuronal activity underlying the observed phenotype, we performed combined LFP recordings and two photon calcium imaging of PCDH19 positive and negative neurons in a mosaic brain in vivo. Preliminary data suggest that some mosaic animals displayed an increased calcium activity likely to be associated to the transient hyperexcitability. However, more data are needed to confirm this indication. No difference was observed between PCDH19 positive and negative neurons in the mosaic animals.

Given the importance of slow wave activity for both the homeostatic and memory functions of sleep, and observations that PCDH19 patients demonstrate abnormal sleep patterns[6], our data could be relevant to patients to increase understanding of the underlying mechanisms of sleep disturbances in PCDH19 Epilepsy.

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# NEURONAL DYSFUNCTIONS UNDERLYING PHELAN−MCDERMID SYNDROME AND THEIRS RESCUE BY ACUTE AND CHRONIC MODULATION OF mGlu5 SIGNALING

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Haploinsufficiency of the Shank3 gene is considered the major cause of neurological symptoms associated with the Phelan McDermid syndrome (PMS), a rare genetic neurodevelopmental disorder (Durand et al., 2007; Phelan and McDermid, 2012). PMS is characterized by severe expressive language and speech delay, hypotonia, global developmental delay, and autistic behaviour and it is often accompanied by epilepsy, thus suggesting an important role for Shank3 for the proper operation of cortical circuitry. Shank3 is a scaffold proteins located in the postsynaptic density (PSD) of excitatory synapses where is involved in formation and plasticity of synaptic connection. We have previously shown that SHANK3 is essential to mediate mGlu5 signaling by recruiting Homer1b/c to the post synaptic density (PSD) specifically in striatum and cortex and that the administration of CDPPB, a mGlu5 positive allosteric modulator (PAM), was able to rescue the behavioral and molecular defects observed in Shank3 KO mice (Vicidomini et al. 2017).
Thus, we explored synaptic connectivity *in vivo* in the visual cortex in Shank3 KO mice anesthetized with urethane. Here, by studying the steady-state activity and the visual evoked potentials obtained in response to stimuli of variable contrast, we have found an altered synaptic transmission resulting from a disruption of the balance between the excitatory input and the inhibitory feedback. Our data are consistent with a reduced recruitment of inhibitory interneurons with consequent hyper-excitability. To assess if mGlu5 pathways was involved in regulating contrast gain, we performed acute treatment with VU0409551, a mGlu5 positive allosteric modulator; we found that this treatment was able to normalize the contrast gain function.
In order to better clarify the role of Shank3 in mediating mGlu5 function, we analyzed mGlu5-mediated signaling pathways in Shank3 KO mice. We found reduced phosphorylation of ERK1/2 in Shank3 KO cortical neurons after DHPG stimulation that was rescued by treatment with the VU0409551. Finally, we showed that acute treatment with VU0409551 was able to rescue behavioral defects due to Shank3 deletion. Because mGlu5 activation modulates protein translation we measured, by SUnSET methodology, protein translation in cortex and striatum of Shank3 KO mice and we found in both areas a significant reduction of protein that was not rescued by acute treatment with VU0409551. On the other hand, when we chronically treated Shank3 KO mice with VU0409551, we normalized protein translation either in cortex and striatum. Because PMS is a neurodevelopmental disorder to revert the phenotype due to Shank3 deletion, we chronically treated young mice from post-natal day 15 (PND15) to PND30 with VU0409551. Chronic treatment significantly ameliorates memory performance and decreased self-grooming. Interestingly, this effect persisted for days after treatment suggesting that chronic activation of mGlu5 causes stable changes in neurons.

# GABA TONIC CURRENTS ARE DEEPLY AFFECTED IN DRAVET SYNDROME MICE

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Dravet Syndrome (DS) is a genetic form of epilepsy associated with de novo loss of function mutations of the SCNA1 gene encoding for α subunit of $Na_{v1.1}$ channels. Early symptoms appear in the first year of life and include different seizure types, often thermally induced, cognitive impairments and ataxia. Preliminary results from our laboratory showed that in a mouse model of DS ($Nav_{1.1}^{+/-}$), which resembles the phenotype of Dravet patients, principal neurons of the entorhinal cortex (EC) exhibit a reduced GABA tonic current. Since GABA tonic current is a persistent form of inhibition that is fundamental for the regulation of network excitability, the main goal in our study is to understand whether an impairment in GABA tonic current is a key player contributing to seizure generation in DS. In particular we investigated the properties of extrasynaptic GABA receptors in neurons from EC/hippocampal slices of $Nav_{1.1}^{+/-}$ mice and GABA transporters (GATs)-mediated currents in astrocytes to clarify if these mechanisms are involved in affecting brain circuit excitability. In order to measure GABA tonic current, we performed whole cell, patch clamp recordings from pyramidal neurons in brain slices containing EC and hippocampus from postnatal day (PD) 15-18 DS mice. Results revealed that there is a significant difference between the two groups, with the DS mice showing a strong decrease in the GABA tonic current of pyramidal neurons in the EC, which may contribute to the network hyper-excitability in this mouse model. The same experiment was performed in the dentate gyrus (DG), known to be involved in the epileptogenesis process. Surprisingly in this area data showed that the GABA tonic current is increased rather than decreased in granule cells of DS mice with respect to age-matched WT littermates. This could explain the lack of spontaneous seizures at this developmental stage. Finally we recorded GABA transporter mediated currents in astrocytes from EC. Our data suggest that astrocyte GATs are not affected in DS. Finally we used a genetic tool to reduce astrocyte $Ca^{2+}$ signals and possibly restore GABA tonic currents by affecting GAT surface expression. The same tool is tested in vivo on hyperthermia induced seizures. In conclusion, our current findings suggest that tonic current is largely altered in DS, with different contribution in different brain areas. Results obtained in this project will provide new insights into the mechanisms of seizure generation in the DS, opening novel therapeutic perspectives for this disorder.

# ALTERED MIGRATION OF INHIBITORY INTERNEURONS IN A MOUSE MODEL OF INTELLECTUAL DISABILITY

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Oligophrenin-1 (OPHN1), a gene associated to intellectual disability (ID), encodes a Rho-GTPase activating protein (RhoGAP) that regulates several developmental events. Here, we studied, in a validate model of ID, i.e. OPHN1-/y mice, the impact of OPHN1 on the postnatal migration of forebrain GABAergic interneurons. We found that migrating cells were deeply perturbed in OPHN1-/y mice. GABA, a key regulator of migration, elicited opposite effect on neuroblasts in OPHN1-/y and WT mice, due to the lower concentration of intracellular Cl-, associated to the premature expression of KCC2 in OPHN1-/y mice. Blocking KCC2 rescued almost completely the speed of mutant cells, with no consequence on their directionality. The latter parameter resulted affected by the overactivation of the OPHN1 downstream-signalling pathway, RhoA-kinase (ROCK), and was completely rescued by blocking ROCK. Here we identified two distinct targets to repristinate normal inhibitory interneurons migration, a key process underlying excitatory/inhibitory balance of neuronal circuits.

# INVESTIGATING IGF-1 AND OXYTOCIN CROSS-TALK IN THE MOUSE MODEL OF RETT SYNDROME

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Rett Syndrome is a severe and progressive neurodevelopmental disorderin the autism spectrum characterized by cognitive and motor deficits. In most cases, this pathology arises from mutations in the gene of the methyl-CpG binding protein 2 (Mecp2). Rett syndrome patients also display autistic-like features, such as impaired sociability and susceptibility to seizures.MeCP2 deletion in mice recapitulates many symptoms of the pathology.Both Mecp2hemizygous knock-out male mice ($Mecp2^{-/y}$ KO) and Mecp2 heterozygous female mice ($Mecp2^{+/-}$) are well-established animal models (Banerjee et al. 2019).Sub-chronic treatment with recombinant Human insulin-like growth factor 1 (rhIGF-1) has been shown to ameliorate the phenotype of these mice, and restore synaptic transmission and plasticity deficits (Castro et al., 2014).
The neuropeptide oxytocin (OXT) in the periphery is pivotal for birth and lactation, whereas in the brain it is involved in the formation of mother-infant bondingand it modulates social behavior. Moreover, it is important in the perinatal period forthe establishment of a correct excitatory/inhibitory (E/I) balancein neurons (Leonzino et al., 2016). OXT is under evaluation as a potential treatment for autism spectrum disorders.
Given that MeCP2 KO mice also show E/I imbalances (Banerjee et al., 2016), we hypothesized the existence of a cross-talk between the IGF-1 and the OXTergic systems in the MeCP2 mice.
Our preliminary data indicates that both IGF-1 and OXT receptors are expressed in the olfactory system and in the hippocampus. We focused our analysis on those regions. We used autoradiography and infra-red immunostaining to characterize the distribution of these two receptors, and to highlight areas of co-localization.Our preliminary results show a similar regional pattern of expression for IGF-1 and OXT receptors in the olfactory system and the hippocampus in all the groups.We are now treating$Mecp2^{-/y}$ male mice and relative controls with rhIGF-1 or OXT to study the impact of such treatments on IGF-1 receptor and OXT receptor levels.
Our goal is to establishif OXT treatment can ameliorate the phenotype of $Mecp2^{-/y}$ mice and if co-treatment of the animals with both rhIGF-1 and OXT has advantages over the two treatments alone. Taken together, these causal investigations will reveal possible mechanistic convergence of factors that critically contribute to the early brain development.

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# ALTERATIONS OF OXYTOCIN RECEPTOR EXPRESSION IN THE BRAIN OF MAGEL2-KO MICE, A MODEL OF PRADER WILLI-LIKE SYNDROME

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Prader Willi Syndrome (PWS) is a complex neurodevelopmental disorder characterized by an abnormal feeding behaviour during infancy, failure to thrive, and altered social skills. It is caused by the lack of expression of several maternally imprinted genes, all located in the 15q11–q13 region. However, the mechanisms by which these genes act to determine the pathologic phenotype of PWS remain largely unknown.

One of the candidate genes contained in this region **is** MAGEL2. MAGEL2 belongs to the family of the melanoma antigen gene expression (MAGE) genes. Truncating mutations of this gene are present in patients affected by Schaaf Yang Syndrome (SYS), a neurodevelopmental disorder similar to PWS characterized by intellectual disability and a high prevalence of autism spectrum disorders. Magel2-KO mice well recapitulate the pathologic phenotype of SYS. In this murine model, different alterations of the oxytocinergic system have already been reported: male KO mice showed a reduction in the number of OT-producing neurons and in OT receptor (OTR) levels (Schaller et al, 2010; Meziane et al, 2015).

To better understand the relationship between the oxytocinergic system and the pathogenesis of SYS, we analyzed oxytocin receptor (OTR) levels in Magel2-KO mice. We used autoradiography to quantify the expression of the OTR in brains of Magel2-KO and WT mice. Moreover, as OT treatment in Magel2 pups can improve not only the abnormal feeding behavior, but also the altered social skills (Meziane et al, 2015), we included in our analysis a group of Magel2-KO mice treated at birth with subcutaneous injections of OT, to understand how the treatment acts on the targeted brain regions. For the analysis, we focused our attention on those brain areas known to be involved in the regulation of social behaviour.

We found a significant increase in OTR expression in the medial anterior olfactory nucleus (AONm) of KO males, and a tendency to increase in the piriform cortex (PC). In females, a strong decrease in receptor levels was detected in the anterior lateral septum. Importantly, an OT treatment at birth was sufficient to restore normal OTR levels in both the AONm and the PC of KO males. Other brain areas are currently under investigation.

Our findings prove that the absence of Magel2 has indeed an impact on the expression of the receptor. Our work will let us identify which are the brain areas targeted by OT treatments in Magel2-KO mice. Also, understanding how these alterations are related to Magel2 loss of function will help to better define the pathogenetic processes involved in neurodevelopmental disorders such as SYS and PWS.

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# MOLECULAR MECHANISMS OF THALLIUM NEUROTOXICITY: ANALYSIS OF OXIDATIVE AND METABOLIC STRESS IN HIPPOCAMPAL NEURONS

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Thallium (Tl) is a heavy metal, very toxic for biological systems. Until now the environmental rarity of Tl has caused a serious underestimation of its potential risk to human health. The nervous system is a critical target of this element and growing evidence supports the link between the exposure to low Tl doses and neurodegeneration. Tl effects on nervous system have been described mainly from a clinical point of view (insomnia, irritability, axonal degeneration, myelin loss, severe central and peripheral neuropathy have been highlighted). Few reports exist about the cellular and molecular mechanisms underlying Tl neurotoxicity since research has been focused mostly on non-neuronal tissues such as liver, kidney, cardiac and skeletal muscle.

In this work we investigated the specific molecular mechanisms of Tl-induced neuronal dysfunction using immortalized hippocampal neurons (HN9.10e cell line), a reliable *in vitro* model of one of the most vulnerable regions of central nervous system.

Neurons were incubated for 48h with three different Tl doses: 1, 10, 100 μg/L (corresponding to 4.9, 49 and 490 nM, respectively). Then, they were washed with fresh culture medium every 24h until 7 days after the Tl treatment. The removed culture medium was collected and analysed by head space - solid phase microextraction -gas chromatography -mass spectrometry (HS-SPME-GCMS) and by RP-HPLC with UV detection. The morphological alterations induced by Tl were quantitatively analysed by confocal microscopy and fluorescent probes.

Tl exposure had significant effects on neuronal growth rate and morphology. An early-onset mitochondrial dysfunction was also evidenced, associated with signs of cellular deregulation such as neurite shortening, loss of substrate adhesion and increase of cytoplasmic calcium. Mitochondrial ROS (mtROS) and transmembrane mitochondrial potential ($\Delta\Psi_m$) were altered even by the lowest Tl dose (1 μg/L), in the absence of changes of neuronal morphology. The treatment with the ATP synthase inhibitor oligomycin revealed immediate and severe impairment of the mitochondrial function, more significant than that measured by the simple quantification of the TMRM fluorescence. These results indicate that mitochondria are a key sub-cellular target of Tl and that the morphological and functional signs of neuronal alteration closely correlate with the energy loss. Furthermore a remarkable shift in the metabolic profile was observed after Tl exposure, indicative of severe alteration of the neuronal bioenergetics. The diagnostic interest of the relationship between level of neuronal stress and metabolic profile will be discussed.

In conclusion, the results herein reported highlight that Tl doses much lower than those considered safe for the human environmental exposure induce significant and long-lasting neuronal alterations.

# PHARMACOLOGICAL STRATEGIES TO SLOW DOWN CONE DEATH AND VISION LOSS IN ANIMAL MODELS OF RETINITIS PIGMENTOSA

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Background: The term Retinitis Pigmentosa (RP) defines a group of inherited dystrophies characterized by progressive degeneration of the visual cells and abnormalities in the retinal pigment epithelium. Vision is lost slowly but inexorably and the outcome is near-blindness. Although the primary mutation responsible for RP is usually in rod-specific genes, the biological mechanisms linking the primary degeneration of rods to the secondary death of cones, ultimately leading to loss of all the useful vision, are still poorly understood. Among the recognized causes of cone secondary death are oxidative stress and release of "toxic" factors from dying rods or intervening glia. Apoptosis is one (although not the sole) mechanism of photoreceptor death.

Here we summarize the efficacy of two different therapeutic approaches undertaken *in vivo*, in two separate animal models of RP, respectively mimicking a recessive mutation of Phosphodiesterase (rd10 mutant mice), occurring on a temporal scale of approximately 30 days; and a dominant mutation of Rhodopsin (Tvrm4 mutant mice), which shows a more rapid phenotype (2 weeks). The goal is to target different biological pathways to slow down the progression of the disease and the loss of vision with a mutation-independent approach.

Methods: Myriocin, a fungal molecule with potent immunosuppressive and anti-inflammatory activity, is known to have high anti-apoptotic activity by inhibiting serine-palmitoyl transferase (SPT) the rate-limiting enzyme that controls ceramide *de novo* synthesis. Myriocin was administered by different routes (intravitreal, as eye drops containing a carrier, and by intraperitoneal injections), to both rd10 and Tvrm4 mutant mice during the period of maximum retinal degeneration. Retinal integrity was assessed morphologically and biochemically and visual function was assessed by ERG recordings.

A different approach takes advantage of the use of nutraceutical molecules such as a flavanone (Naringenin), abundant in citrus fruits such as grapefruits (*Citrus paradisi*) and oranges (*Citrus sinensis*), and a flavonol (Quercetin), abundant in grapes, green tea and apples, both with known anti oxidant properties. Oral, long-term treatment with Naringenin and Quercetin was performed on rd10 mice during the time window of photoreceptor death and the effects tested on retinal structure and function as above.

Results: Myriocin demonstrates an efficacy in slowing down retinal degeneration independently of the route of administration used and of the mutation causing the pathology, being effective in both rd10 and Tvrm4 mutants.

Naringenin and Quercetin also show beneficial effects on retinal structure and function, accompanied by a significant reduction in retinal levels of detoxifying enzymes Sod1 and Sod2, as well as in a decrement of retinal acrolein staining, indicating a reduction of ROS in the cellular environment.

Conclusions: We confirm and extend previous studies on the efficacy of Myriocin to delay RP and we demonstrates the beneficial effects of two nutraceutical, nontoxic, molecules with known antioxidant properties. A combinatorial therapy based on the employment of both Myriocin and the nutraceuticals used here is possible and amenable to human translation.

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# MULTIPLE STRATEGIES TO TARGET INFLAMMATION IN INHERITED RETINAL DEGENERATION

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Background: The term Retinitis Pigmentosa (RP) defines a group of inherited dystrophies characterized by progressive degeneration of photoreceptors and abnormalities in retinal pigment epithelium (RPE). In typical RP, primary degeneration of rods is followed by secondary death of cones, eventually leading to loss of all useful vision. We have recently shown that in rd10 mice, a mouse model of autosomal recessive RP with a mutation in rod-specific Phosphodiesterase, the peak of cone death (postnatal day 45, P45) is associated with a strong retinal inflammatory response. An anti-inflammatory treatment with Dexamethasone, a widely used steroid, administered by general route during the time window of maximum rod and cone degeneration, effectively slows down cone death, concomitantly reducing microglia/macrophages activation in the outer retina and delaying vision loss Here, we describe the rationale and preliminary data of a new pharmacological strategy for RP, specifically exploring the role of the CCL2/CCR2 inflammatory axis on cone death as well as the potential benefits of the NGF analogue “human NGF painless, hNGFp”, endowed with anti-inflammatory properties.
Methods: Groups of rd10 mice receive daily 4mg/kg RS102895 (CCR2 inhibitor) administered sub-cutaneously, from P24 to P45. Control mice receive vehicle. In a parallel study, we use the same mutants for a chronic treatment with 0.54 mg/kg hNGFp administered daily from P24 to P45 by mean of intranasal instillation and/or eye drops. Retinas are harvested at P45, fixed in 4% PFA, stained with cell-type specific antibodies and analysed by microscopy to assess general morphology, rate of photoreceptor survival, number of cones and of microglia-macrophages/retina. RPE morphology and integrity are assessed using whole mount staining for tight junction components. Upon isolation of a protocol leading to succesfull cone rescue, visual behaviour tests and further molecular screening of the retina for inflammation/ immune response pathways are scheduled.
Results: By using RS102895, a selective blocker of the CCL2/CCR2 pathway of signaling, we plan to to decrease massive monocyte-macrophage migration into the retina typical of the acute phase of rod degeneration. By using hNGFp topically, in the same time window, we aim at reaching the retina through a non invasive route to limit the reactivity and aggressiveness of microglial/macrophages .
Conclusions: Converging experimental evidence shows that breakdown of the eye immune privilege and infiltration of macrophages and microglia in the outer retina further contribute to retinal degenerations. Cones, genetically intact, may suffer from side effects of prolonged inflammation and ultimately die out. Timing, route of administration, potential toxicity and pleiotropic effects of the molecules used here make the experimental outcome challenging and yet promising. A combinatorial approach using multiple drugs might be the right choise to ameliorate the RP phenotype.

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# MICROGLIA-DERIVED EXTRACELLULAR VESICLES CARRYING Aβ IMPAIR CORTICO-HIPPOCAMPAL NETWORK

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Alzheimer's disease (AD) is a chronic progressive neurodegenerative disease characterized by memory decline and cognitive dysfunction. AD neuropathological hallmarks are loss of synapses and neurons, extracellular amyloid-beta (Aβ) deposition, and intraneuronal tau aggregation. An extensive literature implicates synaptic dysfunction as an early mechanism affected in AD, with an initial involvement of the Entorhinal Cortex (EC). However, we still lack a full understanding of how synaptic dysfunction starts, propagates, and alters network activity. Among other transfer modes, Aβ dissemination has been suggested to occur through release of Extracellular Vesicles (EVs), which may facilitate delivery of pathogenic proteins over large distances. In previous experiments performed in EC slices we have observed that incubation with microglia derived Aβ-EVs causes an impairment of long-term potentiation (LTP), a form of synaptic plasticity thought to underlie memory. Moreover, we demonstrated the progression of synaptic alterations from the EC to adjacent areas (i.e. hippocampus) caused by stereotaxic injection of Aβ-EVs at the level of the lateral EC (LEC).

In view of determining whether EVs are responsible for changes in neuronal connectivity following Aβ elevation, we performed *in vivo* chronic EEG recordings in freely moving mice at different time points. All mice were recorded daily (for 2hr) during a baseline period and then, they were injected in the LEC with Aβ-EVs, Aβ [1-42], ctrl-EVs or ACSF and EEG monitored from hippocampus and cortex. A second session of EEG recordings was performed soon (1 hour) after EVs injection as well as at 1 day, 1 week and 1 month after the injection (2hr daily for five consecutive days). Moreover, we evaluated the effect of Aβ-EVs on memory using either LEC-dependent associative or hippocampal dependent non-associative tasks based on object recognition. After injection of Aβ-EVs we found a cortico-hippocampal hyper-excitability revealed by a significant higher number of isolated spikes and by increased time spent in clusterized activity. A comparable activity was not observed in mice injected with Aβ [1-42] alone. The behavioural analysis revealed an impairment in memory that was limited to LEC-dependent task shortly after the injection of Aβ-EVs and then involved non-associative hippocampal task at later time points. These findings suggest that EVs carrying Aβ can trigger and spread the cortico-hippocampal network dysfunction contributing to memory impairment.

# MUTATIONS IN PROTEINS RESPONSIBLE OF FAMILIAL CASES OF ALZHEIMER'S DISEASE (FAD) AFFECT MITOCHONDRIALMETABOLISM

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Mitochondrial dysfunction is an important feature of many neurodegenerative disorders, including Alzheimer's Disease (AD).Indeed, mitochondrial functional and morphological alterations have been observed in brain biopsies of Alzheimer's disease patients, both in the sporadic forms and in the familial ones. The familial forms of the disease (FAD) are caused by mutations in proteins composing the catalytic core of the □-secretase, namelypresenilin 1 and 2 (PS1, PS2), or in the amyloid precursor protein (APP), thou the mechanisms by which these mutated proteins contribute to the development of the pathology are still debated. It is known that FAD mutations affect the generation of the amyloid □ (A□) peptides, and there are evidences that A□ increases neuron vulnerability to oxidative stress and impairs mitochondria electron transport chain. We have studied mitochondrial bioenergetics in both brain-cortex isolated mitochondria, and in vitro in neuronal cultures derived from the hippocampi of wt and transgenic mice bearing the FAD-linked PS2N141I mutation, either alone, or in combination with the APP Swedish mutations K670N M671L. We didn't observe statistically significant difference in mitochondria respiration, or membrane potential comparing mitochondria isolated from cortical regions of wt and transgenic animals of different ages form new born up to two years of age. However, in primary neuronal cultures of double transgenic animals mitochondria appear less efficient in maintaining the membrane potential when challenged with drugs inhibiting the respiratory chain (rotenone, and/or antimycin). Also, transgenic neurons appear to have a reduced spare respiratory capacity that might affect their ability to cope with stressful conditions. In fact, when challenged with toxic stimuli, such as an increase in extracellular KCl that cause a partial depolarization of the membrane, a larger proportion of transgenic neurons than wt neurons undergo $Ca^{2+}$ overload.We are interested in clarifying how mitochondrial impairment might contribute in the ultimate neuronal demise which characterizes AD.

# EPILEPSY-CAUSING REELIN MUTATIONS RESULT IN INTRACELLULAR DEGRADATION AND IMPAIRED SECRETION OF MUTANT PROTEINS

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Autosomal dominant lateral temporal epilepsy (ADLTE; OMIM 600512) is a genetic epileptic syndrome 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, including RELN, which encodes Reelin, a well-known extracellular protein involved in the regulation of brain development and functioning. To evaluate the effects of four published disease-causing nonsynonymous Reelin mutations on protein secretion, we previously assayed HEK293T cells transiently expressing Reelin mutants by western blot analysis of cell lysates and media and found that secretion of mutant Reelin to cell culture media was either abolished or significantly reduced compared to wild type (wt) Reelin. Similar results were obtained using COS7 cells as a host. We also showed in COS7 cells that trafficking of Reelin mutant protein from the endoplasmic reticulum marker to the Golgi apparatus was impaired or completely blocked. To test whether the apparent interruption of intracellular trafficking is a consequence of degradation of Reelin mutants within the cell, we analyzed the expression of two markers of autophagy in COS7 cells overexpressing Reelin mutants. We found that expression of Reelin mutant proteins induced formation of cytoplasmic vesicle-like dots immunoreacting with p62, a ubiquitin-binding protein that co-localizes with ubiquitinated protein aggregates and mediates formation of autophagosomes, the vesicles that ensure degradation of abnormal proteins upon fusion with lysosomes. Furthermore, Reelin mutant proteins co-localized with p62-immunoreacting vesicles, suggesting they were embedded into autophagosomes and degraded. In addition, expression of Reelin correlated with a significant increase of LC3-positive autophagosomes, and some Reelin mutants substantially co-localized with LC3-positive vesicles. These experiments clearly indicate that Reelin mutant proteins are recognized as abnormal by the protein quality control machinery, owing to alteration of their three-dimensional folding, and degraded within the cells via the autophagy pathway.
Our results provide evidence for a common loss-of-function mechanism, implying hampered trafficking and subsequent rapid degradation of misfolded mutant proteins, and suggest possible therapeutic approaches to correct Reelin genetic defects.

# 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 form of dementia, in which learning, memory and cognitive function decline simultaneously, dramatically and permanently. $Ca^{2+}$ homeostasis impairment and mitochondrial dysfunction are crucial events associated with several neuropathologies, including AD, ultimately leading to neurodegeneration by still largely unknown mechanisms. Most of the AD cases are sporadic, while only a small percentage are genetic (Familial AD, FAD) and mainly due to mutations in genes encoding presenilin (PS) 1, PS2 and APP (amyloid precursor proteins). These proteins, in addition to their role in β-amyloid plaques formation, directly affect $Ca^{2+}$ signalling.

Here, we aim at uncover the role of $Ca^{2+}$ dyshomeostasis in FAD neuronal dysfunction. Employing a FAD mouse model (PS2-N141I/APPswe) in combination with two-photon time-lapse imaging techniques, we are investigating the effects of FAD mutations on cellular and mitochondrial $Ca^{2+}$ dynamics in hippocampal slices at different ages, exploiting the intracranial injection of adeno-associated virus expressing FRET-based $Ca^{2+}$ probes. Intracellular $Ca^{2+}$ rises induced by ionotropic (NMDA) or metabotropic (DHPG) glutamate receptor stimulation were similar in their peaks in FAD and WT mice at 1.5 month of age, although the duration of the $Ca^{2+}$ increases were substantially longer in FAD mice. The mitochondrial $Ca^{2+}$ uptake ability, in response to the same stimulations, is currently under investigation.

To molecularly characterize $Ca^{2+}$ signal dysfunctions, and their correlation with neuronal death, we exploit RT-qPCR analysis of cortices and hippocampi derived from FAD and WT mice, at different age during disease progression. An upregulation of the Mitochondrial Calcium Uniporter (MCU), and of its several regulatory subunits, has been found in FAD mice, compared to WT controls, at 1.5 month of age. Furthermore, at this age, FAD mice, compared to WT, showed upregulation and downregulation of several genes involved in both inhibitory and excitatory neurotransmission; similar results were obtained concerning genes linked to autophagy, mitophagy, apoptosis and necrosis. Altogether, these results suggest that a substantial rearrangement of gene expression occur early in FAD mice, especially of those involved in $Ca^{2+}$ homeostasis and cell death control. These latter may potentially represent novel therapeutic targets towards the treatment of AD.

# CALCIUM SIGNALLING AND MITOCHONDRIAL FUNCTION IN PRESENILIN 2–KNOCK-OUT MICE: IS THERE ANY LOSS-OF-FUNCTION PHENOTYPE RELATED TO ALZHEIMER'S DISEASE?

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Alzheimer's disease (AD) is the most common form of dementia characterized by progressive cognitive dysfunctions. The histopathological hallmarks of AD brains are extracellular neuritic plaques, formed by amyloid beta (Aβ) peptides and intracellular neurofibrillary tangles of hyper-phosphorylated tau. Familial cases of AD (FAD) are due to mutations in one of three genes encoding amyloid precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2). PS1 and its homologue PS2 form the catalytic core of the γ-secretase complex that, by cleaving APP in concert with β-secretase, produces neurotoxic Aβ peptides. Recently, FAD--PS1 mutations have been associated with a loss-of function phenotype for γ-secretase activity, since a FAD-resembling phenotype was described in mice knock-out (KO) for PS1.
With this project, we intend to verify if the model propose for FAD-PS1 mutants can be extended also to PS2.
In the last years, evidence has accumulated suggesting that AD is linked to an imbalance of cellular $Ca^{2+}$ homeostasis. In particular, FAD-PS2 mutations cause a reduction in the endoplasmic reticulum (ER) $Ca^{2+}$ content, by inhibiting SERCA activity, while increase the physical apposition between ER and mitochondria, favouring ER to mitochondria $Ca^{2+}$ transfer and modulating organelle activity. We thus investigated $Ca^{2+}$ signalling and mitochondrial function in cortical and hippocampal neurons from PS2-KO mice to check whether the previous characterized FAD-PS2-linked phenotypes could be considered loss-of function. For $Ca^{2+}$ measurements, cortical and hippocampal PS2-KO neurons (and control cells from wild type, WT, animals) have been infected in vitro by Aden-Associated viruses expressing the cDNA of FRET-based cytosolic or mitochondria-targeted $Ca^{2+}$ probes. Neurons were stimulated with a mixture of metabotropic or depolarizing stimuli. Despite similar cytosolic $Ca^{2+}$ rises, PS2-KO neurons show a significantly reduced mitochondrial $Ca^{2+}$ uptake, compared to those observed in WT neurons, indicating that the loss of PS2 does not recapitulate FAD-PS2-linked mitochondrial phenotype. The general impairment in mitochondrial $Ca^{2+}$ handling is currently under investigation to understand whether it could be considered a primary defect. Preliminary results indicate no difference in mitochondrial number, morphology and protein content. However, a significant reduction in basal, maximal and ATP-linked respiration is present in PS2-KO neurons, compared to WT cells.

# EXPLORING THE ROLE OF ASTROCYTIC $Ca^{2+}$ SIGNALING IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a chronic, incurable neurodegenerative disease, characterized by severe and progressive memory loss and cognitive dysfunctions.
Over the last decades, research on neurodegenerative diseases has been focused fundamentally on neurons. Many studies provide evidence, however, that brain function and dysfunction are governed by dynamic interactions between neurons and astrocytes.
We here evaluate the involvement of these glial cells in the pathogenesis of AD, focusing on astrocytic $Ca^{2+}$ signaling and its dysregulation along the progression of the disease. Our main goal is to clarify whether a defective astrocytic $Ca^{2+}$ signaling precedes or follows amyloid-beta (Aβ) plaque deposition and neuronal dysfunction. To this aim, we employed three FAD mouse models: single mutants PS2.30H and APPSwe, which express the human PS2-N141I and the human APP Swedish mutation respectively, and the PS2APP (B6.152H) model that expresses both mutations. All the experiments were carried out in female mice at 3 and 6 months of age, before and after, respectively, the onset of plaque deposition in the PS2APP model.
To investigate astrocytic activity, we performed two-photon $Ca^{2+}$ imaging experiments in the somato-sensory cortex (SSCx) of GCaMP6f expressing mice, both in brain slices and *in vivo*. We found that along with the progression of AD, astrocyte $Ca^{2+}$ activity undergoes a sequence of changes: while spontaneous activity is significantly increased in 3-month-old PS2APP mice, 6-month-old PS2APP mice reveal a drastic reduction in both spontaneous activity and the responsiveness to different metabotropic receptor agonists. Although these defects start in concomitance with Aβ plaque deposition, astrocytic hypoactivity appears unrelated to proximity to plaques. Furthermore, these alterations are not present in APPSwe and PS2.30H mice, demonstrating that the expression of APP or PS2 mutation alone is not sufficient to fully recapitulate the astrocytic $Ca^{2+}$ defects observed in PS2APP mice.
Preliminary *in vivo* experiments reveal that spontaneous activity is drastically reduced also in the SSCx of anesthetized 6-month-old mice.
In conclusion, astrocytic $Ca^{2+}$ activity is strongly affected in PS2APP AD model. Additional experiments are currently under development, to assess the relevance of these astrocytic $Ca^{2+}$ defects in the disturbances of synaptic plasticity and of learning/memory processes that characterize AD.

# DEREGULATED MICROGLIAL PRODUCTION OF EXOSOMES IN A MICE MODEL OF ALZHEIMER'S DISEASE

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Alzheimer's disease (AD), the most prominent cause of senile dementia, is clinically characterized by the extracellular deposition of β-amyloid (Aβ), as a consequence of disturbed amyloid homeostasis. Aβ is normally produced at high levels in the brain and is efficiently cleared at an equivalent rate. Aβ is normally removed from the brain by the efflux of soluble Aβ (sAβ) to the peripheral circulation, for example through the blood–brain barrier (BBB), and by the proteolytic degradation of both soluble and fibrillar forms by microglia. In the last years evidence accumulated that microglia could hold the key to understand Alzheimer's Disease (AD). During phagocytosis and degradation of extracellular Aβ peptides, microglia produce and release cytokines, which can be harmful to neurons, and Aβ truncated forms, which may be more prone to aggregation (Mazzitelli, Filipello, Rasile et al. 2016). However, additional processes could contribute to the microglia-mediated deleterious effects in the context of AD. Taking advantage of the double transgenic AD mouse model (APPswe/PSEN1dE9), we analyzed how these human mutations influence microglia response to Aβ1-42 exposure. NanoSight quantification of EVs produced by microglia exposed to Aβ1-42, indicated that WT microglia respond to Aβ1-42 by decreasing the production of exosomes, while APP/PS1 microglia enhance exosome production. Exosomes are known to accumulate near Aβ plaques and are suspected to play a role in the spreading as well as the clearance of Aβ peptides. By MALDI-TOF analysis, we were unable to detect, in WT versus APP/PS1 microglia, different amounts of either Aβ inside exosomes or truncated Aβ fragments in the microglia extracellular medium. However, our results indicated that exosomes derived from APP/PS1 microglia, both at their steady-state or from pre-activated Aβ1-42 exposed cells, have a detrimental effect on Aβ clearance in an in-vitro BBB model which exploits human endothelial cells and astrocytes (Lauranzano et al., 2019). Of note, WT microglia-derived exosomes displayed a similar behavior only upon exposure of microglia cells to Aβ1-42. These findings suggest that microglial exosome dysregulated production can be one of the mechanisms that ultimately lead to Aβ accumulation and plaques burden also in sporadic forms of AD.

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# iPSC MODELLING OF GENETIC PARKINSON'S DISEASE WITH MUTATIONS IN THE GENE POLG

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Mitochondrial dysfunction has been implicated in the pathogenesis of sporadic, idiopathic Parkinson disease. In some cases, mitochondrial DNA primary genetic abnormalities, or more commonly, secondary rearrangements due to polymerase gamma (POLG1) gene mutation, can directly cause parkinsonism. Mitochondrial DNA is replicated by the DNA polymerase gamma complex which is composed of a catalytic DNA polymerase encoded by the POLG gene. Here we report a case of patient carrying the mutation P648R that presented the chronic progressive external ophthalmoplegia, myopatia, peripheral neurophaty, mtDNA multiple deletions and parkinsonism. We reprogrammed skin fibroblasts into transgene-free iPSCs from one patient and unrelated healthy donors. hIPSCs were then differentiated into stable neural progenitor cell (NPC) lines and neurons. Interestingly, PolG mutant NPCs showed a reduced mtDNA content compared to control cells. Moreover, PolG mutant NPCs exhibited heightened oxidative stress and a decrease in cellular respiration, in particular a reduction of complex I and complex IV expression. Moreover, PolG-mutant NPCs have a reduced neuronal differentiation potential and this phenotype is completely rescued using isogenic line where using a CRISPR/Cas9 system we obtained a heterozygous clone. Surprisingly, differentiation of PolG mutant NPC under low oxygen condition is sufficient to rescue the neuronal differentiation potential likely due to HIF1-$\alpha$ expression. Indeed, it is demonstrated that chronic hypoxia leads to a marked improvement in survival, body weight, body temperature, behavior, neuropathology and disease biomarkers in a genetic mouse model with mitochondrial disease. This study establishes a new human in vitro model for neurodegenerative disorders ideal for revealing the relation between mitochondrial dysfunctions and neuronal death as well as particularly attractive for developing new approaches of neuroprotection and translational therapies.

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# EVOLUTION OF EPILEPTIFORM ACTIVITY IN ZEBRAFISH BY STATISTICAL-BASED INTEGRATION OF ELECTROPHYSIOLOGY AND 2-PHOTON $Ca^{2+}$ IMAGING

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The study of sources and spatio-temporal evolution of ictal bursts is critical for the mechanistic understanding of epilepsy and for the validation of anti-epileptic drugs. Zebrafish (Danio rerio) is a powerful vertebrate model representing an excellent compromise between system complexity and experimental accessibility. Here, we performed the quantitative evaluation of the spatial recruitment of neuronal populations during physiological and pathological activity by combining local field potential recordings (LFP) with simultaneous 2-photon $Ca^{2+}$ imaging in models of epilepsy. We developed an unbiased method to extract and quantify electrophysiological transients coupled with $Ca^{2+}$ events. Furthermore, we computed topological maps showing brain regions where $Ca^{2+}$ fluctuations were maximally correlated with the LFP. Our data point to the cerebellum as the main anatomical region involved in the generation of epileptiform signals. Finally, we applied these tools to assess the efficacy of the anti-epileptic drug valproate on a zebrafish model of West syndrome, caused by the loss of function of the inward rectifier K-channel Kir 4.1. This study demonstrates the power of coupling two-photon imaging with LFP recording for the classification and quantification of activity. Unfortunately, while electrophysiology is amenable to be scaled for high-throughput screening, two-photon imaging is not. We wondered if we could predict the extent of $Ca^{2+}$ recruitment from the electrophysiological signature of the epileptiform events thus solving the inverse problem of reconstructing the sources of the LFP signal from the signal itself. To answer this question, we developed an innovative method which uses the $Ca^{2+}$ imaging maps to train an electrophysiology-based algorithm for automated seizure detection built on a mono-dimensional multilayer convolutional neural network (1D-CNN). This deep learning system showed a good features extraction ability, easy scaling and therefore the possibility of being a valid instrument for the cross-over study of $Ca^{2+}$ imaging and electrophysiology. The analysis of the available data set shows that the spatial pattern of $Ca^{2+}$ activation can be broadly described by two set of maps, associated either to interictal-like activity or to generalized seizures and that the network predicts with a large degree of accuracy the associate $Ca^{2+}$ map from the LFP data.

# NUCLEOLIN SUPPRESSES ALS-RELATED TDP-43 TOXICITY IN YEAST AND MAMMALIAN CELL MODELS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by motor neuron degeneration, muscle atrophy, paralysis and, ultimately, death. In most cases (90-95%), ALS occurs sporadically, but 5-10% of patients show a familial history. About 10% of these familial ALS forms involve the inheritance of mutations in the gene encoding TDP-43, a nuclear protein involved in transcription and the regulation of nucleocytoplasmic trafficking, while 90% of them are due to mutations in other genes (e.g., *C9ORF72, SOD1, FUS*, and *NEK1*).. Strikingly, in the vast majority of ALS cases (including both familial and sporadic forms), TDP-43 is depleted from the nucleus of MN and glial cells and mislocalizes to the cytoplasm where it accumulates in insoluble aggregates, suggesting a pivotal role for this protein in ALS pathogenesis. If, on one hand, TDP-43 aggregation can harm cells through a gain of toxicity, a loss of TDP-43 function in conjunction with its nuclear depletion has also been proposed as a pathogenic mechanism. Although the modes of TDP-43 cytotoxicity are far to be fully understood, increasing evidence indicates that specific cellular proteins able to modulate TDP-43-mediated damage could play a crucial role in disease. In this scenario, we unveiled that the nucleolar protein nucleolin (NCL) acts as a potent suppressor of TDP-43 toxicity, as it is able to completely antagonize the devastating effects that human TDP-43 causes in yeast cells. Furthermore, we observed that NCL can also counteract TDP-43-dependent damage in mammalian cells, i.e., in a more consistent patho-physiological context.

# PREVALENCE OF ATRIAL FIBRILLATION SUBTYPES IN THE ITALIAN ELDERLY. PROGETTO FAI

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OBJECTIVE. To evaluate the prevalence of atrial fibrillation (AF) and its subtypes in a large 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.[1] 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. AF subtypes were defined following the European Society of Cardiology criteria.[2]
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. Final prevalence was 7.3% (95% CI, 6.6-8.1) for total AF, 8.6% in men, 6.2% in women. Considering different AF subtypes, prevalence was 3.1% (95% CI, 2.6-3.6) for paroxysmal AF, 3.5% in men, and 2.7% in women; 1.7% for persistent AF (95% CI, 1.4-2.1), 1.8% in men, and 1.6% in women; and 2.5% (95% CI, 2.1-3.0) for permanent AF, 3.3% in men, and 1.9% in women. Total AF prevalence ranged from 3.0% in patients aged 65-69 years to 16.1% in those aged 85+. Figures were higher in men, and increased with age also for the three subtypes of AF.
DISCUSSION AND CONCLUSIONS. Our results indicate a high burden of AF in Italy. Considering that the various types of AF differ in terms of clinical characteristics and prognostic implications, and that they may carry a different risk for vascular and nonvascular comorbidities, including thromboembolic events, reliable epidemiological data on their burden at population level seem essential for both clinicians and policy-makers.

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# NEW LIGHT ON OXYTOCIN RECEPTORS

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The neurohormone oxytocin (OXT) plays a role in various functions including endocrine and immune functions but also parent-infant bonding and social interactions. OXT triggers social behaviors by binding to oxytocin receptors (OXTRs) and both distribution and number of OXTRs in the brain affect the type and degree of behavioral responses. Receptor autoradiography has been used for over 30 years to study the presence of OXTRs in the brain and other organs, and still represents the only possibility to visualize the OXTR physical distribution in tissues. However, this technique presents different limits: reduced anatomical resolution, impossibility to perform double staining, concerns related to the use of radioactive materials and high costs.

As specific OXTR antibodies are difficult to produce and are not available, especially for murine OXTR, we have generated two different fluorescent OXT analogues. These analogues maintain all the pharmacological properties of OXT in term of affinity and selectivity for OXTR. When we used them in cells, we were able to visualize receptor binding and to follow receptor trafficking in response to OXT stimulation. Moreover, we obtained a beautiful staining for OXTR on brain slices. In particular, not only we confirmed the same OXTR distribution and expression levels observed with brain autoradiography but we obtained anatomically high-resolution images. We were also able to compare the distribution of OXTR and dopamine D2R receptor using our OXT-fluorescent analog and an antibody for D2R on the same brain slice.

Our newly developed ligands make possible to precisely determine the level and distribution of OXTRs within the brain and can be applied to study whether the actions of OXT are altered in response to a genetic condition, phenotypic abnormality, disease state or drug treatment. This knowledge can then be used to the development of therapies to treat neurodevelopment and neuropsychiatric disorders associated with dysregulation of the OXT system, such as autism and schizophrenia.

# INCREASED SENSITIVITY TO THE REWARDING EFFECTS OF Δ9-TETRAHYDROCANNABINOL AND MDMA AFTER EXPOSURE TO NICOTINE IN MICE AND ZEBRAFISHZEBRAFISH

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Marijuana and tobacco are substances frequently used by adolescents. Although no direct association has been found between the early onset of smoking and later cannabis use, early nicotine use may increase the risk of developing a cannabis use disorder [1]. Nicotine can therefore act, on the brain, as a "gateway drug". The term "gateway" is used to describe a sequential progression in the use of addictive substances from tobacco and alcohol to cannabis, and then to other illicit drugs. This effect seems to be a response to the drug per se [2], but there are no data regarding its possible gateway effect in relation to amphetamine derivatives.
Zebrafish is a valuable model for high throughput drug discovery and screening and can be used to investigate some aspects of neuropsychiatric disorders, including addiction and reward [3].
On these bases our aim was to investigate the gateway effect of nicotine on the rewarding properties of Δ9-tetrahydrocannabinol (Δ9-THC) and 3,4-methylenedioxymethamphetamine (MDMA), in mice and zebrafish, using the classical conditioned place preference (CPP) task.
Eight-week-old male Balb/c mice (30 per cage) were daily exposed for seven weeks (3 times/day) to standard (cig, containing 0.8 mg of nicotine/cigarette, 10 mg of tar and 10 mg of carbon monoxide smoked), electronic (e-cig, containing 5.6 mg of nicotine dissolved in 1 ml of aqueous solution of propylene glycol (55%), glycerin (35%), flavor and fragrance agents) cigarettes or air, using a mechanical ventilator. This technique mimics the human route and simulates the pharmacokinetic characteristics associated with CIG smoking or e-CIG inhalation. Zebrafish were exposed to nicotine (1 mg/L) or vehicle dissolved in the water tank for two weeks.
At different intervals (2, 30, 60 days) after cig/e-cig/air (mice) or nicotine/vehicle (zebrafish) withdrawal (wdw) animals were tested in CPP paradigm, based on the association of a particular environment with a drug, after treatment with Δ9-THC or vehicle (mice: 0.01 mg/kg/i.p. for 5 days; zebrafish: 0.01 mg/kg/i.m. for 1 day) and MDMA (zebrafish: 0.1 mg/kg/i.m.). 2, 30 and 60 days, after CPP paradigm, animals were sacrificed, brains removed for binding and western blot analysis. Two way ANOVA for repeated measures, followed by Bonferroni test was applied to evaluate the differences among groups.
Mice previously exposed to e-cig/cig showed a greater response to Δ9-THC induced-CPP, an increase of Δ-FosB expression at all the tested intervals. A decreased number of CB1 receptors in the nucleus accumbens compared to control group was shown at 60 days. In zebrafish, an increased response to the rewarding effects of MDMA during wdw, was shown. Experiments are in progress to investigate the effect of Δ9-THC.
Our results show that both mice and zebrafish share an increased sensitivity to drugs of abuse after nicotine exposure suggesting the translational value of nicotine gateway theory.

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# WIDE SPECTRUM OF EFFICACY OF SAIKOSAPONINS (ACTIVE INGREDIENTS OF *BUPLEURUM FALCATUM*) ON ALCOHOL SELF-ADMINISTRATION IN RATS

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Treatment with saikosaponin A (SSA) – an active ingredient of the Asian medicinal herb, *Bupleurum falcatum* – has been reported to suppress operant, intravenous self-administration of morphine and cocaine as well as operant, oral self-administration of alcohol in rats. It has been demonstrated that these *anti*-addictive properties of SSA are mediated, at least in part, by the $GABA_B$ receptor. This lab has recently started a research program aimed at investigating whether ingredients of *Bupleurum falcatum* other than SSA affect alcohol self-administration in rats. Accordingly, the present study investigated whether the *anti*-alcohol properties of SSA extend to saikosaponin $B_2$ ($SSB_2$), saikosaponin $B_4$ ($SSB_4$), saikosaponin C (SSC), and saikosaponin D (SSD; an epimer of SSA); these saikosaponins differ structurally in their glucidic component. Adult, female Sardinian alcohol-preferring rats were trained to lever-respond for alcohol (15%, v/v) on a fixed ratio 5 (FR5) schedule of reinforcement. Once responding had stabilized, rats were tested under the FR5 schedule after treatment with each single saikosaponin (0, 0.25, 0.5, and 1 mg/kg, i.p.). Treatment with SSA and SSD resulted in highly similar, marked reductions (50-60% at the highest dose tested) in number of lever-responses for alcohol and amount of self-administered alcohol. Conversely, treatment with SSC failed to alter lever-responding for alcohol and amount of self-administered alcohol. Treatment with $SSB_2$ and $SSB_4$ produced intermediate results, with reductions of 35-45% at the highest dose tested. The wide spectrum of efficacy of saikosaponins in reducing alcohol self-administration suggests that a structure-activity relationship may be established. To this end, additional data on other saikosaponins are needed. These results confirm that *Bupleurum falcatum* is a valuable source of compounds with *anti*-alcohol potential.

# INTERMITTENT THETA BURST STIMULATION OF THE PREFRONTAL CORTEX IN COCAINE USE DISORDER: A PILOT STUDY

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According to the World Drug Report 2017, 17 million people were past-year cocaine users in 2015 and cocaine seizures were reported in 153 countries during the period 2010-2015 (UNODC, 2017), suggesting that trafficking in cocaine is a global phenomenon. Numerous efforts are being made to target novel molecular and cellular targets and effective strategies against cocaine addiction. To date, though, pharmacological and psychological therapies for treating cocaine dependence have shown only limited success. Hence, research is very active and different experimental approaches are currently under investigation.

Transcranial magnetic stimulation (TMS) has been reported to be useful in the treatment of several neuropsychiatric diseases. A single TMS pulse lasts a few milliseconds, but when pulses are applied repetitively, as in the repetitive TMS protocol (rTMS), they can modulate long-term cortical excitability and affect the function of neuronal circuits in a frequency-dependent manner. TMS is earning a role in the therapeutic arsenal of cocaine use disorder. A widespread and still growing number of studies have reported beneficial use of rTMS in reduction of craving, intake and cue-induced craving in cocaine addicts. In spite of these encouraging findings, many issues are still unresolved such as brain area to be stimulated, laterality of the effects, coil geometry and stimulation protocols/parameters. Intermittent theta burst stimulation (iTBS) is a more tolerable protocol administered at lower intensities and shorter intervals than conventional repeated TMS (rTMS) protocols. Yet, its effects on cocaine craving and length of abstinence in comparison with standard high frequency (10-15Hz) protocols have never been evaluated so far. At present, no study has compared the effects of iTBS treatment sessions vs standard high-frequency TMS protocol (HF) in cocaine-dependent patients. Thus, a key practical question remains unaddressed: does iTBS perform comparably to the existing standard of care in cocaine addicts? This study aimed (i) to evaluate the effectiveness of iTBS (n=25) targeting the prefrontal cortex (PFC) and the insular cortex bilaterally on cocaine craving and intake and (ii) to compare safety and effectiveness for iTBS (3 min) versus PFC-rTMS with 15 Hz (15 min) of the same brain area (n=22) in treatment-seeking cocaine addicts.

The main finding of the present study is the efficacy of PFC-iTBS in reducing cocaine intake and craving in treatment-seeking cocaine addicted patients. Importantly, our iTBS protocol proved to be as safe and effective as a HF (15 Hz) protocol, as both treatments had low numbers of dropouts and similar side-effects, safety and tolerability profiles. In both HF and iTBS protocols, about 80% of urine screen test were negative for cocaine within the fourth week of treatment and the majority (72% and 75%, respectively) of the patients reported to have quitted cocaine consumption by the same time. In line with it, craving significantly decreased by the end of treatment. While larger studies are warranted to confirm these observations, iTBS appears to be a valid approach to be considered in treatment-seeking cocaine addicts, especially in light of its brief duration (3 mins) vs 15 Hz stimulation (15 mins). The use of iTBS would allow increasing the number of patients treated per day with current rTMS devices, thus reducing patient discomfort and hopefully reducing drop-out rates without compromising clinical effectiveness.

# CAN CHRONIC RED BULL TREATMENT DURING ADOLESCENCE AFFECT THE MESOLIMBIC DOPAMINE TRANSMISSION AND THE CARDIOVASCULAR SYSTEM IN ADULT RATS?

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Energy drinks are very popular non-alcoholic beverages among adolescents and young adults for their stimulant effects, mostly due to the high concentration of caffeine and sucrose and to the presence of taurine. The aim of the present research was to investigate, by in vivo microdialysis, the effect of repeated intraoral Red Bull infusion on dopamine transmission in the nucleus accumbens shell and core in adult rats subjected to chronic Red Bull consumption (4 weeks) from adolescence to adulthood. Furthermore, heart rate and blood pressure were weekly recorded in order to monitor the impact of chronic RB consumption on cardiovascular hemodynamic parameters. In addition, the cardiac contractility of Red Bull-Treated rats was studied by testing the biomechanical properties of isolated left ventricular papillary muscle. The main finding of the study was that in Treated animals, Red Bull increased nucleus accumbens shell dopamine by a non-adaptive mechanism, a pattern similar to that observed after administration of drugs of abuse. Moreover, Red Bull increased systolic and diastolic blood pressure but did not change heart rate. No change in isometric and isotonic mechanical parameters were associated with chronic Red Bull consumption. However, a prolonged time to peak tension and half time of relaxation and a slower peak rate of tension fall were observed in Red Bull-Treated rats. It can be hypothesized that Red Bull treatment affects hemodynamic cardiac responses and left ventricular papillary muscle contraction. The neurochemical results here obtained can explain the addictive properties of Red Bull, while the cardiovascular investigation findings suggest a hidden papillary contractility impairment.

# SUPPRESSING EFFECT OF KK-92A, A NEW POSITIVE ALLOSTERIC MODULATOR OF THE $GABA_B$ RECEPTOR, ON ALCOHOL SELF-ADMINISTRATION IN RATS

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Positive allosteric modulators (PAMs) of the $GABA_B$ receptor constitute a class of pharmacological agents with increasing interest in the alcohol research field. KK-92A is a new $GABA_B$ PAM, recently found to reduce operant, intravenous nicotine self-administration and reinstatement of nicotine seeking in rats. It was therefore of interest to test its ability to affect operant, oral self-administration of alcohol. To this end, adult female Sardinian alcohol-preferring rats were trained to lever-respond for alcohol (15%, v/v) on a fixed ratio 5 (FR5) schedule of reinforcement. Once responding had stabilized, rats were treated with KK-92A (0, 5, 10, and 20 mg/kg; i.p.) and exposed to FR5 (Experiment 1) and progressive ratio (Experiment 2) schedules of reinforcement. In Experiment 1, treatment with KK-92A resulted in a dose-dependent suppression of number of lever-responses for alcohol and amount of self-administered alcohol; magnitude of KK-92A-induced suppression averaged ~60% and ~90% at the doses of 10 and 20 mg/kg, respectively. In Experiment 2, treatment with KK-92A resulted in a dose-related reduction in lever-responses and breakpoint for alcohol; magnitude of KK-92A-induced reduction averaged ~65% and ~75% at the doses of 10 and 20 mg/kg, respectively. These results extend to KK-92A the ability of all previously tested $GABA_B$ PAMs to affect several alcohol-motivated behaviors, including alcohol self-administration, in rodents and confirm that these effects are a shared feature of the entire class of $GABA_B$ PAMs. The observed, high efficacy of KK-92A in suppressing alcohol self-administration is likely due to its ago-allosteric profile, combining a distinct intrinsic agonistic activity at the $GABA_B$ receptor with positive allosteric modulation.

# IN VIVO NEUROPHARMACOLOGICAL CHARACTERIZATION OF THE COGNITIVE ENHANCER MODAFINIL AND ITS ANALOGUE CE-123

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Medical treatments for dopamine-related cognitive impairments and neuropsychiatric conditions aim at modulating extracellular dopamine levels in the brain. Indeed, mesocorticolimbic dopamine neurotransmission is involved in processing motivation, salience and reward. The dopamine transporter (DAT) plays a key role controlling synaptic dopamine, being a favorite target for drug development. Unfortunately, commercially available inhibitors of dopamine reuptake, such as the psychostimulants methylphenidate and modafinil, are of limited selectivity for dopamine transporter (DAT) and produce side effects, among others abuse liability.

CE-123 (5-((benzhydrylsulfinyl)methyl) thiazole) is a recently developed selective DAT inhibitor that facilitates cognitive performance and improves spatial memory and motivational symptoms. Moreover, CE-123 shows atypical neurochemical characteristics, which may lower the side effect profile as compared to other psychostimulants.

In this study, we characterized neuropharmacological effects of CE-123 and the analogue R-modafinil in adult male Sprague-Dawley rats. Our results show that the number of 50 kHz ultrasonic vocalizations emitted by rats did not change following acute intraperitoneal administration of neither CE-123 nor modafinil at 1, 5 and 10 mg/kg. In freely moving animal microdialysis experiments, extracellular dopamine levels were reduced in the shell of the nucleus accumbens, and increased in the infralimbic/prelimbic cortex, by intraperitoneal injection of 10 mg/kg CE-123, with no effect of modafinil at the same condition. In vivo single unit extracellular recordings in anesthetized animals showed that cumulative intravenous CE-123 (1.25 to 10 mg/kg), but not modafinil, reduced firing frequency of pyramidal neurons in the infralimbic/prelimbic cortex. At the same doses, neither CE-123 nor modafinil affected the firing frequency or bursting activity of VTA dopamine cells.

Our data support the effects of CE-123 in cognition-associated brain areas, with no significant consequence in reward-related dopaminergic areas. We concluded that CE-123 proves as a potential innovative medication for impairments of cognitive functions with limited abuse liability.

# EFFECT OF N-ACYLETHANOLAMINE ACID AMIDASE INHIBITION ON LOCUS COERULEUS NORADRENERGIC NEURONAL RESPONSES TO MORPHINE

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Morphine is a potent opioid analgesic used to alleviate moderate or severe pain. Nowadays, opioid prescriptions are restricted due to the development of tolerance and the risk to develop addiction. Dopaminergic ventral tegmental area (VTA) and noradrenergic locus coeruleus (LC) are two crucial brain areas that play a pivotal role in addiction. In fact, VTA is important for the acquisition phase, since it is the main actor mediating rewarding properties of opioids. On the other hand, LC is a key component that contributes to the development of withdrawal symptoms.

Emerging evidence suggests that N-palmitoylethanolamine (PEA), an endogenous lipid neuromodulator, delays the development of morphine tolerance. In fact, it has been shown that PEA administration in morphine treated rats prolongs morphine's efficacy as analgesic. An alternative indirect way to increase endogenous PEA bioavailability is the inhibition of N-acylethanolamine acid amidase (NAAA), one of its major hydrolyzing enzymes. Thus, the aim of this study is to assess whether increasing endogenous brain PEA levels is capable to modulate neurophysiological response to morphine.

Therefore, our strategy was to augment endogenous PEA levels through a novel specific brain permeable NAAA inhibitor, AM11095. Then, we employed in vivo electrophysiology recordings in anaesthetized adult male rats that we treated with AM11095 (15 mg/kg, i.p.) or with its vehicle. After 30 minutes from drug administration, we assessed the electrophysiological response to morphine of VTA dopamine cells (cumulative doses 0.5-4.0 mg/kg, i.v.) and LC noradrenergic cells (cumulative doses 0.125-2.0 mg/kg, i.v.).

While preliminary, our results indicate that administration of AM11095: a) does not significantly affect basal electrophysiological properties of VTA DA neurons nor LC NA neurons, and b) does not alter their excitatory or inhibitory response, respectively, to morphine administration. Our next step is to unveil whether increasing endogenous PEA levels in the brain modulates morphine's effects on neuronal responses to nociceptive stimuli or attenuates the development of dependence following chronic morphine administration.

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