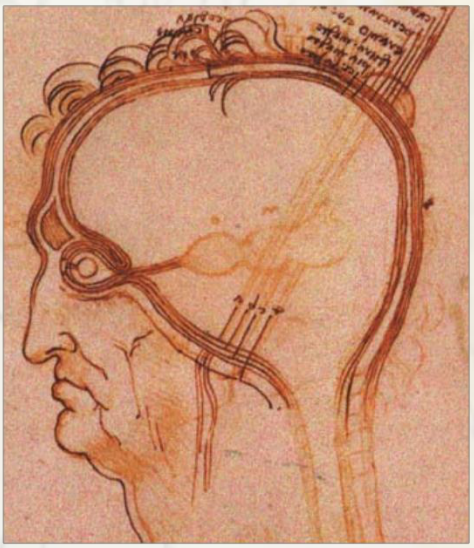

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TALKS

REGENERATION OF THE PERIPHERAL NERVOUS SYSTEM: THE CASE OF THE NEUROMUSCULAR JUNCTION

Cesare Montecucco

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At variance from the central nervous system (CNS) that shows a very limited capacity of regenerating after damage, the complete degeneration of motorneuron terminals can be achieved both in terms of anatomy and function in vertebrates. Several human diseases are due to their degeneration caused by a variety of causes from toxic to autoimmune, from mechanical to genetic alterations. They can be followed by a recovery that results from the interplay among the three cellular components of the neuromuscular junction (NMJ): motorneuron axon, perisynaptic Schwann cells, muscle fibre, and the basal lamina. Recent results on the intercellular signaling at the NMJ during degeneration/regeneration and on drugs that improve functional recovery will be presented. These results may be relevant for regeneration of the central nervous tissue as well.

This lecture is dedicated to the memory of Matteo Caleo.

REHABILITATION AND NEURO-MODULATION AFTER STROKE: NOVEL THERAPEUTIC STRATEGIES IN MURINE MODELS

Cristina Spalletti[1], Livia Vignozzi[2], Francesca Macchi[1], Anna Letizia Allegra Mascaro[1,3], Elena Montagni[3], Maria Pasquini[4], Sara Conti[4], Noemi Barsotti[5], Massimo Pasqualetti[5], Silvestro Micera[4,6], Matteo Caleo[1,2]

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Motor recovery after brain damage induced by an ischemic event is always challenging and often unsuccessful. Despite several innovative approaches have emerged to treat ischemic patients acutely, for most of them the treatment can be applied only in the subacute phase after injury. This time window is extremely precious for its plastic potential and offers the possibility to recover motor function by guiding peri-lesional areas to vicariate what was lost. Unfortunately, this plastic potential, if not properly guided, could led to maladaptive rearrangements and unwanted movement patterns.

Here we use a mouse model of stroke in forelimb motor cortex to test novel and highly translational neurorehabilitative approaches in subacute phase by combining robotic rehabilitation with plasticizing treatments.

We first engaged the serotoninergic system and demonstrated that a selective chemogenetic boosting of the serotoninergic system can increase perilesional plasticity and lead to a significant forelimb recovery without maladaptive movement detected by kinematic analysis. These results were replied by using an FDA approved drug to increase the serotoninergic tone. We are now focusing on non-invasive neurostimulation approaches and on the role of Parvalbumin Interneurons (PV-IN) in post-stroke recovery. We assessed the consequences of the ischemic lesion onto PV-IN activity by electrophysiological recordings and Wide Field Imaging in awake head restrained mice before and after stroke. Based on the results obtained we successfully tested non-invasive brain stimulation approaches, first targeting PV-IN by optogenetics and then using a more translational approach with Non-Invasive Transcranial Alternating Current Stimulation.

Both of the approaches led to a significant improvement of forelimb motor function and pave the way for successful combined approaches in clinical practice.

SOCIAL AND NEUROPHYSIOLOGICAL ASPECTS OF LICKING BEHAVIOR IN MICE

Claudia Alia[1], Nadia Giordano[1,2], Lorenzo Fruzzetti[3,4], Giulia Palla[1], Maria Pasquini[3,4], Raffaele Mazziotti[1], Alberto Mazzoni[3,4], Tommaso Pizzorusso[1,2], Silvestro Micera[3,4,5], Leonardo Fogassi[6], Luca Bonini[6] and Matteo Caleo[1,7]

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Planning and execution of voluntary movement depend on the contribution of distinct classes of neurons in primary motor and premotor areas. However, timing and pattern of activation of GABAergic cells, during specific motor acts remain only partly understood.
By means of electrophysiological and computational techniques we compared directly the response properties of putative pyramidal (PNs) and optogenetically verified GABAergic fast-spiking neurons (FSNs) during licking and forelimb retraction in mice. Recordings from anterolateral motor cortex and rostral forelimb area, reveal that FSNs fire earlier and for a longer duration than PNs, with the exception of a subset of early-modulated PNs in deep layers. Computational analysis reveals that FSNs carry vastly more information than PNs about the onset of movement. However, the informational redundancy was greater among FSNs than PNs. Accordingly, while PNs differently modulate their discharge during distinct motor acts, most FSNs respond with a stereotyped increase in firing rate.
These data suggest that a global rise of inhibition contributes to action initiation.
Beyond the self-driven motivation, the action initiation can we also be socially influenced. Therefore, in addition to specific neuronal contribution to movement, focusing on licking behaviour, we settled a social facilitation test. We measured spontaneous licking in an observer mouse, with or without a licking demonstrator. Preliminary results show a positive influence of social observation, resulting in an increase of time spent to lick, synchronized with the observed licking, suggesting the presence of neurophysiological correlates, mediating social influence in mice.

BRAIN CIRCUITS FOR FEAR ATTENUATION

Bianca A. Silva

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How are consolidated memories modified on the basis of experience? Understanding this biological process allows us to decipher how new information is constantly incorporated into existing memory, how a newly formed memory is integrated into previous knowledge and how the fine balance between memory stability and memory flexibility is maintained.
By using fear memory extinction as a model of memory update, we combined neuronal circuit mapping, fiber photometry, chemogenetic and closed-loop optogenetic manipulations in mice, and showed that the extinction of remote (30-day old) fear memories depends on thalamic nucleus reuniens (NRe) inputs to the basolateral amygdala (BLA). We find that remote, but not recent (1-day old), fear extinction activates NRe to BLA inputs, which become potentiated upon fear reduction. Both monosynaptic NRe to BLA, and total NRe activity increase shortly before freezing cessation, suggesting that the NRe registers and transmits safety signals to the BLA. Accordingly, pan-NRe and pathway-specific NRe to BLA inhibition impairs, while their activation facilitates fear extinction.

These findings identify the NRe as a crucial BLA regulator for extinction, and provide the first functional description of the circuits underlying the experience-based modification of consolidated fear memories.

ASTROCYTIC Ca^{2+} SIGNALING IN THE PROGRESSION OF ALZHEIMER'S DISEASE

Annamaria Lia[1], Gabriele Sansevero[2], Angela Chiavegato[3], Miriana Sbrissa[3], Diana Pendin[1,3], Tullio Pozzan[1,3], Nicoletta Berardi[2], Cristina Fasolato[3], Giorgio Carmignoto[1] and Micaela Zonta[1]

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[3]University of Padova, Italy

Alzheimer's disease (AD) is a chronic incurable neurodegenerative disorder characterized by progressive memory loss and cognitive dysfunctions.
The evidence that brain function requires dynamic interactions between neurons and astrocytes implies that both cell types can contribute to brain dysfunction. We here evaluate the involvement of astrocytes in the pathogenesis of AD, focusing on astrocytic Ca^{2+} signaling and its dysregulation during AD progression. 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 mouse model of AD expressing the human PS2-N141I and APP Swedish mutations.
To investigate astrocytic activity, we performed two-photon Ca^{2+} imaging experiments in brain slice and *in vivo* preparations of somatosensory cortex (SSCx) astrocytes expressing GCaMP6f. We found that astrocyte Ca^{2+} activity exhibits a sequence of changes along time: while spontaneous activity increases in 3-month-old PS2APP mice, both spontaneous activity and the response to different metabotropic agonists are drastically reduced in all astrocytic territories in 6-month-old PS2APP mice. Although these defects start in concomitance with plaque deposition, we show that they are unrelated to plaque proximity. We evaluated the consequences of these alterations for SSCx long-term memory processes and, importantly, we found that astrocytic Ca^{2+} hypoactivity is associated to a strong impairment of long-term potentiation in SSCx circuits in 6-month-old PS2APP mice, anticipating the specific loss of tactile memory retention occurring at 8 months of age.
We then explored the molecular mechanisms underlying Ca^{2+} dysregulation in PS2APP astrocytes and reveal that this deficit is closely associated with a reduction in Ca^{2+} concentration in the endoplasmic reticulum (ER) and in the expression of the ER Ca^{2+} sensor STIM1. Noteworthy, we provide evidence of a full rescue of astrocytic Ca^{2+} signaling upon specific STIM1 overexpression in astrocytes.
In conclusion, our data identify the dysregulation of astrocytic Ca^{2+} activity as a functional hallmark of early AD stages and point to the ER protein STIM1 as a target to rescue AD memory deficits.

NEURONAL CIRCUITS ACTIVITY AND DYNAMICS: FUNCTION AND DYSFUNCTION

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Neuronal circuits exhibit distinct patterns of activity that support a wide range of functions, from sensation to cognition. In this scenario, a growing body of evidence indicates that correlated activity among neurons within local and wider networks exerts a key role in coding neuronal information. Having said that, it is not surprising that alterations in synaptic function and network dynamics are among the first sign of neuronal disorders. We aim to understand how neuronal circuits generate patterns of activity which underly specific behaviour and how these rhythms are disrupted during neurological disorders.
Here, I will provide an overview of the topics we are interested in and recent data obtained by analysing neuronal circuits activity and dynamics in model organisms, combining behaviour, optical and electrophysiological recordings *in vivo*.

THE VALUE OF CORTICOSPINAL EXCITABILITY AND INTRACORTICAL INHIBITION IN PREDICTING MOTOR SKILL

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Objectives The observation of other's actions represents an essential element for the acquisition of motor skills [1,2]. While action observation is known to induce changes in the excitability of the motor cortices [3], whether such modulations may explain the amount of motor improvement driven by action observation training (AOT) remains to be addressed.
Methods: By using transcranial magnetic stimulation (TMS), we first assessed in 41 healthy volunteers the effect of action observation on corticospinal excitability, intracortical inhibition, and transcallosal inhibition. Subsequently, half of the participants (AOT group) were asked to observe and then execute as quickly as possible a right-hand dexterity task, while the control group had to observe a no-action video before practicing the same task.
Results: AOT participants showed greater performance improvement relative to controls. More importantly, the amount of improvement in the AOT group was predicted by the amplitude of corticospinal modulation during action observation and, even more, by the amount of intracortical inhibition induced by action observation. These relations were specific for the AOT group, while the same patterns were not found in controls.
Discussion and conclusion: Taken together, our findings demonstrate that the efficacy of AOT in promoting motor learning grounds on the capacity of action observation to modulate the trainee's motor system excitability, and even more its intracortical inhibition. Our study not only enriches the picture of the neurophysiological effects induced by action observation onto the observer's motor excitability, but linking them to the efficacy of AOT, it also paves the way for the development of models predicting the outcome of training procedures based on the observation of other's actions.

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INTRACELLULAR CHLORIDE AS MASTER REGULATOR OF CORTICAL EXCITABILITY DURING THE CIRCADIAN CYCLE

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The main inhibitory synaptic currents, gated by gamma-aminobutyric acid (GABA), are mediated by Cl^--conducting channels, and are exquisitely sensitive to changes in the chloride electrochemical gradient. As GABAergic activity dictates neuronal firing, the intracellular chloride concentration ($[Cl^-]_i$) plays a major role in the regulation of fast inhibition and neuronal activity and, therefore, is ideally placed to be a regulator of neuronal excitability. Chloride levels have been thought to be stable in adult cortical networks, except when associated with pathological states and thus little attention has been paid to Cl^- regulation in physiological conditions, in part because of the difficulty in measuring $[Cl^-]_i$ *(Lodovichi et al. 2022)*. In the last few years, we have developed a genetically encoded sensor, LSSmClopHensor, that, when coupled with 2-photon imaging, allows the ratiometric measurement of $[Cl^-]_i$ *in vivo (Sulis Sato et al. 2017)*. We have measured $[Cl^-]_i$ in the visual cortex of anaesthetized young adult mice, and surprisingly we found a large physiological diurnal fluctuation of baseline chloride inside pyramidal cells, equating to an ~15 mV positive shift in of its equilibrium potential at times when mice are typically awake (midnight), relative to when they are usually asleep (midday). The diurnal redistribution of $[Cl^-]_i$ reflects changes in surface expression and phosphorylation states of the cation-chloride-co-transporters, KCC2 and NKCC1, leading to a greatly reduced chloride-extrusion capacity at night (awake period).

This diurnal rhythm of $[Cl^-]_i$ should modulate the excitation/inhibition dynamics of the cortex and network processing as it relies on the fine regulation of the inhibitory feedback on pyramidal neurons. We studied the oscillations of the extracellular field potential in response to patterned visual stimuli that depend on the inhibitory feedback of somatostatin interneurons *(Veit et al. 2017)*. During the period of high $[Cl^-]_i$ (at night) oscillations are less well synchronized that during the day, as expected by the reduced strength of inhibition. Importantly, at night synchronisation is restored by the local application of bumetanide, a blocker of NKCC1, that causes a reduction of $[Cl^-]_i$. Furthermore, the chloride cycle affects the stability of cortical network, as demonstrated by a greater susceptibility to epileptic seizures induced by 4- aminopyridine at midnight, compared to midday. Also in these experiments, seizures were prevented by inhibition of NKCC1.

These results open a novel scenario in which the diurnal oscillations of $[Cl^-]_i$ affect brain function and possibly represent a determinant of the recognized circadian dependency of epilepsy and other neurological and psychiatric manifestation *(Karoly et al. 2021, Singla et al 2022)*.

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HUMAN CONNECTOMICS AND DISCONNECTOMICS: INSIGHTS FROM INSIDE THE BRAIN

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Several worldwide, national initiatives witnessed hot the investigation of the brain has in connectomics one of the major pillar nowadays. While connectivity and its multiple facets can be tackled from multiple perspectives, spanning from purely anatomical to functional characterizations, major limitations can still be identified in the use of indirect measurements of the neural activity (e.g. metabolic or diffusion indices) as well as in the virtual impossibility to collect meaningful longitudinal evidence of how connectivity changes upon the occurrence of brain damages. Starting from an exceptional single case, here we will show how intracranial recordings in humans have the potential to overcome these limitations, documenting two fundamental aspects: first, that brain connectivity can propagate pathological activity throughout the brain, leading to a mulfunctioning of the spared brain region and a potential mislocalization of the epileptogenic zone. Second, that radio-frequency thermocoagulations can serve to disconnect the previous vicious loops, giving immediate clinical benefits and further providing unprecedented insights into the neuroscience of brain damages due to the possibility to monitor spontaneous and evoked activity changes before and after the focal lesion induction.

PHYSICAL ACTIVITY AND CORTICAL PLASTICITY

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Physical activity has been recently shown to enhance adult visual cortical plasticity, both in human subjects and animal models, emerging as an effective treatment for amblyopia, one of the major causes of impaired vision.
While it is known that physical activity enhances mitochondrial oxidative metabolism leading to a transient production of reactive oxygen species, whether and how this process is involved in the plasticizing effects elicited at the visual cortical level is still an open question.
We investigated the possibility that modulation of oxidative stress through a dietary intervention with antioxidants (vitamins E and C) interferes with the impact of physical exercise on visual cortex plasticity in adult rats. Our results show that antioxidant supplementation beyond the closure of the critical period was sufficient to block ocular dominance plasticity in response to eye deprivation, which is normally induced by physical activity.
Proteomic and biochemical analyses demonstrated that the antioxidants exerted their action through a mithormetic effect that involved a brain dampening of mitochondrial biogenesis and an increased IGF-1 signaling.
Altogether, our data underscore the relevance of mild stress induced by physical activity as a powerful tool to modulate brain plasticity.

MCT1-DEPENDENT ENERGETIC FAILURE AND NEUROINFLAMMATION UNDERLIE OPTIC NERVE DEGENERATION IN WOLFRAM SYNDROME MICE

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Wolfram syndrome 1 (WS1) is a rare genetic disorder caused by mutations in the *WFS1* gene leading to a wide spectrum of clinical dysfunctions, among which blindness, diabetes and neurological deficits are the most prominent. *WFS1* encodes for the endoplasmic reticulum (ER) resident transmembrane protein Wolframin with multiple functions in ER processes. However, the *WFS1*-dependent etiopathology in retinal cells is unknown. Herein, we showed that *Wfs1* mutant mice developed early retinal electrophysiological impairments followed by marked visual loss. Interestingly, axons and myelin disruption in the optic nerve preceded the degeneration of the retinal ganglion cell bodies in the retina. Transcriptomics at pre-degenerative stage revealed the STAT3-dependent activation of proinflammatory glial markers with reduction of the homeostatic and pro-survival factors Glutamine synthetase and BDNF. Furthermore, label-free comparative proteomics identified a significant reduction of the monocarboxylate transport isoform 1 (MCT1) and its partner Basigin that are highly enriched on retinal astrocytes and myelin-forming oligodendrocytes in optic nerve together with Wolframin. Loss of MCT1 caused a failure in lactate transfer from glial to neuronal cell bodies and axons leading to a chronic hypometabolic state. Thus, this bioenergetic impairment is occurring concurrently both in the axonal regions and cell bodies of the retinal ganglion cells, selectively endangering their survival while impacting less on other retinal cells. This metabolic dysfunction occurs months before the frank RGC degeneration suggesting an extended time window for intervening with new therapeutic strategies focused on boosting retinal and optic nerve bioenergetics in WS1.

ORAL NANO-DELIVERY OF NASCO POMACE EXTRACT EXERTS NEUROPROTECTIVE AND ANTI-INFLAMMATORY EFFECTS IN THE MPTP MOUSE MODEL OF PARKINSON'S DISEASE

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Grape pomaces, a waste by-product of wine production, have received great attention for their richness in polyphenols, compounds known to exert anti-inflammatory and antioxidant effects in animal models of neurodegenerative diseases, including Parkinson's disease (PD). Nonetheless, their oral use is limited by their low brain bioavailability and extensive first-passage metabolism.

To overcome these limitations, in the present study we incorporated grape pomaces extract from *Vitis vinifera* Nasco into nutriosome (Nasco nutriosome), a novel nanovesicle system composed of the S75 phospholipid and of the maltodextrin Nutriose® FM06. To investigate the neuroprotective and anti-inflammatory properties of Nasco nutriosome in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, we administrated Nasco nutriosome intragastrically in mice that received MPTP sub-acutely.

Degeneration of dopaminergic neurons was assessed through the immunohistochemical evaluation of tyrosine hydroxylase (TH) in the caudate-putamen (CPu) and substantia nigra pars compacta (SNc), along with the dopamine transporter (DAT) in the CPu. In the same brain areas, the immunoreactivity for the glial fibrillary acidic protein (GFAP, marker of astroglia), and for the ionized calcium-binding adaptor molecule 1 (IBA1, marker of microglia) was also evaluated to assess the occurrence glial activation and reactivity. Additionally, the pro-inflammatory interleukin (IL)-1β was co-localized with IBA1, to gain additional information about the microglia phenotype.

Immunohistochemical analysis revealed that Nasco nutriosome significantly contrasted the MPTP-mediated reduction of TH and DAT-positive fibres in the CPu as well as the number of TH-positive cells in SNc. Additionally, Nasco nutriosome significantly prevented both MPTP-induced astrogliosis in the SNc and CPu, and microgliosis in the CPu, along with the microglial production of the IL-1β. Altogether, these results highlight the promising neuroprotective and anti-inflammatory effects exerted by Nasco nutriosome treatment in the preclinical MPTP-mouse model of PD.

MICROGLIAL EXTRACELLULAR VESICLES IN MOTION AT THE NEURONAL SURFACE: IMPLICATION IN THE PROPAGATION OF AMYLOID-RELATED SYNAPTIC DYSFUNCTION IN THE MOUSE BRAIN

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Extracellular vesicles (EVs) are important mediators of microglia-to-neuron communication and are responsible for the spreading of misfolded proteins in the diseased brain. However, how EVs move across the extracellular space to reach target neurons and spread pathological signals remain largely elusive. Using optical tweezers combined to time-lapse imaging, we studied EV-neuron interaction dynamics *in vitro* coming to the unexpected observation that a large fraction of EVs move along the surface of axons and dendrites, scanning actin protrusions. Motion of most EVs is driven by EV binding to a surface receptor that drift on the plasma membrane following cytochalasin-sensitive and nocodazole- resistant cytoskeleton rearrangements. In addition, a minor fraction of EVs have an independent capacity to move along a gradient of neuronal receptors in an actin-dependent manner. These data unveil a complex trafficking of microglial EVs at the neuron surface and suggest that EVs may exploit axonal projections as highways to reach target neurons and spread pathological signals in the diseased brain. We tested this hypothesis in a neurodegenerative context, by exploring whether EV motion may be involved in the rise and propagation of amyloid-related synaptic dysfunction. In fact, synaptic malfunctioning is a very early and crucial pathological event in Alzheimer's disease, that involves progressively larger areas of the brain over time, but how it starts and propagates is still unknown.

Our data show that microglial EVs carrying Aβ (Aβ-EVs) alter synaptic plasticity both *in vitro* and *in vivo* the entorhinal-hippocampal circuit. Aβ-EVs impair long-term potentiation (LTP) upon injection in the enthorinal cortex (EC), and 24h later they propagate LTP impairment to the dentate gyrus. Importantly, when Aβ-EV motion is decreased, no propagation of LTP deficit occurs along the EC-DG circuit, implicating large EV extracellular motion in the spreading of LTP impairment. Accordingly, proteomic analysis displays differences in the composition of Aβ-EVs vs. EVs from microglia not exposed to Aβ. The influence of mesenchymal stem cell (MSC) indirect co-colture with microglia primed with Aβ on cell phenotype, EVs and functions is currently being explored.

Our data unveil a new mechanism controlling the diffusion of large EVs and related pathogenic signals in the brain parenchyma, and implicate large microglial EVs in the rise and propagation of synaptic dysfunction in AD.

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MECHANISMS OF SYNAPTIC DYSFUNCTION IN THE ANGELMAN SYNDROME

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The *UBE3A* gene codes for the E3 ubiquitin ligase E6-AP and is critical to ensure a proper brain function. Indeed, genetic defects of *UBE3A* result in pathological phenotypes. The loss of *UBE3A* causes the Angelman Syndrome (AS), a severe neurodevelopmental disorder characterized by intellectual disability, motor delay and seizures, while increased *UBE3A* copy number or *gain-of-function* mutations are associated to autism. Although considerable efforts have been put to dissect the molecular underpinnings of UBE3A function in neurons, the pathogenic mechanisms of these neurodevelopmental disorders are still poorly understood. For this reason, current therapies only aim at mitigating symptoms.

In this project, we study the effects of *UBE3A* loss (thus mimicking the genetic alterations of the AS) on the regulation of synaptic development at single-cell level *in vivo*. To this aim, we combine cortex-directed *in utero electroporation* to inactivate *UBE3A* in sparse pyramidal neurons with confocal and super-resolution microscopy to investigate the role of *UBE3A* on the formation, maturation and functional organization of excitatory and inhibitory synapses up to the nanometer scale. As already suggested by other groups, our results indicate that *UBE3A* critically regulates the formation of excitatory synapses. In addition, our evidence also suggests that *UBE3A* controls the assembly and the maturation of specific subtypes of inhibitory synapses, namely those located in the perisomatic region and in the axon initial segment.

A crucial aspect of *UBE3A* function and AS pathophysiology concerns the molecular diversity of *UBE3A*. It encodes multiple isoforms generated by alternative splicing. Human isoforms 1 and 3 are the most abundant and differ in their N-terminus, ultimately resulting in a distinct subcellular distribution, nuclear and cytosolic, respectively. The *in utero* replacement of endogenous *Ube3a* with individual human *UBE3A* isoforms indicates that the development of specific subtypes of synapses is selectively controlled by distinct isoforms. Using specific *UBE3A* mutants, in which either the catalytic domain or the subcellular localization is selectively abolished, we are currently carrying out an *in vivo* structure-function analysis and a proximity-dependent proteomic screen to dissect the molecular mechanisms underlying the *UBE3A*-dependent synaptic regulation.

Together, our results suggest for the first time that the *UBE3A* gene may be critical to set the number of excitatory and inhibitory synaptic connections through cell-autonomous mechanisms, thus contributing to regulate (in an isoform-specific fashion) the ratio between excitation and inhibition.

IMPACT OF EARLY LIFE STRESS AND SOCIAL ENRICHMENT ON BEHAVIOR IN ADOLESCENT AND ADULT RATS: FOCUS ON SEX DIFFERENCES

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Social experiences during the early phases of life are thought to be critical for proper emotional and cognitive development. Exposure to early-life social isolation is known to cause long-lasting behavioral impairments and increase the risk for psychopathologies, with males and females showing a different vulnerability and neuroendocrine reactivity to stress.

Early social stress (ESI) interferes with neurodevelopmental processes and can lead to long-lasting emotional, cognitive, and hormonal alterations in adulthood. Communal nesting (CN) is a form of alloparenting in which 2 or more lactating female conspecifics rear their offspring within a common nest while sharing share milk and caregiving behavior from birth to weaning. This phenomenon occurs in many social species and, similarly to their wild counterparts, also laboratory rodents preferentially rear their young in communal nests when the opportunity is provided. CN provides a socially stimulating environment that was shown to affect social and anxiety-like behaviors. Yet, only limited studies have investigated whether the offspring of laboratory rodents reared in CN show important differences in physiology and behavior when adolescents or adults as compared with conspecifics reared in a single nest.

This study investigates whether pre-weaning ESI affects reward-related processing for natural rewards, compulsive tendencies, sensorimotor gating, and basal corticosterone levels in adolescence and adulthood, and whether CN could reverse the impact of ESI on behavior and hormonal plasma levels. Both male and female rats were used to detect potential sex-dependent differences. In a food self- administration paradigm, both adolescent and adult rats reared in CN conditions showed a significantly slower acquisition and lower active responding than standard housed (SH) animals, while ESI led to a steeper curve in adulthood. During the maintenance period of the training, CN led to a general decrease in active responding in adolescence, while ESI increased responding in adolescent and adult females, an effect that was reversed by CN in adult females. Under a progressive ratio protocol, non-stressed CN animals showed lower breakpoints than SH animals, and ESI increased the breakpoint in all groups, although to a greater extent in females than in males. Notably, ESI-induced effect was reverted by CN. The Marble Burying test revealed an obsessive-compulsive trait in ESI adolescent males, but also this effect was fully prevented by the CN condition. The Prepulse Inhibition (PPI) test showed lowered the PPI in ESI adolescent animals, an effect that was long-lasting in males and reverted by CN. Finally, female rats showed higher plasma corticosterone levels, independently from housing and stress conditions.

Altogether, our findings indicate that social isolation and communal nesting have long-lasting effects on sensorimotor gating, reward-seeking, and compulsive-like behaviors that could significantly differ between males and females. Specifically, we demonstrated that an early life socially enriched condition, like the CN, may exert a “protective” effect toward early stress-induced reward-related behaviors and prevent the development of an obsessive-compulsive trait and the impairment of PPI in socially stressed adolescent males without inducing significant alterations in hypothalamic-pituitary-adrenal axis activity. This study therefore (i) supports the need of sex-tailored intervention strategies to face the behavioral, emotional and cognitive alterations induced by early-life social stress, (ii) reveals a protective role of CN against early life social insults and (iii) highlights the importance of translational investigations taking the social environment, development and sex into account.

HOW THE IMMUNE SYSTEM AFFECTS SYNAPTIC FUNCTION

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In the last years, the old concept that the brain is an immunological privileged organ has been shaken from the ground and replaced by the notion that a continuous crosstalk occurs between the nervous and the immune systems. This crosstalk is particularly relevant during development and aging. Common routes of communication between nervous and immune systems include the direct access of immune cells and peripheral cytokines into the brain, a process which can affect synapse formation and function (1). Furthermore, a continuous communication occurs between immune cells and neurons, where the starring actor is recognized as microglia, the main brain residential myeloid cells. Besides representing the first line of defense against pathogenic insults, microglia contribute to physiological neurodevelopment, by regulating neurogenesis and neuronal survival, favoring synapse formation, and participating in the widespread elimination of exuberant synaptic connections generated during the early phases of development. Thanks to the recent introduction of single-cell sequencing techniques, it is now recognized that these widely heterogeneous roles are supported by distinct subtypes of microglia, characterized by broad genetic diversity, and residing in distinct brain regions at different times during CNS development. Among the molecular players shaping the state of microglia, a key role is played by triggering receptor expressed on myeloid cells 2 (TREM2), an innate immune receptor of the immunoglobulin superfamily expressed in the brain solely by microglial cells. TREM2 plays a key role in several microglial functions including the microglia-dependent pruning in the developing brain. By impairing the normal shaping of microglia states and affecting synapse homeostasis, TREM2 defects result in derangements in circuit formation, accompanied by behavioral defects (2, 3). The talk will present recent results pointing to the central role of microglial TREM2 in shaping synapse homeostasis. It will describe the molecular interactors involved in the microglia-to-neuron TREM2-mediated communication and will delineate conditions where TREM2 expression and function are altered. Finally, the talk will introduce to possible strategies to modulate TREM2 function and aimed at rescuing brain damages caused by the reduction of levels and/or function of the protein.

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POSTERS

MULTIOME TECHNOLOGY TO EXPLORE THE ROLE OF NUCLEAR UBE3A IN ANGELMAN SYNDROME

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Angelman syndrome is a rare neurodevelopmental imprinting disorder arising in 1/12.000 to 1/20.000 liveborn. The symptomatology includes microcephaly, seizures, ataxia, muscular hypotonia and motor and developmental delay with intellectual disabilities. Patients are also characterized by frequent laughing, smiling and a happy behavior. Affected individuals lack the expression of *UBE3A*. This gene is expressed by both alleles in non-neuronal cells while in mature neurons the paternal one is imprinted. When the maternal one is deleted or mutated, the pathological status arises. UBE3A protein displays three known isoforms, two of which are expressed predominantly in the nucleus, while the remaining one localizes mainly in the cytoplasm. Interestingly, loss of the nuclear-specific isoforms but not of the cytoplasmatic one has been found to be causative of Angelman Syndrome related phenotype in mice. In line with this, it has been demonstrated that the majority of mutations targeting *UBE3A* gene causes a loss of its nuclear localization or loss of catalytic activity of its nuclear isoform, thus suggesting the pivotal role of UBE3A in the nucleus where it possibly interacts with epigenetic regulators. Hence, we decided to dissect the function of this yet poorly characterized protein in this cellular compartment.
For this purpose, cerebral cortices from mice lacking *Ube3a* only on the maternal chromosome and relative were isolated for comparative analysis. Nuclei were extracted from the cortical tissues and subjected to 10x Multiome technology combining ATAC-seq and RNA-seq for concurrent transcriptional and epigenetic analyses. Thus, the gene expression alterations were compared to epigenetic changes in the *Ube3a* affected brains. Computational data analysis identified the major cellular subtypes populating the cerebral cortex in both pathological and control brains. Interestingly, preliminary data of RNA velocity analysis suggested that mature neurons, but also other cellular subtypes developed faster in the Angelman Syndrome mice compared to the control. Most importantly, we identified and validated by Western blot differences in the expression of a pool of genes and their related proteins, part of which are strongly related to the clinical presentation of Angelman Syndrome. Some of the differentially expressed genes identified in this analysis presented differential open chromatin profile. This multiomic analysis allowed for a precise characterization of the different cellular subtypes in Angelman syndrome brains and shed light on a group of deregulated genes that are possibly related to Angelman Syndrome. Currently, we are testing an epigenetic mechanism and its responsible molecular player that can be responsible for the observed transcriptional and epigenetic dysregulations.

PHOX2B REGULATES NEURONAL EXCITABILITY BY MODULATING THE EXPRESSION OF K^+, NA^+ AND CA^{2+} CHANNEL GENES

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PHOX2B encodes for a transcription factor, characterised by the presence of two polyalanine repeats of 9 and 20 residues in the C-terminus, and it is a "master gene" of the development of the autonomic nervous system (ANS). Moreover, *PHOX2B* is essential for the noradrenergic specification and neuronal differentiation by regulating cell cycle exit. Whereas the role of *PHOX2B* during neurodevelopment is well established, the exact role of *PHOX2B* in adulthood is still an open question. *PHOX2B* expression persists in important structures in the hindbrain of adult rats, among which chemoreceptors and it may also maintain the function of the noradrenergic neurons. However, very little is known about the genes regulated by PHOX2B [1]. Heterozygous mutations in the PHOX2B gene, consisting of 4 to 13 triplet expansion of the 20-alanine tract (PARM), lead to congenital central hypoventilation syndrome (CCHS), a rare life-threatening condition characterized by sleep-related hypoventilation and impaired CO_2 chemosensitivity [1]. In addition, other ANS dysfunctions are present, including cardiac and thermoregulatory abnormalities. Non-PARM mutations within exon 1, 2 or 3 that include rare missense, nonsense and frameshift mutations may occur in 5% of patients, frequently associated with the onset of neuroblastoma or Hirschprung disease. Consistent with its role as transcription regulator, transcriptional dysregulation might be an important mechanism of CCHS pathogenesis.

No pharmacological intervention is currently available, and recently progestins showed to provide partial recovery of chemoreflex impairment [1].

By means of a CRISPR-CAS9 Knocked-down PHOX2B IMR32 cells model, here we show that ion channels are newly identified PHOX2B target genes, and the different modulation of their expression by wild-type or mutant PHOX2B proteins and by progestins, through PHOX2B expression modulation, contribute to regulate the cell excitability.

These data prompt the idea that ion channels may be promising therapeutic targets in CCHS.

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SMALL MOLECULES TO FIX THE GENETIC DEFECTS OF PATHOGENIC REELIN MUTATIONS IN ADLTE

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Autosomal Dominant Lateral Temporal Epilepsy (ADLTE; OMIM 600512) is a genetic epileptic syndrome, clinically characterized by focal seizures with auditory or aphasic symptoms, originating from the temporal lobe lateral region, and negative MRI results. It is inherited with autosomal dominant pattern with reduced penetrance (about 70%) and is genetically heterogeneous. Our research group identified three genes, LGI1, RELN and MICAL-1 that overall cause ADLTE in about 60% of affected families. The RELN gene, encodes for Reelin, a glycoprotein synthesized and secreted by neurons. Recently, we demonstrated that pathogenic mutations in the RELN gene inhibit or reduce notably Reelin secretion due to a three-dimensional structure alteration of mutated proteins, which are degraded through the autophagic pathway. These data allowed us to design new experimental approaches based on the use of small molecules, known as CFTR correctors, able to recover the defective folding of Cystic Fibrosis mutant proteins. Therefore, we tested 8 CFTR correctors, and 4 chemical correctors approved by the Food and Drug Administration for clinical use in other diseases. We performed the preliminary experiments to test the efficacy of these molecules on cells transiently expressing a Reelin mutant protein, which inhibits its secretion (p.G2783C). These pilot experiments revealed that four CFTR correctors are able to restore mutant Reelin secretion. This experimental evidence constitutes an excellent starting point to evaluate the effectiveness of correctors and to lay the foundation for a specific therapy for ADLTE patients carrying mutations in the RELN gene. In addition a library of nearly 800 FDA-approved drugs it will be tested in cells expressing mutant Reelin for their capacity to restore autophagy levels back to control conditions. This further approach will allow us to identify already known molecules that could be used in combination with molecular correctors thus increasing their effectiveness.

MODELING NATIVE AND SEEDED SYNUCLEIN AGGREGATION AND RELATED CELLULAR DYSFUNCTIONS IN DOPAMINERGIC NEURONS DERIVED BY A NEW SET OF ISOGENIC iPSC LINES WITH SNCA MULTIPLICATIONS

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Triplication of the *SNCA* gene, encoding the protein alpha-Synuclein (aSyn), is a rare cause of aggressive and early-onset parkinsonism. Herein, we generated iPSCs from two siblings with a recently described compact *SNCA* gene triplication and suffering for severe motor impairments, psychiatric symptoms and cognitive deterioration. Using CRISPR/Cas9 gene editing, each *SNCA* copy was inactivated by targeted indel mutations generating a panel of isogenic iPSCs with decremental number from 4 down to none of functional *SNCA* gene alleles. We differentiated these iPSC lines in midbrain dopaminergic (DA) neuronal cultures to characterize aSyn aggregation in native and seeded conditions and evaluating its associated cellular dysfunctions. Utilizing a new nanobody-based biosensor combined with super-resolved imaging, we were able to visualize and measure aSyn aggregates in early DA neurons in unstimulated conditions. Calcium dysregulation and mitochondrial alterations were the first pathological signs detectable in early differentiated DA neuronal cultures. Accelerated aSyn aggregation was induced by exposing neurons to structurally well-characterized synthetic aSyn fibrils. 4x*SNCA* DA neurons showed the highest vulnerability which was associated with high levels of oxidized DA and amplified by TAX1BP1 gene disruption. Seeded DA neurons developed large aSyn deposits whose morphology and internal constituents resembled Lewy bodies commonly observed in Parkinson's disease (PD) patient brain tissues. These findings provide strong evidence that this isogenic panel of iPSCs with *SNCA* multiplications offers a remarkable cellular platform to investigate mechanisms of PD and validate candidate inhibitors of native and seeded aSyn aggregation.

DISENTANGLING THE SIGNALING COMPLEXITY OF NERVE GROWTH FACTOR RECEPTORS BY CRISPR/Cas9

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The binding of Nerve Growth Factor (NGF) to the tropomyosin–related kinase A (TrkA) and p75^{NTR} receptors activates a large variety of pathways regulating critical processes as diverse as proliferation, differentiation, membrane potential, synaptic plasticity and pain. To ascertain the details of TrkA-p75^{NTR} interaction and cooperation, a plethora of experiments, mostly based on receptor overexpression or downregulation, have been performed. Among the heterogeneous cellular systems used for studying NGF signaling, the PC12 pheochromocytoma-derived cell line is a widely used model. By means of CRISPR/Cas9 genome editing, we created PC12 cells lacking *TrkA*, *p75^{NTR}*, or both. We found that TrkA-null cells become unresponsive to NGF. Conversely, the absence of p75^{NTR} enhances the phosphorylation of TrkA and its effectors. Using patch-clamp, we demonstrated that the individual activation of TrkA and p75^{NTR} by NGF results in antagonizing effects on the membrane potential. These newly developed PC12 cell lines can be used to investigate the specific roles of TrkA and p75^{NTR} in a genetically defined cellular model, thus providing a useful platform for future studies and further gene editing.

TAU HYPERPHOSPHORYLATION AFFECTS AXONAL TRANSPORT *IN VITRO* AND *IN VIVO*

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Tau is a protein abundantly expressed in neurons where it has the main role of stabilising axonal microtubules contributing to the regulation of axonal transport of organelles.
Tau is found aggregated in a group of neurodegenerative diseases named tauopathies, which include frontotemporal dementia (FTD) and Alzheimer's disease.
Recent *in vitro* work has described the existence of tau "islands", a microtubule-bound "aggregation" state of tau, distinct from pathological aggregates and liquid-liquid phase condensates. These "islands" appear to protect microtubules from severing enzymes and to act as roadblocks for motor proteins on reconstituted microtubules. However, whether these structures exist in neurons remains unclear.

We used cultured mouse neurons overexpressing human tau either wild type or containing FTD-linked mutations. We found that tau displays a non-homogenous distribution along axons with regions of higher density reminiscent of tau islands. The presence of the FTD-linked mutations, known to cause increased phosphorylation and pathological aggregation of tau, induced larger islands.

Functionally, the FTD-linked mutant tau caused defects in the anterograde transport of BDNF-secretory granules compared to wild type tau, with reduced speed due to more frequent pausing events, which could be due to the greater size of tau islands roadblocks. Interestingly, these defects were reversed by inhibition of the p38α MAPK, known to phosphorylate tau at multiple sites.

We then moved *in vivo* and analysed axonal transport of BDNF-secretory granules in anesthetised mice by using two-photon microscopy. rTg4510 mice, which overexpress human tau carrying the FTD-linked mutation P301L, presented defects in axonal transport compared to control mice. Interestingly, this impairment is present very early on in this model, before the appearance of neuronal activity deficits and overt tau aggregation. Inhibition of p38α was able to partially rescue the defects in axonal transport also *in vivo*.

Our data suggests that hyperphosphorylation of tau, which occurs before formation of pathological aggregates, is sufficient to affect axonal transport, possibly due to the increased size of tau islands. This may represent a very early event in the pathogenesis of tauopathies. An inefficient transport of organelles and protein complexes along the axons may have severe consequences on the connectivity, activity and plasticity of neuronal circuits. The evidence that reducing tau phosphorylation levels by inhibiting the key kinase p38α potentiated axonal transport and restored the normal size of tau islands, points towards pharmacological or genetic inhibition of p38α as a promising therapeutic strategy in tauopathies.

MITOTRAP: A NOVEL TOOL DESIGNED TO STUDY MITONUCLEAR COMMUNICATION

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Mitochondria, classically regarded as the "powerhouse of the cell", in addition to producing ATP generate key metabolites and are central to apoptosis and metabolic processes. Recent advances in research have significantly further expanded our view of their roles, from semi-independent organelles to signalling hubs strategically integrated in multiple signal transduction pathways.

Most mitochondrial proteins are encoded in the nuclear genome, thus nuclear transcription controls mitochondrial biogenesis and function (nucleus-to-mitochondria anterograde signalling). To prevent divergence between the mitochondrial performance and the metabolic demand of the cell, the expression of nuclear and mitochondrial genes need to be coordinated. An efficient mitonuclear coordination, however, requires a bi-directional communication, involving also signals from mitochondria, reflecting their bioenergetics and biosynthetic status, that influence nuclear function (mitochondrial retrograde response). Molecules serving as retrograde signals are continuously discovered, and include cytochrome c, TCA metabolites, AMPK activation, calcium uptake and release, changes in mitochondrial membrane potential, ROS, mtDNA, sncRNA and an increasing number of peptides with nuclear/mitochondrial double localization, including TCA enzymes.

To unveil the relationship between the nuclear function and the positioning of mitochondria with respect to the nucleus, we designed and constructed "mitotrap", a molecular tool that, when expressed on the surface of the outer mitochondrial membrane (OMM), drives the repositioning of mitochondria all around the nucleus, trapped to the nuclear envelope. Our trap exploits the ability of Epac1, a major cAMP effector, to bind RanBP2, a component of the nuclear pore complex.

We show here that trap-labelled mitochondria effectively localize in close apposition to the nuclear envelope, that the molecular anchor is indeed RanBP2, and that such a relocalization has no major effects on the nuclear envelope permeability, nor on mitochondrial protein expression and morphology, indicating that mitotrap is a suitable tool to investigate the mitonuclear signalling. We also preliminary show the effect of the perinuclear mitochondrial localization on the Ca^{2+} handling of different intracellular compartments.

UNRAVELING THE ROLE OF MITOCHONDRIAL METABOLISM IN TUMORS OF THE PERIPHERAL NERVOUS SYSTEM

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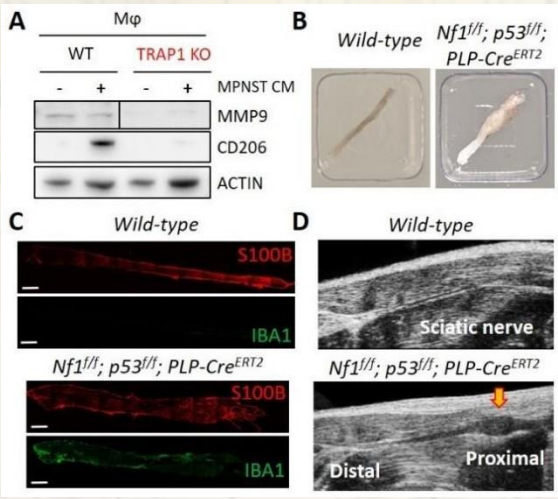
Neurofibromatosis type 1 (NF1) is a genetic disease caused by germline inactivating mutations in the gene encoding for neurofibromin, a negative RAS regulator. The loss of this tumor suppressor predisposes NF1 patients to the development of many neoplasms among which neurofibromas. These grow along peripheral nerves and are characterized by a heterogeneous tumor microenvironment (TME) where mutated Schwann cells (SCs) interact with infiltrates of multiple cell types such as fibroblasts, mast cells and macrophages. An inflammatory gene signature distinguishes neurofibroma lesions from normal peripheral nervous system (Sci. Rep. 7, 43315), and neurofibroma-associated macrophages can exert pro-tumor roles whereby tumor progression to malignant peripheral nerve sheath tumors (MPNSTs) positively correlates with macrophage infiltration.

We and others have elucidated that NF1-related tumors adopt several metabolic changes: **a)** a repression in oxidative phosphorylation (OXPHOS) through SDH inhibition (Cell. Rep. 18, 659-672) and NADH dehydrogenase (respiratory complex I) downregulation (Cell Death Differ. 2022 Apr 7); a master regulator of this mitochondrial rewiring is the RAS/MEK/ERK-dependent activation of the mitochondrial chaperone TRAP1, which drives succinate accumulation and elicits the ensuing stabilization of the pro-neoplastic transcription factor HIF1α (Cell Metab. 2013 17(6):988-999) and whose targeting with a newly identified and selective inhibitor (cmpd5) impairs neoplastic growth of MPNST cells (Cell. Rep. 31, 107531); **b)** an increased utilization of glutamine for biosynthetic purposes (Oncotarget 8, 94054-94068) whereby the pharmacological inhibition of glutaminase (GLS) (*e.g.* with JHU395, CB839) exerts anti-neoplastic effects (Mol. Cancer. Ther. 19, 397-408).

In this framework we are investigating the metabolic adaptations in NF1-related malignancies with the aim of spotting relationships between autonomous traits of cancer cells (*i.e.* Warburg phenotype, glutamine dependency) and non-autonomous features (*i.e.* alternative macrophage polarization, tumor tolerance and maintenance) of the TME.



To this purpose we are exploiting co-cultures techniques where macrophages with different TRAP1 genotypes are exposed to conditioned media (CM) from MPNST cells and we perform MPNST-inducing sciatic nerve crush in genetically engineered NF1 mouse models that lack Nf1 and p53 in the Schwann cell compartment.

Our data indicate that TRAP1 absence in macrophages impedes the acquisition of a pro-tumoral phenotype (Fig. A). Furthermore, nerve damage in the context of neoplastic Schwann cells leads to an enlargement of the nerve at the level of the crushed area where macrophage infiltration is still retained after nerve regeneration (Fig. B-D).

Figure. A) MMP9 and CD206 induction in macrophages exposed to MPNST CM. B) Sciatic nerve explants from genetically engineered mice at 60 days post crush. C) IBA1 (macrophages) and S100B (SCs) staining and D) echography of crushed sciatic nerves at 3 months post crush. Scalebar: 20μm.

Our goal is to study the potential metabolic crosstalk mechanisms between MPNSTs and tumor associated macrophages (TAMs) bringing to light targetable signals in non-tumoral cells of the neoplastic environment that could be leveraged for repressing MPNST growth.

THE OXYTOCIN SYSTEM CONTRIBUTES TO BRAIN MICROVASCULAR DEVELOPMENT

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The interplay between vascular and neuronal systems is critical for the normal growth and function of neurons [1]. Indeed, brain development relies heavily on proper cerebrovasculature, that not only supports the proliferation, differentiation and migration of neural progenitors but also ensures brain homeostasis, and the supply of oxygen and nutrients for healthy neuronal functions [2]. Alterations of the brain microvascular network has already been reported in diverse neurological and psychiatric conditions like Alzheimer [3], Parkinson Diseases [4] and chronic pain [5] and very recently also in ASD [6, 7, 8].

The oxytocin (OXT) system, regulating postnatal neuron maturation is strongly implicated in pathological conditions affecting the social sphere, and can also affect angiogenesis and vascular functions [9,10], an aspect generally overlooked.

Using high-energy X-ray computed tomography (XR-CT), we studied the vascular network in Oxtr knock-out mice (Oxtr -/-), that completely lack the OXT receptor and display deficits in autism-related behavior [11]. We analysed the primary somatosensory cortex (S1) and the medial prefrontal cortex (mPFC), at three different brain development stages, postnatal day (PN) 13, corresponding to childhood, PN 21 corresponding to adolescence, and PN 90, corresponding to adulthood.

We found a general and significant reduction in the number of blood microvessels that is more evident at PN 13 and that persists in all the developmental stages.

These exciting results, although very preliminary, confirm the contribution of the OXT system in the brain development during the first days after birth [12] and suggest an unexpected new important role of the OXT system in brain microvascular development. Moreover, our data support the hypothesis that brain vascular deficiency can contribute to the etiopathogenesis of neurodevopmental disorders, opening the way to novel therapeutic strategies.

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OXYTOCIN AMELIORATES BEHAVIORS AND PROMOTES IN VITRO BLOOD-BRAIN BARRIER FORMATION IN A 22q11.2 DELETION SYNDROME MOUSE MODEL

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The 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge syndrome, is characterized by developmental social and intellectual disabilities, high prevalence of ADHD and ASD during childhood, and early onset schizophrenia in adolescence / young adulthood (Schneider et al, 2014). The deletion in the 22q11.2 region contains about 50 genes, including Claudin 5, a component of the tight junctions specifically enriched in the brain endothelial cells and necessary for the maintenance of the BBB. An impairment in BBB permeability has been indeed observed in endothelial cultures differentiated from hiPSCs derived from 22q11.2DS patients (Crockett et al, 2021).

The LgDel/+ mouse, embedding the full 22q11.2 hemideletion, recapitulates several key aspects of DiGeorge syndrome, including a compromised BBB (Crockett et al, 2021.) We also found that LgDel/$^{+}$ mice have reduced endogenous oxytocin (OT) levels in the hypothalamus and that the intranasal supplementation of OT in the first post-natal days of life led to a long-lasting behavioral ameliorations of sensorimotor gating deficits, social interactions and social memory deficits later on in adolescent/adult mice.
To explore the mechanisms of this rescue action of OT in the perinatal brain, we focused on the role of OT on endothelial cells, a key component of the BBB, which we have shown to express oxytocin receptors (Cattaneo et al, 2008).
We tested primary brain microvascular endothelial cells (BVEC) isolated from WT and LgDel/$^{+}$ mice as well as two immortalized cellular lines BEND-3 (of mouse origin) and hCMEC (of human origin), largely used as BBB *in vitro* models. We found that OT increases the transepithelial/ transendothelial electrical resistance (TEER) of the cellular monolayer and promotes the localization of Claudin5 into the cell membrane in BVEC WT, hCMEC and BEND-3 cells. Interestingly, BVEC cells isolated from the LgDel/$^{+}$ mice express lower levels of Claudin5, as expected, and form an altered endothelial monolayer with reduced TEER that was partially restored by OT treatment.
Our results suggest that a perinatal OT treatment can ameliorate the behavioral deficits in LgDel/$^{+}$ mice by promoting a sealed endothelial monolayer. OT could represent a new pharmacological strategy to rescue BBB alterations in 22q11.2DS animal models and, hopefully, in 22q11.2DS patients.

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EFFECTS OF THE PHENETHYLAMINE 2-CI-4,5-MDMA AND THE SYNTHETIC CATHINONE 3,4-MDPHP IN ADOLESCENT RATS: FOCUS ON SEX DIFFERENCES

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The illicit drug market of Novel Psychoactive Substances (NPS) is expanding, becoming an alarming threat due to increasing intoxications cases and insufficient (if any) knowledge of their effects. The phenethylamine 2-chloro-4,5-methylenedioxymethamphetamine (2-CI-4,5-MDMA) and the synthetic cathinone 3',4'-methylenedioxy-α-pyrrolidinohexanophenone (3,4-MDPHP) are new emerging NPS suggested to be particularly dangerous. This study verified whether these two new drugs (i) possess abuse liability, (ii) alter corticosterone plasma levels, (iii) induce microglial proliferation in limbic areas (iiii) interfere with dopaminergic transmission and included male and female adolescent rats to evaluate potential sex-differences in the drug-induced effects. Findings showed that the two NPS are not able to sustain reliable self-administration behaviors in rats, cumulative earned injections of drugs being not significantly different from cumulative earned injections of saline in control groups. Yet, at the end of the self-administration training, females (but not males) exhibited higher corticosterone plasma levels after chronic exposure to low levels of 3,4-MDPHP (but not of 2-CI-4,5-MDMA). In male rats low levels of both drugs were able to induce microgliosis, compared to saline. Similar levels of activation were found in female rats, but also in saline control group. Finally, electrophysiological patch-clamp recordings in the rostral ventral tegmental area (rVTA) showed that both drugs are able to increase the firing rate activity of rVTA dopaminergic neurons in males, but not in females, confirming the sex dimorphic effects of these two NPS. Altogether, this study demonstrates that 3,4-MDPHP and 2CI-4,5-MDMA are unlikely to induce dependence in occasional users but can induce other effects at both central and peripheral levels that may significantly differ between males and females

BEHAVIORAL CHARACTERIZATION OF CO-EXPOSURE TO CANNABINOIDS AND HORMONAL CONTRACEPTIVES IN FEMALE RATS

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Chronic treatment with ethinyl estradiol (EE) and levonorgestrel (LNG), two of the synthetic steroids used in the hormonal contraceptive pill, decreases brain and plasma levels of progesterone and its neuroactive metabolite allopregnanolone in female rats. Likewise, hormonal contraceptives prevent the increase in plasma allopregnanolone concentrations that occurs during the luteal phase of the menstrual cycle in women. Allopregnanolone is involved in several brain functions including regulation of emotions and reward; in fact, its levels are also altered by several drugs of abuse.

In the last 10 years a decrease in the gender gap for drug abuse has been reported, due to an increase in the number of women consuming drugs, especially alcohol, tobacco and cannabis. Such increase is even more evident among young women, who are also more likely to use hormonal contraceptives. Given that chronic treatment with hormonal contraceptives decreases brain and plasma levels of allopregnanolone and that this neurosteroid influences reward, we evaluated whether co-exposure to hormonal contraceptives and cannabis might affect the behavior of female rats and might alter plasma levels of progesterone.

Young female Sprague-Dawley rats (PND 52) were treated with the EE-LNG combination (0.020-0.060 mg/rat, s.c., once daily), or its vehicle, for 4 consecutive weeks. During the last 2 weeks of hormonal treatment, rats also received daily intravenous (i.v.) infusions of the CB1 receptor agonist WIN 55,212-2 (12.5 µg/kg) to mimic the self-administration pattern of drug intake described in our previous studies, i.e. administering, through an i.v. catheter, an increasing number of infusions (up to 25 during the last 5 days) at the same rate of infusion (100 µl/5 s) and over 2 hours of passive administration session. A separate batch of animals underwent similar passive treatment with saline and served as control group. At the end of the cannabinoid treatment (i.e., after 4 weeks of hormonal treatment), they underwent a series of behavioral tests to assess locomotor activity, emotional/motivational state, and cognition. At the end of the behavioral tests, rats were sacrificed and progesterone levels were measured in plasma using a commercially available ELISA kit.

Co-exposure with hormonal contraceptives and cannabinoids did not significantly alter spontaneous locomotor activity, anxiety-like behavior in the elevated plus maze test, neophobia in the marble burying test, depressive-like behavior in the forced swim test, cognitive performance in the novel object recognition test or sensorimotor gating in the prepulse inhibition test. Chronic EE-LNG treatment decreased plasma progesterone levels as expected, while WIN 55,212-2 did not alter progesterone levels in vehicle-treated rats and did not affect the EE-LNG-induced decrease in plasma levels of this neurosteroid.

In conclusion, our study showed that hormonal contraceptives do not interact with low, rewarding doses of cannabinoids at behavioral level in young female rats, suggesting that occasional marijuana smoking is unlikely to induce significant behavioral alterations in young women using hormonal contraceptives.

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THE INTERPHOTORECEPTOR MATRIX: INVESTIGATING THE ROLE OF IMPG2 IN ZEBRAFISH RETINAL DEVELOPMENT AND FUNCTION

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Retinitis pigmentosa (RP) is one of the most commonly inherited retinal dystrophies, characterized by progressive degeneration of photoreceptors. Recent studies have reported that nonsense mutations in the interphotoreceptor matrix proteoglycan 2 (IMPG2) gene are associated with autosomal recessive RP in humans. This gene encodes the proteoglycan IMPG2 expressed in the interphotoreceptor matrix that surrounds retinal photoreceptor outer segments and ellipsoids. We chose zebrafish to investigate Impg2 expression and function, as its retinal structure is similar to humans. Zebrafish has two Impg2 paralogues, Impg2a and Impg2b. Phylogenetic analysis showed that not all teleosts have two paralogues, even it theirs is the only vertebrate group with two paralogues, consistently with the whole genome duplication that occurred in their common ancestor. Homology modelling of IMPG2 conserved domains in human and in zebrafish highlighted a high structure similarity of the domains in the two species. Expression analyses revealed that impg2a and impg2b start to be expressed at 3 days post fertilization and their expression is eye-specific in the adult. Interestingly, we observed that Impg2 localization changes over time. Microinjection of antisense morpholino oligonucleotides, specific for impg2a and impg2b, provided preliminary evidence that Impg2 is involved in eye development. Finally, we are generating a zebrafish line carrying the human IMPG2 mutation, by using CRISPR/Cas9 technology. Preliminary experiments on Impg2a -/- mutant fish showed alteration of the photoreceptor layer with respect to the WT. We plan to characterize the phenotype of single and double mutants at a functional level and perform large-scale testing of therapeutic compounds on this new inherited retinal dystrophy model.

INFANTILE CEROID NEURONAL LIPOFUSCINOSIS: NEW POSSIBLE APPROACHES TO INTERFERE WITH LYSOSOMAL ACCUMULATION

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Infantile neuronal ceroid lipofuscinosis (INCL) is a lysosomal storage disorder characterized by mutations in the Cln1 gene. This gene encodes for the lysosomal enzyme palmitoyl-protein thioesterase- 1 (PPT1), whose defect causes the massive death of neurons, because of accumulation of waste products as lipofuscin.

Patients lacking Cln1 expression show epilepsy, blindness, and progressive neurodegeneration with premature death during childhood. Although the INCL model has been used to describe changes to retinal and cortical anatomy during the onset of the disease, the effects of the mutation on retinal and cortical processing is still far from being completely clarified. To reach this aim, we have developed several tools to characterize retinal degeneration, and hyperexcitability in animal models during the progression of the disease. Here, we will show preliminary results involving the characterization of the murine model of Cln1/Ppt1 deficiency related to heterozygous mice, that have not been previously characterized. The Cln1/Ppt1 KO mice are often used as a model of INCL but that is at odds with the human disease that occurs in heterozygous patients. Cln1/Ppt1 +/- mice present a milder phenotype that has not been yet described, but it should represent a better model for the human pathology.

Specifically, flash electroretinogram shows alterations in retinal computation in heterozygous mice with longer latency of b-wave response at different luminance, indicating an alteration in retinal pathways downstream to photoreceptors. Moreover, susceptibility to epilepsy in Cln1/Ppt1 +/- at midday unmasked by focally microinjecting 4-AP in the visual cortex of anesthetized mice is enhanced respect to control animals; heterozygous mice seems to show open and prolonged seizures soon after 4AP release opposite to their control mice, and to previous experiments recorded at this time of the day (Pracucci et al., BioRXiv, 2021).

This study is part of an ongoing Regione Toscana-Bando Salute 2018 project and of a Telethon proposal devoid to explore the effect of a chronic treatment in Cln1 mutant mice with a molecule developed by the Department of Pharmacy, University of Pisa. This molecule, called SG2, is a diphenyl-methane molecule already used in vitro and in vivo in Zebrafish, and capable of promoting autophagic processing, probably by acting on Akt and mTOR; moreover, it has been shown to ameliorate cognitive deficits in a model of Alzheimer disease, 5XFAD mice.

A better understanding of the physiological mechanisms underlying the progression of INCL is essential to design future therapeutic approaches and the time window in which to act.

SUMOYLATION OF OPHN1 CONTROLS SPINE DENSITY AND ARCHITECTURE BY TUNING ACTIN DYNAMICS

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OPHN1 gene encodes Oligophrenin-1 (OPHN1), a Rho-GAP protein highly expressed in neurons. In humans, all OPHN1 mutations cause the loss of function of OPHN1 leading to syndromic intellectual disability (ID) characterized by cerebellum abnormalities. In neurons, OPHN1 regulates dendritic spine density and architecture, actin dynamics and AMPA receptor (AMPAR) trafficking. Interestingly, we identified for the first time Oligophrenin-1 (Ophn1) as a novel target of sumoylation. Sumoylation is a post-translational modification essential to the modulation of several neuronal functions, including neurotransmitter release and synaptic plasticity. Altered sumoylation has been associated with neurological disorders. Here, we combined molecular biology with live imaging and super resolution microscopy to address the role of sumoylation in controlling OPHN1 function in hippocampal neurons. Furthermore, since the sumoylation site is located close to the novel missense mutation (G412D) identified in ID patients, we explored thrilling hypothesis that compromised sumoylation may lead to synaptic dysfunction associated to the ID phenotype. Altogether, our results clearly demonstrate that sumoylation is a novel regulatory mechanism tuning OPHN1 activity. Furthermore, since the ID-linked G412D mutation impacts OPHN1 sumoylation and affects spine density and morphology, AMPAR surface expression as well as actin polymerization rate to a similar extent as the non-sumoylatable OPHN1 mutant, our data support the hypothesis that impaired OPHN1 sumoylation may participate to the etiology of ID in patients carrying the G412D mutation

NEURONAL NETWORK ACTIVITY AND CONNECTIVITY IS IMPAIRED IN A CONDITIONAL KNOCKOUT MOUSE MODEL WITH PCDH19 MOSAIC EXPRESSION

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Developmental and Epileptic Encephalopathy 9 (DEE9, OMIM # 300088) is a debilitating neurological condition with no effective cure, characterized by early-onset seizures, intellectual disability and autism. DEE9 is due to mutations in the X-chromosome gene PCDH19 that encodes protocadherin-19 (PCDH19), a calcium dependent cell-cell adhesion molecule highly expressed in the limbic system and cortex.
Unlike other X-linked disorders, DEE9 mainly affects females with heterozygous mutations while it generally spares males with hemizygous mutations, except for few males with somatic mutations. For this reason, a cellular interference mechanism has been hypothesized according to which PCDH19 mosaic expression in the brain arising from the coexistence of PCDH19-negative and PCDH19-positive neurons would scramble cell-cell communication and neuronal network functioning.
To validate this hypothesis and investigate DEE9 pathogenic mechanisms, we generated a *Pcdh19* conditional knockout (cKO) mouse model by exploiting the Cre-LoxP technology. We delivered the Cre recombinase in *Pcdh19* floxed mice by either crossbreeding with hSyn1-Cre transgenic mice or intracerebroventricular (ICV) injection of adeno-associated vectors (AAVs) in order to obtain a mosaic expression of PCDH19 in the brain.
Pcdh19 mosaic mice, which recapitulate behavioral traits of DEE9, display a reduced density of hippocampal excitatory synapses, with altered structure and function.
Functionally, patch-clamp experiments on acute hippocampal slices revealed that the hippocampus of mosaic mice is characterized by the presence of a population of hyperexcitable neurons, corresponding to PCDH19-negative neurons. At network level, we observed a global reduction of firing rate associated with increased neuronal synchronization, as inferred from multielectrode array (MEA) recordings *ex-vivo*.
Finally, network activity analysis in freely behaving mice revealed a decrease in excitatory / inhibitory ratio and functional hyperconnectivity within the limbic system of *Pcdh19* mosaic mice.
Altogether, these results indicate that PCDH19 mosaic expression profoundly affects circuit wiring and functioning, and provide new key to interpret DEE9 pathogenesis.

TONIC SOMATOSENSORY RESPONSES AND DEFICITS OF TACTILE AWARENESS CONVERGE IN THE PARIETAL OPERCULUM

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Although clinical neuroscience and the neuroscience of consciousness have long sought mechanistic explanations of tactile-awareness disorders, mechanistic insights are rare, mainly because of the difficulty of depicting the fine-grained neural dynamics underlying somatosensory processes.

Here, we combined the stereo-EEG responses to somatosensory stimulation with the lesion mapping of patients with a tactile-awareness disorder, namely tactile extinction.

Whereas stereo-EEG responses present different temporal patterns, including early/phasic and long-lasting/tonic activities, tactile-extinction lesion mapping co-localizes only with the latter. Overlaps are limited to the posterior part of the perisylvian regions, suggesting that tonic activities may play a role in sustaining tactile awareness. To assess this hypothesis further, we correlated the prevalence of tonic responses with the tactile-extinction lesion mapping, showing that they follow the same topographical gradient. Finally, in parallel with the notion that visuotactile stimulation improves detection in tactile-extinction patients, we demonstrated an enhancement of tonic responses to visuotactile stimuli, with a strong voxel-wise correlation with the lesion mapping.

The combination of these results establishes tonic responses in the parietal operculum as the ideal neural correlate of tactile awareness.

CONTINUOUS TRACKING AS A PROBE FOR PERCEPTUAL MOTION EXTRAPOLATION

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Humans are highly proficient in interacting with dynamic, moving objects when navigating the environment and performing tasks like driving or playing sports. This requires the ability to extrapolate trends and trajectories from moving targets, and to anticipate where an object will be based on evidence from its behavior in the recent past. Continuous psychophysics offers convenient tools for investigating these dynamic tasks. In this work, we explore the performance of human volunteers in a visuo-motor task requiring continuous tracking of a trajectory of drifting dots which erratically changed in elevation following a random walk. We varied both the amount, temporal proximity and reliability of prior information to be used in the tracking task by limiting the participants' view of the dots' trajectory, by only showing an anticipated preview window of the trajectory and by introducing spatial discontinuities on the dots path, respectively. We show that when providing ample and reliable prior information about how the dots will move, the participants exhibit excellent tracking performance; the highest performance benefits arise when providing perceptual evidence in a preview window presented 250 ms before time of contact; observers can flexibly buffer information presented up to 800 ms before the actual time of contact of the trajectory; tracking lag arises from impairments in the ability to internalize the spatial structure of the dots' path. In conclusion, this work shows that tracking is a viable tool to characterize the action-perception loop, which is remarkably flexible and can adapt to the quality of available perceptual information.

ANATOMICAL AND ELECTROPHYSIOLOGICAL BIOMARKERS TO PREDICT SPONTANEOUS RECOVERY AFTER MCAO IN MICE

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Stroke is a devastating pathology and the main cause of long-term disability, despite many patients show a partial spontaneous recovery due to a reorganization of spared areas and connections. Nowadays, it is not possible to predict the final degree of recovery, making difficult to stratify stroke survivors and address them towards their optimal therapeutic protocol in order to maximize its own restoration potentiality. Here, we used a mouse model of middle cerebral artery occlusion (MCAO) to investigate novel prognostic tools in preclinical models. To mimic clinical scales used in stroke patients, we implemented a novel "Global Motor Score" (GMS) comprehensive of the sensorimotor performance assessed in behavioral tests. GMS was able to detect a global deficit after MCAO but showing a partial overall recovery 4 weeks post-stroke. However, going deeper in individual performances, we found a large variability among stroke mice, allowing a distinction between poor and good recoverers. We found that chronic deficit can be explained by acute performance (as in humans) and by the structural integrity of the descending motor system. Mice were also implanted with chronic electrodes in the caudal forelimb area (CFA) to record local field potentials from both hemispheres during the retraction task. In particular, we analysed post-stroke alterations in the Event Related Potential (ERP), in the Perievent Spectograms and in the Event Related Synchronization/Desynchronization (ERS/ERD). These measures were also correlated with the individual degree of spontaneous recovery. Findings from this study will contribute to the Neurorehabilitation quest in identifying early, reliable neurophysiological biomarkers of recovery, which would allow a better tailoring of rehabilitation pathways and allocation of resources in an ageing world destined to a sharp increase in stroke disability.

THE DYNAMIC EXPERIENCE OF VIRTUAL ENVIRONMENTS INFLUENCES MOTOR AND ATTENTIONAL MECHANISMS DURING THE OBSERVATION OF EMOTIONAL BODY POSTURES

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Built environments represent the surrounding stage of our everyday social interactions. Although recently researchers showed that different virtual elements modulate individuals' inner states (Vecchiato et al., 2015; Jelic et al., 2016), less is known about the influence of the dynamic experience of the environment on the perception of other's affective states. To this aim, we recorded event-related brain potential (ERPs) to characterize the time course of emotional body postures processing modulated by a dynamic experience of virtual environments.

Two virtual environments were designed to generate low and high arousing states according to specific variation of Forms (Low, High arousing) (Presti et al., 2022). Textures were provided in two Colors (Cold, Warm). Emotional body postures were represented by virtual avatars conveying three possible arousal levels (Low, Middle, High) (Presti et al., 2021). The electroencephalographic (EEG) activity of twenty-five subjects was recorded while they made a dynamic experience of the designed environments in virtual reality. Each trial started with a virtual promenade within the environment. Afterward, an avatar appeared and subjects were asked to judge the arousal level conveyed by the body posture. Subjective ratings were analyzed through 2x2x3 repeated measure ANOVA, with within factors Form, Color, and Body. ERPs waveforms were firstly analyzed using a factorial mass univariate analysis with a permutation-based clustering correction to identify significant electrodes within restricted time windows. Then, we performed a cluster mass permutation test on mean difference wave amplitude. Finally, we localized the source of the ERPs by solving the inverse problem with the Tikhonov-regularized minimum norm and computing the cortical current density map with dipole orientations that are normal to the cortex via Brainstorm software. As a forward model, we adopted the symmetric boundary element method from the open-source software OpenMEEG using three realistic layers based on the head model provided by the FreeSurfer template (ICBM152).

Subjective ratings on avatars' bodily arousal were higher after the experience of low arousing enviroments ($F(1,24)=8.183$, $p=0.008$). The ERPs analysis returned a higher P200 amplitude over centro-parietal electrodes during the observation of avatars presented within low arousing architectures when compared to high arousing ones ($p=0.001$). The source localization of the P200 showed that this cerebral feature is generated by the activation of the supplementary motor area (SMA) and the left angular gyrus (AG).

Overall, we found that the dynamic experience of virtual environments influenced motor and attentional mechanisms during the perception of emotional body postures. The higher behavioral ratings and P200 amplitude in low arousing architectures reflected a higher attention level towards emotional body postures, possibly pointing to broaden attentional resources generated by the dynamic experience of relaxing environments. The increased P200 amplitude corresponds to an enhance of activity of the SMA and left AG, representing the involvement of motor preparation, attention and emotion regulation mechanisms during the observation of emotional body postures after the dynamic experience of low arousing environments.

These findings could guide the design of virtual and real environments favoring the social events expected to occur inside them. A better understanding of how environmental features influence and possibly support social interactions will also bring us a step closer to designing for neurodiversity.

Reference

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THE EPILEPSY-ASSOCIATED PROTEIN PCDH19 UNDERGOES NMDA RECEPTOR-DEPENDENT PROTEOLYTIC CLEAVAGE AND REGULATES THE EXPRESSION OF IMMEDIATE EARLY GENES

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Protocadherin-19 (PCDH19) is a cell-adhesion molecule encoded by the *PCDH19* gene (Xq22.1). *PCDH19* mutations cause Developmental and Epileptic Encephalopathy 9 (DEE9; OMIM # 300088), a neurodevelopmental disorder characterized by seizures, cognitive impairment and autism spectrum disorder (ASD).

It is well accepted that cell-adhesion molecules (CAMs), in addition to a structural role, also play functional roles and are directly involved in both Hebbian and homeostatic forms of plasticity. In fact, several CAMs undergo a two-step proteolytic cleavage. First, matrix metalloproteases or other proteases such metalloproteases containing a disintegrin domain (ADAM) cut the target protein extracellularly causing the ectodomain shedding. Next, the resulting membrane-bound protein stump is cut within the transmembrane region or at the inner membrane surface by presenilin, the catalytic component of the gamma-secretase complex, thus releasing the cytoplasmic domain. The cytoplasmic domain can exert a signaling function in the cytoplasm or in the nucleus, where it can modulate gene expression directly or indirectly, for instance via protein-protein interaction with transcriptional regulators.

Epigenetics is emerging as a mechanism to regulate synaptic plasticity and cognitive processes. In fact, transcription of genes such as immediate early genes (IEGs) allows translating patterns of neuronal activity into structural and functional long-term synaptic changes. The nuclear translocation of synaptic proteins is one of the mechanisms proposed to transmit information from synapses to nucleus. However, the mechanisms by which synaptic proteins can influence epigenetic mechanisms to transfer information are largely unknown.

We found that PCDH19 undergoes a NMDA receptor (NMDAR)-dependent proteolytic processing and unveiled a nuclear role of PCDH19, which is conserved from rodents to humans. In particular, we investigated the crosstalk between PCDH19 and the chromatin remodeler Lysine-Specific Demethylase 1 (LSD1). LSD1 is a transcriptional corepressor of the Corepressor for RE1-Silencing Transcription factor (CoREST)/HDAC2 complex that provides a bridge between neuronal activity and IEGs. Through the interplay with LSD1, we found that PCDH19 is able to regulate IEGs expression in a way that suggests a homeostatic control of neuronal activity.

RARE MISSENSE MUTATIONS OF THE hβ4 SUBUNIT ALTER α3β4 nAChRs TRAFFICKING

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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 (Slimak et al, 2014).

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. Indeed by means of immunofluorescence and biochemical assays we could demonstrated that the presence in the fifth position of a β4 subunit bearing the mutation alters the expression of α3β4 nAChRs at the plasmamembrane. In particular we demonstrated that the presence of the mutation D444Y in the β4 subunit, which has been shown to increase the nicotine evoked currents in hippocampal neurons expressing α3-β4D444Y receptors (Slimak et al, 2014), increases the amount of surface nAChRs. On the contrary, the presence of the mutation R349C deeply interferes with nAChRs exposure on the cell surface in accordance with the functional data published of decreased amplitude of the nicotine-evoked currents.

THE lncRNA PHOX2B-AS1 IN THE PATHOGENESIS AND AS POTENTIAL DRUG TARGET IN CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

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Heterozygous polyalanine expansion mutations (PARM) in the *PHOX2B* gene cause Congenital Central Hypoventilation Syndrome (CCHS), a genetic disorder affecting the Autonomic Nervous System (ANS) and central chemosensitivity. PHOX2B is a transcription factor that plays a crucial role in autonomic nervous system development. *In vivo* and *in vitro* studies suggest that a loss of function mechanism, combined with a dominant-negative effect and/or toxic gain of function of the mutated proteins, is responsible for the entire disease spectrum. No pharmacological intervention is available for treating the disease or its symptoms. Fortuitous clinical observation that the progestin desogestrel can improve respiratory functions in two CCHS patients, opened the possibility for a relief of the respiratory symptoms and a reduction of risks of death during sleep in CCHS patients [1]. *In vitro* and *in vivo*, we showed that desogestrel downregulates the expression of both wild-type and mutant *PHOX2B* and some of its target genes thus suggesting that the clinical effect might be mediated by the limitation of the toxic effect of the mutant protein [1,2].
The recent mapping of a natural antisense lncRNA, *PHOX2B-AS1*, in the *PHOX2B* locus, and our data showing that it acts at favouring PHOX2B translation, pave the way for validating whether modulating its expression can be used as a new therapeutic strategy aimed at reducing the expression of mutant PHOX2B proteins. Due to the unavailability of viable CCHS mice models to study the function of *PHOX2B-AS1*, we have recently generated iPSC lines from two CCHS patients with PHOX2B PARM mutation (20/25 genotype), but with different onset of the disease (birth vs adulthood), to be differentiated into autonomic sympathetic neurons [3]. The early-onset CCHS patient line has shown an ectopic expression of *PHOX2B* and *PHOX2B-AS1* already at the undifferentiated level, despite the expression of the pluripotency markers, thus confirming that dysregulated *PHOX2B-AS1* and *PHOX2B* transcription at earlier developmental stages may be involved in CCHS pathogenesis. Indeed, the *AS1* regulatory region lies within the expanded poly-alanine tract. Here we provide evidence of the expression of an antisense transcript that positively influences *PHOX2B* gene expression, and suggest that PHOX2B mutations alter antisense transcription.

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MOLECULAR BASIS AND BEHAVIOURAL SIGNIFICANCE OF A SEX SPECIFIC CIRCUIT SWITCH IN DROSOPHILA

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Sex-specific behavioural differences are driven by differences in circuit architecture established through development and genetically controlled. However finding a causal link between genetic, circuit and behavioural differences is challenging. The circuit responding to the male pheromone 11-cis-vaccenyl acetate (cVA) in *Drosophila melanogaster* offers a powerful entry point to this question. cVA has been shown to promote courtship in females and aggression in males (Kurtovic A, et al, 2007, Wang L & Anderson DJ, 2010). cVA information flow is redirected by a switch formed by two types of interneurons, aSP-g and aSP-f, with sexually dimorphic dendritic projections (Cachero S et al, 2010; Kohl J et al, 2013), whose sex is cell-autonomously controlled by the master regulator of sex-differentiation fruitless.

We investigated the molecular underpinnings of sex-specific connectivity by transcriptionally profiling aSP-gs and aSP-fs in females, males and masculinised females during pupation. We identified a set of differentially expressed genes, whose function was addressed by a loss-of-function screen.

Thanks to the molecular profiling we additionally found cell-type specific molecular markers, which gave us genetic access to these neurons. Through optogenetic activation experiments we showed that aSP-gs increase female sexual receptivity, while aSP-fs reduce male copulation success, demonstrating that the circuit switch has the hypothesised impact on behaviour. Additionally, in line with the idea that cVA induces attraction towards males by activating aSP-g neurons, their masculinisation resulted in reduced female receptivity.

Together our work is revealing causal links between sex-specifc circuit architecture and behaviour, as well as the molecular genetic basis of these circuit differences.

BINGE-LIKE ALCOHOL DRINKING IN SARDINIAN ALCOHOL-PREFERRING RATS LIVING IN AN ENRICHED ENVIRONMENT

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Recent work from this lab demonstrated that exposure of selectively bred, Sardinian alcohol-preferring (sP) rats to an enriched environment (EE) reduced different aspects of operant oral alcohol self-administration, including lever-responding for alcohol under fixed and progressive ratio schedules of reinforcement (Maccioni et al., Physiol. Behav. 249:113771, 2022). These data were interpreted in terms of the reinforcing and motivational properties of the main components of EE (i.e., social interactions, physical activities, exploration, and novelty) substituting, at least partially, for those of alcohol; they were also consistent with multiple lines of experimental evidence suggesting that exposure of rats and mice to EE prevented and reversed different behaviors related to alcohol and drugs of abuse.

The present study was aimed at expanding investigation of the effect of EE exposure upon a singular model of binge drinking comprised of daily 1-hour drinking sessions with unpredictable access to multiple alcohol concentrations; this procedure is known to promote binge-like, intoxicating alcohol intakes in sP rats when the drinking session occurs over the final hours of the dark phase of the light/dark cycle (Colombo et al., Alcohol 48:301-311, 2014).

Starting from Postnatal Day (PND) 21, male sP rats were kept under 3 different housing conditions: impoverished environment (IE; single housing in shoebox-like cages with no environmental enrichment); standard environment (SE; small colony cages with 3 rats and no environmental enrichment); EE [large colony cages (standardized Marlau® Cages) with 6 rats and multiple elements of environmental enrichment, including ladders, maze, running wheels, and shelter]. From PND 69, and over a period of 12 consecutive days, rats were exposed daily to a 1-hour drinking session under the 4-bottle "alcohol (10%, 20%, and 30%, v/v) *vs* water" choice regimen, occurring during the dark phase, and with timing of alcohol exposure changed each day and unpredictably to rats.

In all 3 rat groups (IE, SE, and EE), alcohol intake increased progressively from an average of approximately 1 g/kg, when the drinking session occurred during the 1st hour of the dark phase, to an average equal to or higher than 2 g/kg, when the drinking session occurred during the 11th and 12th hours of the dark phase. Slope of regression line was steeper in EE than IE and SE rats, suggestive of higher intakes of alcohol in EE than IE and SE rats when the drinking session occurred over the last hours of the dark phase. Alcohol drinking of EE rats at the last hours of the dark phase was also characterized by a clear preference for the highest alcohol concentration (30%; i.e., the alcohol preparation likely resulting in a more rapid alcohol absorption and faster perception of alcohol central effects). Blood alcohol levels (BALs), assessed at the end of a final drinking session occurring at the 12th hour of the dark phase, did not differ among the 3 rat groups and averaged approximately 150 mg%.

These relatively unexpected results are discussed hypothesizing that the stressful attributes of alcohol expectation, typical of this specific alcohol drinking procedure, were potentiated by the increased "emotionality" that rats living in a comfortable environment (i.e., EE) may experience when facing new, challenging events or environments. Finally, data on BALs confirm that this experimental procedure, comprising of daily 1-hour drinking sessions with unpredictable access to multiple alcohol concentrations, may generate binge-like, intoxicating levels of alcohol drinking in sP rats.

TOWARDS A CURE FOR CREATINE TRANSPORTER DEFICIENCY: PROMISES AND CHALLENGES OF GENE THERAPY

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Creatine Transporter Deficiency (CTD) is an X-linked neurodevelopmental disorder caused by mutations in the Creatine Transporter (CrT) gene. It typically presents with brain creatine (Cr) depletion, intellectual disability, epilepsy and behavioural problems, and no treatment is currently available. To evaluate gene replacement therapy as a possible solution to reverse CTD pathology we developed an adeno-associated viral vector carrying a functional copy of the CrT gene and administered it to newborn CrT knockout mice through intracerebroventricular injection. After six weeks we found that treatment induced transgenic CrT expression throughout the whole brain, increased brain Cr levels and improved cognitive function. However, we also observed dose-dependent toxicity, neuroinflammation and neurodegeneration likely caused by Cr overload. This suggests that therapeutic Cr levels are a matter of a fine balance, and we are now optimising the vector design to obtain a more tolerable CrT expression. Our results provide proof-of-concept evidence that gene therapy has potential applications for treating CTD and suggest that further steps of vector engineering to finely tune CrT expression may be pivotal for maximising safety and efficacy.

INTERLEUKIN 10 PROTECTS FROM DOPAMINERGIC CELL LOSS BY GUIDING THE FATE OF INNATE AND ADAPTIVE IMMUNE CELLS

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Inflammation, including the role of both innate and adaptive immunity has gained attention in the pathophysiology processes underling the occurrence of neurodegenerative disease. Despite the role in multiple sclerosis is considered as a priming event, the involvement of the immune system in Alzheimer and Parkinson's disease (PD) is still controversial among researchers and clinicians. Nearly 20 years elapsed before there was a more detailed study demonstrating and quantifying the infiltration of T lymphocytes (CD4 and CD8) but not of B lymphocytes in the affected brain regions in PD (1). Studies confirmed that neuroinflammation is associated with the PD pathological process, with increased concentrations of proinflammatory cytokines in blood, cerebrospinal fluid (CSF), and even the brain post-mortem of PD patients (2) Evidence for brain inflammation in PD has also been obtained using neuroimaging approaches, primarily with ligands binding to translocator protein 18KD (3). Moreover, neuroinflammation is observed across a broad range of PD model systems, including models based on neurotoxins such as 6-hydroxydopamine and MPTP and a variety of α-synuclein-based models including transgenic animals, models based on viral delivery of α-synuclein, and models induced by administration of misfolded α-synuclein fibrils (4). However, one open question is whether neuroinflammation represent a primary event or is only a consequence in response to cell death. To answer this question, our group recently generated a unique mouse model, by which we have been able to dissect the role of inflammation in the cell death process from the cell autonomous degeneration due to intraneuronal alpha-synuclein accumulation (5). In our model we also observed the presence of T-cells infiltration, in particular CD8+ lymphocytes, which is in line with what has been observed in the brain of patients in early stages of the disease (6). Due to the involvement of innate and adaptive immune cells in the process determining the neurodegeneration in PD we sought to design a novel gene therapy strategy based on the delivery of the immune modulator cytokine interleukin 10 (IL-10). Indeed, signalling through the IL-10 receptor regulates several steps of the immune response, from decreasing cytokine gene expression to down-regulating the expression of major histocompatibility complex class II (MHC-II) and thus antigen presentation to T cells (7).

In different studies performed in PD animal models, IL-10 It has been described to protects ventral mesencephalic neurons in LPS induced neuroinflammation, to inhibit TNF-α production and reduce caspase-3 and caspase-9 neuronal mediated apoptosis (8). We experimented the neuroprotective effect of human IL-10 released by resident microglia in a model of alpha-synuclein accumulation in the substantia nigra. The selective targeting of microglia as recipient cells has been made possible by using a novel LV vector based on the miRNA detargeting strategy. Our results clearly show the ability of IL-10 overexpression to counteract dopaminergic degeneration elicited by nigral alpha-synuclein accumulation after 10 weeks. We found that neuroprotection is mediated by the immune modulation exerted by IL-10 on innate and adaptive immunity. Interestingly, through the use of single cell RNA sequencing technique, we observed that IL-10 drives the immune cells specialization toward either phagocytic or inhibitory microglia phenotype, by favoring the exhaustion of CD8+ cells and the generation of regulatory T cells. Therefore, IL-10 seems to contribute to the neuroprotection following different routes. On one hand, it can enhance the aggregates clearance and restrains the role of microglia in sustaining immune activation. On the other hand, the exhaustion of CD8+ adjuvate by the regulatory T cells culminate in the reduction of the cytotoxic response. Overall, the data obtained with this study, candidate IL-10 as a potential neuroprotective molecule able to control the immune response in neurodegenerative diseases as PD.

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50-kHz ULTRASONIC VOCALIZATIONS IN HEMIPARKINSONIAN RATS REPEATEDLY TREATED WITH DOPAMINOMIMETIC DRUGS AS A POSSIBLE BEHAVIORAL MARKER OF THE AFFECTIVE PROPERTIES OF DOPAMINOMIMETIC DRUGS USED IN THE THERAPY OF PARKINSON'S DISEASE

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The current leading therapeutic strategy used to manage Parkinson's disease (PD) is the so-called dopamine replacement therapy (DRT). However, prolonged use of DRT is associated with the onset of both motor and non-motor complications. The latter include alterations in the emotional state and the manifestation of iatrogenic psychiatric-like disturbances. As of today, the preclinical investigation of these disturbances is limited due to a substantial lack of effective experimental models that allow studying the affective properties of dopaminomimetic drugs in parkinsonian animals. In this regard, we evaluated the emission of 50-kHz ultrasonic vocalizations (USVs), a behavioral marker of positive affect, in rats bearing a unilateral lesion with 6-hydroxydopamine (6-OHDA) in the medial forebrain bundle. Apomorphine (2 or 4 mg/kg, i.p.), L-3,4-dihydroxyphenilalanine (L-DOPA, 6 or 12 mg/kg, i.p.), or pramipexole (2 or 4 mg/kg, i.p.) were administered in a test cage (× 5 administrations) on alternate days. Seven days after treatment discontinuation, rats were re-exposed to the test cage to measure conditioned calling behavior and thereafter received a drug challenge. Hemiparkinsonian rats treated with either apomorphine or L-DOPA, but not pramipexole, markedly vocalized during repeated treatment and after drug challenge, and showed conditioned calling behavior. Moreover, apomorphine, L-DOPA and pramipexole elicited different patterns of 50-kHz USV emissions and rotational behavior, indicating that calling behavior in hemiparkinsonian rats treated with dopaminomimetic drugs is not a byproduct of motor activation. Taken together, these results suggest that measuring the emission of 50-kHz USVs may be a relevant experimental tool for further characterizing at the preclinical level the effects that dopaminomimetic drugs used in the DRT of PD elicit on the emotional state.

MICROANGIOARCHITECTURE EFFICIENCY IN A SWINE ALS MODEL: TOWARD A POSSIBLE DIAGNOSTIC MARKER?

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It is growing knowledge that vascular alterations accompany or precede, human neuronal degenerations. This was observed in Alzheimer's Disease, Parkinson's Disease, Diabetes, and Multiple Sclerosis. Possibly, also Amyotrophic Lateral Sclerosis (ALS) presents this behavior.
Quantitative analysis of a tissue micro-angioarchitecture is a difficult task. However, one of us recently proposed a semi automatic, image analysis, approach that summarizes the 3D layout of a micro-angioarchitecture. It quantifies how proficient is the angioarchitecture itself in providing cargo (through larger vessels) and in releasing it (smaller vessels) to every corner of a volume.
This approach was originally tested in an animal model for Krabbe Disease (*twitcher* mouse) and very recently we tried to adapt it to a published swine model overexpressing a human, mutated SOD1 gene and recapitulating the genesis of human ALS together with its pre-symptomatic lag phase.

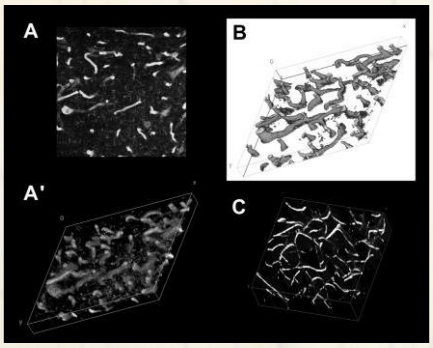

Figure 1

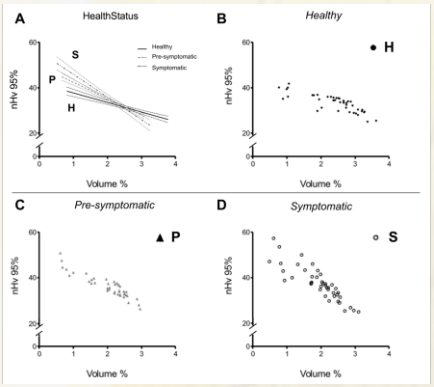

Figure 2

In this preliminar approach we observed vascular alterations in spinal samples at different extents according to tissutal sources: cervical, thoracic or lumbar.

Re-considering data according to this new source of variability (Figure 3), we obtained graphs with a high level of internal consistency. Absence of inter-animal variabilities spoiled our results of significance with respect to pathology. Conversely, it confirmed the ability of the approach we used to identify and parametrize vascular differences in an ALS, swine model engineered to present a pre-symptomatic silent phase of the disease. In this pathology, our data focus attention to alterations in microangioarchitectures, even if we were not yet able to demonstrate a direct correlation with pathological status and disease progression.

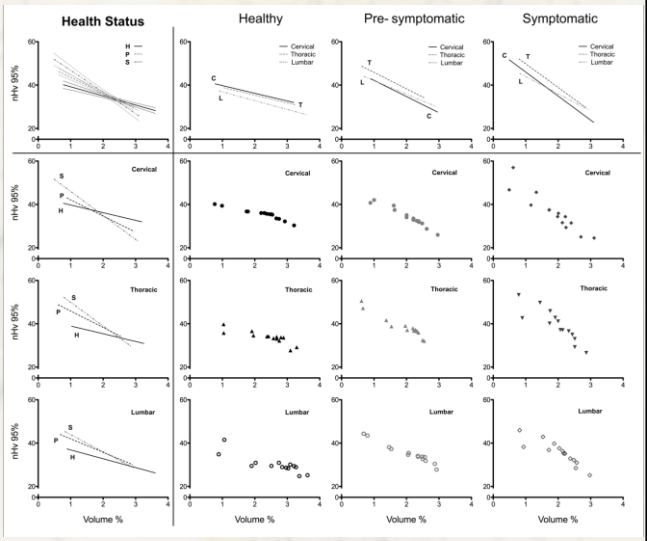

Figure 3

MULTI-LEVEL CHARACTERIZATION OF THE KNOCK-OUT MOUSE FOR *Pcdh9*, A CELL-ADHESION MOLECULE IMPLICATED IN NEURODEVELOPMENTAL DISORDERS

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Protocadherin 9 (PCDH9) is a cell-adhesion molecule member of the cadherin superfamily and implicated in brain development and functioning. *PCDH9* gene has been associated with Autism Spectrum Disorder (ASD) after the finding of copy number variations (CNVs) in autistic individuals, and was recently identified as a risk gene for Major Depression Disorder (MDD) by GWAS analysis. In the mouse brain, *Pcdh9* deletion induces cellular and synaptic defects in the somatosensory cortex accompanied by behavioral deficits, thus strengthening the link of *PCDH9* with neurodevelopmental disorders. However, the molecular mechanisms underlying the developmental and behavioral abnormalities in *Pcdh9* KO mouse, as well as *PCDH9* linkage with ASD and MDD, remain to be elucidated.

To this aim, we planned a comprehensive characterization of *Pcdh9* KO mice at the biochemical, ultrastructural, functional, transcriptomic, metabolic and behavioral level. We focused on hippocampus and prefrontal cortex (PFC), two brain areas critically implicated in depression. Electron Microscopy (EM) analysis revealed increased PSD length and spine head area in the CA1 of *Pcdh9* KO hippocampus compared to WT, while no alterations in the levels of glutamate receptors and synaptic markers were detected. Recordings from CA1 acute slices showed a significant increase in the frequency of spontaneous excitatory post-synaptic currents (EPSCs) in *Pcdh9* KO mice, denoting a defect in glutamatergic transmission. No structural or electrophysiological abnormalities were observed in the medial PFC, indicating a specific role for *Pcdh9* in CA1 pyramidal neurons. Single-nucleus RNA-seq analysis will shed light on the molecular pathways altered in *Pcdh9*-depleted hippocampal neurons, and will identify potential druggable targets to rescue the behavioral and synaptic defects observed in *Pcdh9* KO mice.

Furthermore, here we describe the neuronal activity-induced and NMDA receptor (NDMAR)-dependent proteolytic cleavage of PCDH9 in cultures of primary neurons. PCDH9 processing leads to the generation of a soluble intracellular PCDH9 C-terminal fragment (CTF), with potential signaling activity in response to neuronal stimulation. Interestingly, preliminary data show PCDH9 cleavage in P7 and P14 hippocampus *in vivo*, suggesting a specific role for PCDH9 processing during early post-natal brain development, a critical time window for neuronal and circuits maturation.

INVESTIGATION OF THE ROLE OF PARVALBUMIN-POSITIVE INTERNEURONS IN A CONDITIONAL MODEL OF PCDH19-RELATED DISORDER

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Pcdh19 gene encodes for the Protocadherin 19 (PCDH19) protein and is located on the X chromosome, its mutations lead to Developmental and Epileptic Encephalopathy 9 (DEE9), a severe neurodevelopmental disorder characterized by early-onset seizures, combined with autism features and intellectual disability. In vivo mosaic expression of *Pcdh19* impairs the maturation and migration of neurons and the GABA circuitry. Moreover the altered expression of PCDH19 cause defects in the E/I balance in DEE9 mouse model(see Mazzoleni poster). Considering that GABAergic neurons, and in particular (PV)-positive interneurons (PVIs), play a fundamental role in regulating cortical E/I balance, we propose to investigate the effects of the deletion of Pcdh19 specifically in PVIs subpopulation.

Starting from this leading point, we generated two transgenic mouse models. First we obtained the *PV-Pcdh19 floxed* mouse by crossing female homozygous mice *flox-Pcdh19* with a male homozygous *PV*$^{tm1(cre)Arbr/J}$ mouse. Females and males from this first generation were crossed till reaching homozygosity for both transgenes, to obtain a mouse colony in which *Pcdh19* expression is specifically deleted in all PVIs. To reach the second model, the *PV-Pcdh19;Ai14,* we crossed a *PV-Pcdh19 floxed* mouse with a reporter mouse *Ai14,* and we eventually obtained the triple homozygous mice for the three transgenes. This model will permit to directly highlight the PVIs, in which Pcdh19 is deleted, by the expression of the fluorescence protein tdTomato and to perform morphological, electrophysiological and transcriptomic studies.

Therefore, we are validating our mouse models through a multidisciplinary approach. Promising preliminary results demonstrate that PCDH19 is strongly expressed in PVIs in the cerebral cortex in control mice, while its expression is lost in all PVIs in the *PV-Pcdh19 floxed* mice. From a functional point of view, continuous electroencephalogram (EEG) recordings (i.e., ≥24 h) and post-Pentylenetetrazol (PTZ) analysis hinted at a higher susceptibility to epileptic seizures in *PV-Pcdh19 floxed* mice compared to the control littermates. These data are very intriguing since it seems that PCDH19 plays a specific role in the GABAergic system.

In order to further validate our novel models, we will evaluate it at the structural, functional and transcriptional levels.

We will study the temporal expression of PCDH19 in PVIs at different time-points through immunofluorescence. Then we will perform morphological analysis through the analysis of dendritic arborization and by taking advantage of electron microscopy high resolution technique to reveal possible defects in synaptic structures. In parallel, we will carry out electrophysiological and calcium imaging analysis to study how the single neuron activity or the entire network activity is affected upon deleting *Pcdh19* in PVIs.

Moreover, we will disclose altered gene expression in target PVIs by single-nucleus RNA sequencing analysis.

By focusing on the role of PVIs, our study will provide a new insight into DEE9 pathogenic mechanisms and aid the identification of new therapeutic targets.

PARALLELING THE SHORT-TERM EFFECT OF ACTION OBSERVATION WITH THE LONG-TERM EFFICACY OF ACTION OBSERVATION TRAINING

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AIMS: Action Observation Training (AOT), exploiting the properties of the mirror mechanism, represents an effective tool to promote the acquisition of new motor abilities [Rizzolatti et al., 2021; Bazzini et al., 2022]. However, whether the motor improvement depends on the convergence of the observer's motor pattern onto the observed model remains unsettled. Thus, we designed an EMG experiment to investigate whether responsiveness to AOT depends upon a gain of similarity in term of temporal patterns of muscular activity between the trainee and the model.
MATHERIALS AND METHODS: 72 right-handed participants were enrolled in the study and subdivided into two groups (AOT and Control). All participants had to learn positioning 15 marbles on a board with holes using chopsticks. The training consisted of six sessions composed of an observation and an execution phase. During the observation phase, the AOT group observed an expert performing the task, while the Control group observed landscapes videos. For each participant behavioural indices were measured, such as the number of grasping attempts, the number of failed liftings and the mean duration of the reach-to-place action. Furthermore, during the 6 executions, the EMG pattern of 3 hand muscles (opponent pollicis- OP, first digital interosseus- FDI and abductor digiti minimi- ADM) was collected and compared with that of the expert (R^2). The gains in similarity were correlated with behavioural improvement for both groups.
RESULTS: While both AOT and Control groups improved upon the training, participants receiving AOT presented a larger improvement, especially in terms of decrease of grasping attempts (-25% AOT group and -16% Control group). Moreover, a significant correlation was found between behavioural improvements and the degree of convergence toward the muscular pattern of the model. Interestingly, this applies to FDI and ADM in the reaching and holding phases of the action, i.e., the muscles and motor act surrounding the grasping event. Of note, these correlations were specific for AOT participants.
DISCUSSION: These results outlined that AOT can promote the acquisition of skills that are not part of the observer's cultural motor repertoire. Furthermore, the behavioural improvement due to AOT appears mediated by the similarity of EMG patterns between the observer and the model. To better investigate the effect of AOT during the acquisition of novel motor skills, starting from preliminary evidence that TMS modulation induced by action observation predicts the amount of motor improvement induced by AOT, future TMS studies should aim at evaluating the temporal dynamics of corticospinal excitability (CSE) and short intracortical inhibition (sICI) during AOT and their relationship with time-wise behavioural outcomes.
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KINEMATIC CHARACTERIZATION OF MOTOR PHENOTYPES IN WHOLE-BODY ACTIONS: CLAIMING THE ROLE OF MOTOR SIMILARITY IN OBSERVATION-BASED MOVEMENT LEARNING

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Recent studies highlighted how unique kinematic features characterized individual movements, representing specific motor signatures [1]. This between-subjects strategy variability can mean a determining factor in understanding and learning actions. Within this framework, no studies have manipulated kinematic similarity between observational models and observers in motor training based on action observation (AOT, [2]), exploring its potentiality in influencing learning efficacy.

The following study puts the basis for this investigation and has two main aims: a) identify and cluster motor strategies for whole-body movements in a population of healthy subjects and b) investigate whether, during action observation, the grade of motor similarity with the model could be distinctly perceived by the observer and facilitate action recognition.

We recorded the kinematics of 45 participants while performing whole-body reach-to-manipulate movements of spheres located at different distances. For each manipulation task (rotation – squeezing) and each position (9 combinations between lower, middle, and upper heights with left, middle, and right sides), the movement trajectories of 10 body segments were extracted and submitted to a Principal Component analysis. The resulted output was implemented in a cluster analysis to group individual motor strategies in performing each movement. Then, participants' kinematics was assessed and statistically compared through MMGA (Method for Movement and Gesture Assessment, [3]) to characterize each strategy for its ergonomic goodness and level of injury riskiness. Significant differences emerged for four positions (lower left, central and right positions plus the upper central) for both tasks, evidencing a gradient of ergonomic quality between the identified motor patterns.

In the second phase of the experiment, we built a sequence of videos for each participant proposing three versions of the same actions, without displaying the manipulation phase, showing movements belonging to participants who revealed a high, intermediate, or low grade of similarity with the observer. Each participant was required to observe the videos and a) judge the grade of perceived similarity and b) guess the final goal of the action: rotation or squeezing. Results showed that participants can distinguish motor patterns similar to their ones and that this perception was related to the correctness of the goal prediction.

The study results open to a future neurophysiological assessment of the relation between motor reactivity and learning during the observation of actions performed by models chosen with different grades of similarity. Moreover, the ergonomic characterization allows identifying “at-risk motor phenotypes”, exploiting action observation training for motor refinement and injury prevention purposes.

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KICKING IN OR KICKING OUT? THE ROLE OF INDIVIDUAL MOTOR EXPERIENCE IN PREDICTING THE OUTCOME OF RUGBY PLACED KICKS.

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AIMS: The mirror mechanism (Rizzolatti et al., 2014) i.e., the neural mechanism making active the same motor areas involved during action execution also during action observation, underlies our abilities to understand others' actions and intentions. In sports, these abilities are fundamental to predict the opponents' actions and their outcomes, so to prepare more rapidly and efficiently a complementary reaction. While it is well known that the observation of an action belonging to the observer's motor repertoire determines a stronger activation of the mirror mechanism (Calvo-Merino et al., 2005), how to apply this principle to team sports training remains to be established. Indeed, players have different roles, requiring different motor repertoires which, intrinsically, equip the teammates with different levels of others' behaviours decoding. To test this hypothesis, we designed a behavioural experiment to evaluate whether a specific rugby action (placed kick) is better predicted by rugby kickers (RK) compared to their not-kicking teammates (RNK) and controls (CTRL).
MATHERIALS AND METHODS: We enrolled 134 participants (34 RK, 36 RNK, 64 CTRL) asking them to observe 144 videos of placed kicks (stopped at the foot-ball contact) performed from 3 positions and recorded from 3 perspectives. They had to predict each kick outcome. The accuracy of each participant was computed as d' and ANOVAs were calculated considering Group as between-subject factor, Position and Perspective as within-subject factor. Finally, the motor (i.e., the number of placed kicks executed) and visual experiences (i.e., the number of rugby match watched) of the rugby players (RK+RNK) was correlated with their abilities to predict the kicks outcome.
RESULTS: A main effect of Group emerged on overall prediction accuracy ($p=.044$), with RK outperforming RNK and CTRL. Such results were confirmed also by mixed ANOVAs modelling the position and perspective, which indicated an advantage also when the whole-body movement was visible, and the position was along the central midline of the field. Furthermore, partial correlation analyses revealed that the motor experience play a key role in the outcome prediction ability ($p=.027$, $R^2=.081$), more than the visual experience ($p=.313$, $R^2=.017$).
DISCUSSION: Watching only the initial phase of the action executed by another player, RK are better in predicting the placed kick outcome compared to RNK and CTRL.
CONCLUSION: In sports, individual motor skills serve not only to improve the own direct performance, but also to enrich the cognitive abilities needed to decode the opponents and teammates behaviours. Traditional training sessions should then be integrated with training of the actions mostly performed by opponents, thus helping players putting themselves in their opponents' shoes.

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EXPLORING THE ACCURATE DEFINITION OF ACUTE STROKE IMAGING OUTCOMES WITH CIRCULATING BIOMARKERS

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Background and aims: Not all patients clinically improve after acute ischemic stroke early recanalization therapy: the restoration of blood flow (reperfusion) to suffering brain tissue could lead to a paradoxical worsening of brain damage through a complex series of biochemical and cellular pathophysiological mechanisms, the so-called reperfusion injury. The disruption of the neurovascular unit, namely the cellular and extracellular components involved in the regulation of cerebral blood flow and Blood Brain Barrier (BBB) function (endothelial cells, basal lamina matrix, astrocyte, pericytes, neurons, and supporting cells) is responsible of the major clinical-radiological complications of acute ischemic stroke, cerebral edema, and hemorrhagic transformation. At variance of intracerebral hemorrhage, there are fewer data on clinical-anamnestic and neuroimaging predictors for the development of cerebral edema after ischemic stroke revascularization therapies. This could be probably due to the minor availability of classification strategies and reliable neuroimaging quantification measurements. Harston et al. recently developed a direct and automated quantification method of cerebral edema on MRI images, proposing the new concept of Anatomical Distortion (AD). The hypodense area on follow up brain CT scan is the result of the Final Infarct Volume (FIV) and the Anatomical Distortion (AD) due to edema and hemorrhage, that are the effects of BBB disruption. Current literature suggests that cerebral edema and hemorrhagic transformation share both pre-transcriptional and transcriptional factors and should be considered to belong to the same physio-pathological continuum, pointing out that blood components extravasation could represent the result of a complex process triggered by ischemic cascade. Cerebral edema could be categorized by a pathophysiological classification, that distinguishes between cytotoxic, ionic and vasogenic edema, but unfortunately it lacks applicability in clinical practice. We used an objective and automated method to obtain a separate quantification of the two distinct pathological processes, FIV and AD. Our aim is to investigate the possible association between AD and a panel of circulating biomarkers reported in literature to be involved in parenchymal damage following acute ischemic stroke treatments.
Methods: We used the linear and non-linear registration method described by Harston et al. to measure AD. Relative AD (rAD; AD/FIV ratio) was also calculated to control AD for infarct size. We included in our retrospective analysis anterior circulation acute ischemic stroke patients treated with systemic thrombolysis, endovascular therapy, or both, that had both baseline and 24h circulating biomarkers blood levels available for analysis.
Results: Both FIV and rAD resulted associated with higher baseline values of MMP-8 (respectively p=0.01, 95% CI 0.003-0.021 and p=0.01, 95% CI 0.000-0.002). Higher baseline levels of MMP-7 and their pre-post variation resulted associated with rAD (respectively p=0.01, 95% CI 0.01-0.10; p=0.003, 95% CI -0.09- -0.02). Higher baseline levels of Metalloproteinases (MMP-7, MMP-8) and inflammatory cytokines, are related with AD development.
Conclusions: Higher baseline levels of Metalloproteinases (MMP-7, MMP-8) and inflammatory cytokines, are related to AD development, that is the effects of neurovascular unit and BBB disruption. We quantified brain swelling with a novel neuroimaging method first applied on brain CT. If replicated on larger studies and confirmed by experimental studies, MMP-7 and MMP-8 might be a druggable target in future neuroprotection strategies. Moreover, they might predict outcome before reperfusion treatments, and therefore support decision making with a precision-medicine approach.

TEMPORAL MANIPULATION OF *Scn1a* GENE EXPRESSION IN DRAVET SYNDROME

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Dravet syndrome (DS) is a severe infantile epileptic encephalopathy, characterized by drug-resistant epilepsy, severe cognitive and behavioral deficits and a high risk of sudden unexpected death (SUDEP). In the 80% of cases, it is caused by haploinsufficiency of Scn1a gene, that encodes for the α subunit of the voltage-gated sodium channel $Na_v1.1$. Fast-spiking interneuron dysfunction whose activity strongly relies on this channel is considered to be the underlying mechanism of disease onset.
Gene therapy is a promising treatment for DS but whether the reinstatement of physiological levels of Nav1.1 is sufficient to revert the pathology after symptom onset remains unknown. To address this question, we generated a Scn1a conditional knock-in mouse model (Scn1aStop/+) in which Scn1a expression can be re-activated on-demand during the mouse lifetime. we showed that Scn1a gene re-activation when symptoms were already manifested (P30) led to a complete rescue of both spontaneous and thermic inducible seizures and a marked amelioration of behavioral abnormalities including social deficits, hyperactivity and cognitive impairment. Normalization of fast-spiking interneuron firing in the CA1 of the hippocampus was observed upon Scn1a reinstatement. We also identified dramatic gene expression alterations, including those associated with astrogliosis in Dravet syndrome mice, that, accordingly, were rescued by Scn1a gene expression normalization at P30. Interestingly, regaining of Nav1.1 physiological level rescued seizures also in adult Dravet syndrome mice (P90) after months of repetitive attacks. Overall, these findings represent a solid proof-of-concept highlighting that disease phenotype reversibility can be achieved when Scn1a gene activity is efficiently reconstituted in brain cells. In a therapeutic perspective, it would be important to determine whether *Scn1a* is also required after a critical developmental time-window or whether ensuring physiological levels of $Na_v1.1$ during early post-natal life is sufficient to prevent or mitigate DS symptoms.
To achieve temporal control of *Scn1a* gene expression, we crossed a conditional model of DS carrying a missense mutation in the coding sequence (floxed stop Scn1a*A1783V) with UBC-Cre-ERT2 mice. By tamoxifen injections, we induced the expression of *Scn1a* mutant allele at two different post-natal (P) time points, P30 and P60 and compared their phenotypes to those of perinatally (P2) inactivated mice (Dravet model). P30-inactivated mice show a mortality rate for SUDEP comparable to Dravet mice and video-EEG analysis detected the occurrence of spontaneous seizures. When tested for behavioral alterations, P30-inactivated mice revealed to be anxious and hyperactive and display impairment in social memory similarly to Dravet mice. Interestingly, in the water maze test, they showed to be more skilled in comparison to Dravet mice. When *Scn1*a mutant allele expression was induced at P60 (P60-inactivated) mice showed seizure at a frequency comparable to the other experimental groups, but, conversely, they were protected from SUDEP. Our data show that the maintenance of physiological levels of $Na_v1.1$ until P30 is not sufficient to prevent DS symptoms but ameliorated cognitive performance. These data suggest that continuous administration of any treatment aiming to increase levels of *Scn1a* gene is required as also late induction Scn1a gene haploinsufficiency manifest in DS phenotype.

A NOVEL PHOTOTHROMBOTIC MODEL OF OCCLUSION–RECANALIZATION OF MCA IN MICE

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Stroke is the leading cause of adult disability and the third cause of death. Reperfusion treatment indications are expanding, but there is still no explanation why a large proportion of patients (54.5%) experience futile recanalization: poor outcomes despite successful recanalization. A preclinical stroke model that resembles the ischemic progression allowing to retrace clinical processes is really urgent.
Here we developed and characterized a novel mouse model of photothrombotic occlusion and light-induced recanalization of the distal branch of the middle cerebral artery (MCA).
In order to characterize the lesion induced by the stable occlusion of the distal branch of the MCA one week after photothrombosis, we performed an ex vivo immunostaining that showed a region of necrotic tissue localized in the mouse cortex, with an overall lesion volume of 6.9 ± 0.1 mm^3 in stroked mice. By evaluating the diffusion of the serum albumin dye Evans Blue, we revealed the insurgence of extravasation 24 hours after the damage. Moreover, we observed at the same time point the increment of the water content in the ipsilesional hemisphere with respect to the contralesional one and Sham mice. The behavioral assessment performed by the clasping test revealed that the functional impairment of mouse forelimb decreased from two to seven days after stroke. Finally, through ex vivo immunohistochemical investigation of astrocytes morphology, we observed a strong upregulation of the levels of glial fibrillary acidic protein (GFAP), indicating the activation of reactive astrogliosis during the acute phase after the lesion. In a different experimental group, 30 minutes after the photothrombotic occlusion of the MCA we illuminate the same blood vessel with a UV LED in order to induce the breakage of the bond inside the clot and to induce temporal vasodilatation that fosters the recanalization of the blood vessel and the consequent reperfusion of the tissue downstream. Preliminary experiments showed the reduction of the water content of the affected hemisphere with respect to mice without recanalization. Moreover, the motor and cognitive impairment, revealed by a wide range of behavioral tests, highlighted a complete recovery of motor function 24 hours after stroke.
Since half of all clinical ischemic strokes occur in MCA territory, the development of a reproducible mouse model of stroke mimicking large thromboembolic stroke in humans is crucial in preclinical animal studies. Indeed the development of the occlusion-recanalization model of the distal branch of the middle cerebral artery in mice has a great translational value allowing in future studies thus mimicking the clinical treatment and fostering the investigation of the neurovascular mechanisms underneath the ischemic progression.

ASTROCYTES MODULATE SOMATOSTATIN INTERNEURON SIGNALING IN THE VISUAL CORTEX

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At glutamatergic synapses, astrocytes respond to the neurotransmitter glutamate with intracellular Ca^{2+} elevations and the release of gliotransmitters that modulate synaptic transmission. While the functional interactions between neurons and astrocytes have been intensively studied at glutamatergic synapses, the role of astrocytes at GABAergic synapses has been less investigated. In the present study, we combine optogenetics with 2-photon Ca^{2+} imaging experiments and patch-clamp recording techniques to investigate the signaling between Somatostatin (SST)-releasing GABAergic interneurons and astrocytes in brain slice preparations from the visual cortex (VCx). We found that an intense stimulation of SST interneurons evokes Ca^{2+} elevations in astrocytes that fundamentally depend on $GABA_B$ receptor ($GABA_BR$) activation, and that this astrocyte response is modulated by the neuropeptide somatostatin. After episodes of SST interneuron hyperactivity, we also observed a long-lasting reduction of the inhibitory postsynaptic current (IPSC) amplitude onto pyramidal neurons (PNs). This reduction of inhibitory tone (i.e., disinhibition) is counterbalanced by the activation of astrocytes that upregulate SST interneuron-evoked IPSC amplitude by releasing ATP that, after conversion to adenosine, activates A_1Rs. Our results describe a hitherto unidentified modulatory mechanism of inhibitory transmission to VCx layer II/III PNs that involves the functional recruitment of astrocytes by SST interneuron signaling.

BRAIN SEX-DEPENDENT ALTERATIONS AFTER PROLONGED HIGH FAT DIET EXPOSURE

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Obesity represents a risk factor for mental health. To understand underlying mechanisms, we longitudinally evaluated the effects on brain and periphery of 33 weeks exposure of female and male C57BL/6 mice to two high fat diets (HFD). Males fed with either diet were more vulnerable than females in periphery, displaying higher and faster increase in body weight and more elevated cholesterol and liver enzymes levels, despite glucose intolerance was similar in both sexes. [^{18}F]-FDG PET imaging showed higher glucose metabolism in the olfactory bulbs of both sexes. However, males also displayed altered metabolism in anterior cortex and in cerebellum, accompanied by a more prominent brain inflammation relative to females. Although both sexes displayed reduced transcripts of neuronal and synaptic genes in anterior cortex, only males had decreased protein levels of AMPA and NMDA receptors. Oppositely, to anterior cortex, cerebellum of HFD-exposed mice displayed hypometabolism and transcriptional up-regulation of neuronal and synaptic genes. These results indicate that male brain is more susceptible to metabolic changes induced by HFD and that the anterior cortex versus cerebellum display inverse susceptibility to HFD.

DEXAMETHASONE OCULAR IMPLANTS FOR RETINITIS PIGMENTOSA

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Retinitis pigmentosa (RP) is a family of heterogeneous genetic disorders causing progressive degeneration of photoreceptors eventually leading to blindness. Clinical symptoms reflect the degeneration patterns of photoreceptors: rods, responsible for night vision, degenerate first because typically genetic mutations affect rod-specific genes; cones, necessary for colour vision and photopic acuity, die later. The biological causes of cone secondary degeneration are an active research topic worldwide. Recently, inflammation responses are emerging as key players in the pathogenesis and progression of RP and in cone death, thus suggesting a potential efficacy of anti-inflammatory therapies for attenuating the severity of the RP phenotype.
In a previous study, we found that the peak of cone death in retinal degeneration mice is accompanied by a retinal overexpression of inflammatory genes that is lowered by dexamethasone systemic administration. Considering that long-term systemic treatment might have side effects and that intravitreal dexamethasone implants are already used in clinical practice to treat other ocular disorders complications (such as edema secondary to non-infectious uveitis, diabetic macular edema and macular edema secondary to RP), we implemented a long-term administration of this steroid in RP mutant mice. Here we demonstrate that this treatment is able to modulate inflammatory responses at retinal level, as assessed by molecular tools, at the same time promoting a rescue of cones. rd10 mice, a well-known model of RP with a mutation of the rod-specific phosphodiestarase (PDE) gene, are treated by local delivery of human dexamethasone implants adapted to mouse eyes. The treatment outcome is then assessed evaluating the expression of molecular inflammatory markers by qPCR, and preservation of retinal cones and blood retinal barrier with immunohistology analyses. The physiological relevance is finally tested by visual behaviour and electroretinogram. Further investigation will complete this preclinical study supporting possible repurposing of ocular dexamethasone implants for reducing human RP progression.

BRAIN-STATE DEPENDENT FUNCTIONAL (DYS)CONNECTIVITY OF EXCITATORY CIRCUITS IN SHANK3B MICE

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Phelan-McDermid syndrome is a neurodevelopmental disorder with non-specific features including global developmental delay, intellectual disability, and autism spectrum disorder (ASD). In humans, hemi-deletion of SHANK3 is found in a large fraction of PHMDS cases. Animal studies have provided insight into the behavioral dysfunctions, like motor stereotypies and socio-communicative impairment, in Shank3 mutant mice. Systemic modifications in functional connectivity (FC) are prevalent in neurodevelopmental disorders and have been regarded to account for the complex repertoire of symptoms exhibited by ASD patients. A leading hypothesis is that a generalized hyper-excitability in the brains of individuals with autism may drive alterations in connectivity. Here we tested this hypothesis by using a combination of advanced imaging, genetic engineering and behavioral tests in Shank3B mutant mice. Results show that haploinsufficiency of Shank3B determines a global rewiring of the cortical excitatory circuits, as shown by hierarchical cluster analysis of the FC matrices. This alteration of intra and inter-hemispheric connectivity is strongly dependent on the brain state: indeed the significant difference in the awake state between mutated and wild type mice is reduced in light and disappears with deep anesthesia. Male are more affected than female Shank3B mice by the rewiring of the functional circuits. The alteration is age-dependent and does not ameliorate or compensate from post-natal age 45 (P45) to P90. Finally on the same mice we tested the excitability of the cortical network with peripheral sensory stimulation of the whiskers and found large-scale alterations in the sensory response, primarily affecting the late response to the stimulus. In conclusion, Shank3B mice are characterized by global alterations in the excitability and in the FC of cortical excitatory neurons. Future investigations using optogenetic will allow dissecting the causal role of the altered functionality of specific cortical regions in the behavioral dysfunction of Shank3B mice.

EXPLORING THE ROLE OF RLF PROTEIN IN NEURODEVELOPMENTAL DISEASES THROUGH IPSC-BASED AND ORGANOID MODELS

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Re-arranged L-Myc Fusion (RLF) is a DNA binding protein found fused with L-MYC, impairing the function of the latter in cancer. Later, murine *Ifl* gene was identified in a ENU screening as an epigenetic modifier of a metastable epiallele, through keeping low DNA methylation, especially at distal regulatory regions. Altered levels of enhancer-specific mark H3K4me1 were also identified in *Rlf* mutants; recently, *Rlf* loss-of-function has also been associated with heart defects. Details on RLF functions and its characterization in human settings are still missing.

The objects of this project are newly identified *de novo RLF* mutations in patients presenting developmental delay, mental retardation, behavioral deficits.

We aim to generate two human isogenic iPSC lines carrying two of the new *RLF* mutations each (pGlu519Lysfs*10 and p.Val827*) by CRISPR/Cas9 editing. Since the mutations are predicted to have a dominant-negative effect, we propose to tag the resulting proteins with V5 peptide for downstream purposes. As controls, we will use the parental line, both (i) unmodified and (ii) modified to have V5-tagged WT *RLF* and (iii) the complete *RFL* KO.

We will exploit our iPSC models to evaluate the effect of RLF truncated proteins as well as complete LoF on neural derivatives, through the differentiation of neural progenitors, post-mitotic neurons and cerebral organoids. To investigate the molecular role of RLF and its disease-inducing variants in neurological context, we will perform genomic studies, including the profiling of DNA methylation (MeDIP-seq), RLF binding on the genome (ChIP-seq for V5), epigenetic marks (e.g. H3K4me1, ChIP-seq), and RNA-seq. Through our iPSC-based in vitro modelling, we foresee to identify those loci on the genome that are (i) sensitive to *RFL* LoF and/or mutations, (ii) important for transcription in neural cells, and (iii) at the basis of the related phenotypes. We expect to gain information on RLF involvement in proliferation/differentiation dynamics as well as on the neuronal properties (e.g. neuronal shape and activity). These data may provide hints on the role of RLF for the pathophysiology of the associated neurological diseases.

GENERATION OF A PATIENT SPECIFIC HIPSC-DERIVED NEURONAL MODEL FOR CONGENITAL CENTRAL HYPOVENTILATION SYNDROME (CCHS)

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Congenital Central Hypoventilation Syndrome (CCHS), is a rare neonatal disorder of the autonomic nervous system (ANS), characterized by a deficient control of autonomic ventilation and a global autonomic dysfunction. Indeed, the disease-defining gene, *PHOX2B,* encodes a master transcription factor whose role is essential during development of the neural lineages of the ANS.
Polyalanine repeat expansion mutations (PARMs), occurring within the sequence stretch coding for the 20-alanine tract in exon 3 of *PHOX2B*, have been identified in ~ 90% of patients affected by CCHS [1]. Importantly, *in vivo* and *in vitro* models of the disease that have been generated so far provide a limited representation of human pathophysiology. Therefore, the use of human-induced pluripotent stem cell (hiPSC) technology is essential to obtain patient-specific cell type models relevant to the disease that could be otherwise unobtainable.
To clarify the pathogenesis of CCHS, we have generated a hiPSC-derived autonomic neuronal model that fully recapitulates the patient's entire genetic profile. By using a non-integrating Sendai Virus (SeV), we reprogrammed fibroblasts from two CCHS patient's carrying the same genetic mutation but with different clinical manifestation of the disease. By means of a specific autonomic neural differentiation protocol, iPSC lines have been differentiated to neural crest stem cells (NCSCs), which bear the potential to develop into different lineages, among which PHOX2B positive peripheral autonomic neurons that carry the patient's specific mutation.
Here we show a complete characterization of both patient-derived iPSC lines [2] by karyotyping, morphology study, immunocytochemistry, and qPCR analysis to confirm the presence of gene and protein expression of markers of pluripotency (e.g., Nanog, Oct4 and SSEA4). Moreover, we derived PHOX2B+ sympathetic neurons from both patient-derived iPSC lines.
This new personalized disease-in-a-dish model of CCHS opens numerous possibilities to identify molecular and cellular defects induced by the mutations as well as modelling for drug discovery/screening for therapeutic perspectives.

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ZIKA VIRUS INDUCES FOXG1 NUCLEAR DISPLACEMENT AND DOWNREGULATION IN HUMAN NEURAL PROGENITORS

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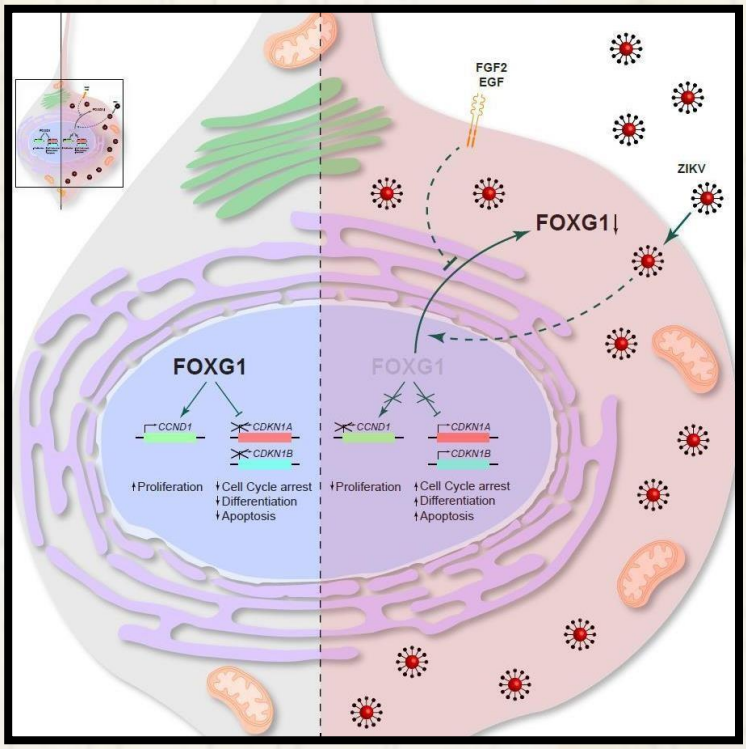
Congenital alterations in the levels of the transcription factor Forkhead box g1 (FOXG1) coding gene trigger "FOXG1 syndrome," a spectrum that recapitulates birth defects found in the "congenital Zika syndrome," such as microcephaly and other neurodevelopmental conditions. Here, we report that Zika virus (ZIKV) infection alters FOXG1 nuclear localization and causes its downregulation, thus impairing expression of genes involved in cell replication and apoptosis in several cell models, including human neural progenitor cells. Growth factors, such as EGF and FGF2, and Thr271 residue located in FOXG1 AKT domain, take part in the nuclear displacement and apoptosis protection, respectively. Finally, by progressive deletion of FOXG1 sequence, we identify the C-terminus and the residues 428-481 as critical domains. Collectively, our data suggest a causal mechanism by which ZIKV affects FOXG1, its target genes, cell cycle progression, and survival of human neural progenitors, thus contributing to microcephaly.



ADVANCING STEM CELL-BASED MODELING OF THE PROPRIOCEPTIVE NEURONAL CIRCUIT WITH DORSAL ROOT GANGLIA ORGANOIDS AND ITS IMPAIRMENT IN FRIEDREICH'S ATAXIA

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Friedreich's ataxia (FRDA) is an autosomal-recessive neurodegenerative and cardiac disorder which occurs when transcription of the FXN gene is silenced due to an excessive expansion of GAA repeats into its first intron. Herein, we generated dorsal root ganglia organoids (DRG organoids) by in vitro differentiation of human iPSCs from FRDA affected individuals and normal donor for an improved in vitro modeling of the FRDA pathology. Bulk and single-cell RNA sequencing showed that DRG organoids present a transcriptional signature similar to native DRGs and display the main peripheral sensory neuronal and glial cell subtypes. Furthermore, when a patterned iPSC-derived sensory neuronal circuitry was established by co-culture of DRG organoids with human intrafusal muscle fibers, sensory neurons contacted their peripheral targets and reconstituted the muscle spindle proprioceptive receptors. FRDA DRG organoids model some disease-specific deficits in survival and morphology, such as a severe impairment in axonal spreading and in mitochondria mass compared to controls, particularly affecting the proprioceptive neurons. The targeted excision by CRISPR/Cas9 of the GAA repeats with two different genomic deletions in patient iPSCs, strongly ameliorated the molecular and cellular disease phenotype in DRGOs. These results strongly suggest that removal of the repressed chromatin flanking the GAA tract might contribute to rescue FXN total expression and fully revert the pathological hallmarks of FRDA DRG neurons.
We showed that DRGO cultures can be coupled with microfluidic technologies to more finely organize and compartmentalize DRGO neuronal networks. In the future these microdevices will be exploited to spatially organize connections between DRGOs, spinal motor neurons and muscle cells to reconstitute more complex neuronal circuits by which studying functional properties of DRGO neurons after stimulation of the connected muscle spindles.

COMMUNAL NESTING ATTENUATES THE EFFECTS INDUCED BY EARLY LIFE SOCIAL STRESS ON SENSORIMOTOR GATING, REWARDING AND COMPULSIVE-LIKE BEHAVIORS IN MALE AND FEMALE RATS

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The Communal nesting (CN) paradigm is a form of pre-weaning environment enrichment in which three female mice are mated with the same male, gestate together, and rear their litters together until weaning. The Early Social Isolation (ESI) paradigm, conversely, is considered a valid adverse environmental manipulation, which consists in a 30-min period of social isolation from both mother and peers during the 3rd postnatal week. This study investigates whether early social enrichment/deprivation i) affects reward-related processing for natural reward, using self-administration of palatable food under a continuous (FR-1) and progressive (PR) schedules of reinforcement, ii) alters sensory motor gating abilities, using a pre-pulse inhibition of acoustic startle reflex test, and iii) induces obsessive-compulsive tendencies, using the marble-burying test. Moreover, since the early social environment has crucial effects not only on the offspring's behaviour but also the hormonal milieu of the adolescent and adult individual, and in particular on the physiological stress system of the Hypothalamic–Pituitary–Adrenal (HPA) axis, we investigated whether plasma corticosterone levels are affected by housing conditions and social isolation. Adolescent and adult rats of both sexes, reared at the different conditions (SH, CN, SH+ESI, CN+ESI), were used to detect potential sex-dependent and/or age-related differences in the role played by early life housing conditions in the abovementioned behaviours and hormonal levels. Results showed that adolescent and adult CN rats took longer to acquire operant behaviour and showed lower food intake than SH animals, with ESI leading to a steeper curve in adulthood. During the maintenance period of the training, CN led to a general decrease in active responding in adolescence, while ESI increased responding in adolescent and adult females, an effect that was reversed by CN in adult females. Under a PR protocol, non-stressed CN animals showed lower breakpoints than SH animals, and ESI increased the breakpoint in all groups, although to a greater extent in females than in males. Notably, ESI-induced effect was reverted by CN. In the marble burying test, differences were noted only in adolescent males, with an increase in the number of marbles buried in SH-ESI condition with respect to SH-CTRL corresponding group; again, the CN condition completely reverted the ESI-induced effect. Finally, ESI reduced the %PPI in adolescent SH male and female rats, an effect that in males was long-lasting and reverted by CN. Notably, the CN condition per sè was able to increase PPI in adolescent females but not in males with respect to corresponding SH groups. In adult animals, ESI lowered PPI performance in males only, an effect fully prevented by the CN condition, while had no effect on females. Finally, responsivity of the HPA axis appeared to vary noticeably depending on the sex and the age of the animals. The social isolation procedure used in the present study was not associated with significant alterations in corticosterone levels, suggesting that enhanced food self-administration in isolate rats as well as altered burying behaviour and sensorimotor gating abilities are unlikely to reflect enduring alterations in HPA axis activity. Independently from housing and stress conditions, females presented higher corticosterone levels than males, a finding in line with the well-established sexual dimorphism of rodent HPA axis, with higher plasma corticosterone levels consistently reported for female as compared with male rats.

Altogether, our findings indicate that early life social isolation and communal nesting have long-lasting effects on sensorimotor gating, reward-seeking, and compulsive-like behaviors, with males and females showing different vulnerabilities in these domains. Specifically, we demonstrated for the first time that a socially enriched condition exerts a “protective” effect toward early stress-induced reward-related responses, emotional changes and cognitive deficit. This study therefore (i) reveals a protective role of CN against early life social insults, (ii) supports the need of sex-tailored intervention strategies to face the behavioral, emotional and cognitive alterations induced by early-life social stress, and (iii) highlights the importance of translational investigations taking the social environment, development and sex into account. Patch clamp recordings in whole-cell configuration in dopamine neurons of the ventral tegmental area (VTA) are in progress to evaluate in all the experimental groups the potential modifications induced by the different social environmental conditions on their basal as well evoked firing and hyperpolarization-activated (Ih) currents. Analytical techniques such as High-Performance Liquid Chromatography (HPLC) on brain tissue homogenates will be used to determine dopamine and glutamate concentrations in selected brain areas, namely the VTA, nucleus accumbens, prefrontal cortex, dorsal and ventral hippocampus, hypothalamus and bed nucleus of the stria terminalis.

LONG-TERM EFFECT AFTER INTRAVENOUS SELF-ADMINISTRATION (IVSA) OF THE SYNTHETIC CANNABINOID RECEPTOR AGONIST 5F-MDMB-PICA IN ADOLESCENT MICE

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Synthetic cannabinoids (SC) are the largest group of new psychoactive substances monitored worldwide (EMCDDA, 2022). 5F-MDMB-PICA is a recently SC classified as a potent agonist of CB receptors able to activate the mesolimbic dopamine (DA) transmission in adolescent (0.01 mg/kg ip) but not adult mice (Musa et al, 2020). We investigated the reinforcing properties of 5F-MDMB-PICA in adolescent mice and characterized the behavioral effects induced in the same animals at adulthood. The response-study to varying doses showed an inverted U-shaped trend. Adolescent mice acquired operant behavior for 5F-MDMB-PICA at the dose of 2.5 (µg/kg/25ul). This dose was then used under different ratio of responding (FR1-3, PR), thus showing that the behavior was directed to obtain the SC. Behavioral differences were identified in relation to the amount of 5F-MDMB- PICA intake during adolescence. We observed that a total intake higher than 15 ug/kg (over 15 sessions) during adolescence induced a propensity for aggressive behavior and reduced social interaction at adulthood, whilst no differences were observed in the behavioral taste responsiveness to a rewarding stimulus (i.e. intraoral chocolate). Moreover preliminary data shown a delayed medial prefrontal cortex (mPFC) DA response to an olfactory stress and a tendency to opposite releasing patterns in the mPFC and nucleus accumbens DArgic transmission. This study provides the first evidence that 5F-MDMB-PICA IVSA is acquired and sustained by adolescent mice at lower doses than that of the prototypical SC JWH- 018, confirming an higher abuse liability of this newer SC. Moreover, 5F-MDMB-PICA IVSA during adolescence induced long-term behavioral changes and it's consumption during adolescence could cause a probable hypo frontality after chronic use, confirming the detrimental consequences related to the use of SC by adolescents.

EFFECTS OF ENVIRONMENTAL ENRICHMENT ON ALCOHOL SELF-ADMINISTRATION AND MESOLIMBIC DOPAMINERGIC NEURONAL ACTIVITY IN SARDINIAN ALCOHOL-PREFERRING RATS

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Background. Living in an enriched environment (EE) produces brain structural and functional effects that are associated with changes in behavior, including those motivated by drugs of abuse. However, while several studies support a protective effect of EE against addiction-related behaviors from psychostimulants, the picture appears more complex when referring to alcohol. To further disentangle the relationship between EE and alcohol-motivated behaviors, this study aimed to assess the effect of EE in a validated animal model of alcohol use disorder, the Sardinian alcohol-preferring (sP) rats. In particular, we investigated the influence of an EE exposure on i) operant oral alcohol self-administration and ii) functionality of the mesolimbic dopamine system, key in the mechanisms underlying alcohol consumption and dependence.

Methods. Starting from postnatal day 21 (PND 21), male sP rats were housed in 3 different conditions: impoverished environment (IE; single housing and no environmental enrichment), standard environment (SE; small colony and no environmental enrichment), and EE (large colony and multiple elements of environmental enrichment). From PND 60, rats were subjected to different phases of shaping and training of alcohol self-administration. IE, SE, and EE rats were then compared under (a) fixed ratio (FR) 4 (FR4) schedule of reinforcement for 20 daily sessions and (b) progressive ratio schedule of reinforcement in a final single session. In a separate set of experiments, extracellular electrophysiological recordings from ventral tegmental area (VTA) dopamine neurons were carried out in anesthetized rats (PND 60) belonging to the different experimental groups (IE, SE, and EE).

Results. We found that, compared to IE rats, EE rats required a higher number of shaping and training sessions to acquire the alcohol self-administration behavior; SE rats displaying intermediate values. A similar ranking order (IE>SE>EE) was also observed in number of lever-responses for alcohol, amount of self-administered alcohol, and breakpoint for alcohol under FR4 and PR schedules of reinforcement. In addition, analysis of the electrophysiological properties of VTA dopamine neurons revealed a higher number of cells/track, coupled with a higher mean spikes/burst, in EE rats compared to SE and IE rats (EE>SE>IE), thus suggesting that exposure to EE was able to increase both tonic and phasic mesolimbic dopaminergic neuronal activity.

Conclusions. Altogether our results indicate that living in enriched environments reduced the reinforcing and motivational properties of alcohol in sP rats. Importantly, the tendency toward an increased in mesolimbic dopamine neuron functionality might provide a mechanism for these behavioral responses.

ADOLESCENT JWH-018 SYNTHETIC CANNABINOID VAPING IN MALE RATS INCREASES THE AVERSIVE STATE AND WITHDRAWAL SYMPTOMS AFTER CESSATION AND DYSREGULATES DOPAMINE RESPONSIVENESS TO HEDONIC TASTE STIMULUS AT ADULTHOOD

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Since 2015, vaping synthetic cannabinoids (SC), commonly known as *Spice/K2* drugs, with electronic cigarette has been gaining popularity among adolescents worldwide. Differently from THC, the SC are able to induce more adverse reactions and psychiatric consequences, most likely due to their higher potency and affinity at cannabinoid (CB) receptors. Previous studies in adult male rodents showed that the prototypical SC, JWH-018, has cannabinoid CB1 receptor-dependent reinforcing properties and acutely increases dopamine (DA) transmission selectively in the NAc shell, with a lower dose than THC (De Luca et al., 2015). Recently, we demonstrated that repeated JWH-018 exposure in adult male rats alters DA transmission and its responsiveness to motivational stimuli, and induces abnormalities of emotional state and withdrawal signs together with glial alterations (Pintori et al., 2021). Despite the growing use of *Spice/K2* drugs, there is a lack of data concerning the consequences of repeated SC exposure by vaping during a critical neurodevelopmental period such as adolescence.

In the present study, adolescent male SD rats were exposed once a day for 21 consecutive days (from PND 35 to PND 55) to JWH-018 (0.3 or 0.6 mg/ml, 30-min session by LJARI vapour chamber) or vehicle vapour. During vaping period, at different time-points (after 1st, 7th, 14th, 21st vaping session) rats weight gain and locomotor activity were assessed in order to evaluate animal welfare and possible motor impairments induced by JWH-018 inhalation. In addition, 24 hours after last JWH-018 vaping session, spontaneous somatic withdrawal signs and anxiety-like (Eleveted Plus Maze, EPM) and repetitive-like behaviors (Marble Burying, MB) were scored in order to evaluate alterations of emotional state and acute withdrawal symptoms. At adulthood, in order to evaluate a possible encoding modification of the motivational value of stimuli, rats were repeatedly exposed to a natural rewarding stimulus (i.e. intraoral chocolate) and the pattern of DA responsiveness was estimated by in vivo brain microdialysis in the NAc shell and in the mPFC.

Our preliminary results showed that JWH-018 inhalation alters the growth curve and the locomotor activity (i.e. ambulatory and rearing activity) of adolescent rats during the first 30 minutes of test. Moreover, 24 hours after the last JWH-018 vaping session, JWH-018 treated rats exhibited spontaneous sign of withdrawal (e.g., facial rubbing, licking, forepaw fluttering and chewing) and increased anxiety-like and repetitive-like behaviors as revealed by a decrease of time spent in the open arms of the EPM and by the higher number of marbles buried in the MB test, respectively. Moreover, at adulthood, adolescent JWH-018 vaping induced dysregulation of DA responsiveness to repeated chocolate exposure.

These results showed that adolescence JWH-018 inhalation induces behavioral abnormalities suggesting the risk associated with recurring vapes of Spice/K2 drugs during a critical neurodevelopment period.

IRON-FED MICROGLIA: AN IN VITRO SYSTEM TO MODEL MICROGLIAL PHENOTYPE IN VITRO AND TEST NEW THERAPY IN NEURODEGENERATIVE DISEASES?

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Neuronal survival and function are highly dependent on microglia, the brain immune cells. In several neurodegenerative diseases microglia display a Disease Associated Microglia (DAM) signature, that may initially protect neurons, but then lose their homeostatic properties, contributing to neuronal loss. Microglia normally lose their protective function during senescence; senescent microglia exhibit cell cycle arrest, impaired metabolism, a Senescence-Associated Secretory Phenotype (SASP) and deficits in phagocytosis. Recently, a subcluster of late-stage DAM has been reported to display increased ferritin and iron content in the human brain. Despite the transcriptional profile of microglia accumulating iron have been defined, whether these cells are senescent and how they impact the brain environment is unknown. Aim of this study was to develop an in vitro model of iron-loaded microglia and to study their function. We explored whether murine microglia chronically (30 days) exposed in vitro to high iron concentration (500 µM) become senescent. Iron-fed microglia were de-ramified and acquired a senescent-like phenotype, characterized by proliferation arrest, decreased phagocytosis, increased Senescence Associated ß-Galactosidase activity and upregulation of SASP markers, p16 and RPL32, a ribosomal protein overexpressed in human microglia accumulating iron. Biochemical and immunofluorescence analyses showed a decrease in Nicotinamide Adenine Dinucleotide (NAD) content and in the expression of NAD dependent deacetylases Sirtuins 1 and 6, which are downregulated in aged/senescent cells. In preliminary experiments we analysed the impact on cultured neurons of iron-fed microglia, finding that their secretome is neurotoxic. By next-generation sequencing we are currently investigating whether iron-loaded microglia may recapitulate the transcriptional changes of human DAM accumulating iron, thus offering a useful model to study and modulate the function of senescent microglia.

FENOFIBRATE MITIGATES INFLAMMATION-INDUCED FUNCTIONAL ALTERATIONS IN THE MEDIAL PREFRONTAL CORTEX IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

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Background

Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder. In addition to amyloid beta (Aβ) peptides and neurofibrillary tangles formation, neuroinflammatory processes are attracting interest in the etiopathogenesis and progression of AD[1]. We hypothesized that an inflammatory insult might worsen disease progression in a validated mouse model of AD, the 3xtg-AD mice[2], assessed by electrophysiological recordings from medial prefrontal cortex (mPFC) neurons. Secondarily, we evaluated whether activation of nuclear ligand-regulated receptors (PPARs) would mitigate the effects of the inflammatory challenge. PPARs are promising targets to modulate inflammation in the CNS[3]. Hence, among the members of the PPAR family, PPAR-α are abundantly expressed in the brain[4] and act by modulating gene expression to attenuate neuroinflammation[5]. Thus, we investigated whether inflammation altered the mPFC neuronal electrical activity in 3xtg-AD mice and evaluated the potential effects of PPAR-α agonist fenofibrate on the modulation of inflammation-induced effects.

Methods

In 4-months old female 3xtg-AD mice (APPswe, Tau_{P301L} and $PS1_{M146V}^{+/-}$), we induced inflammation by a single injection of polyriboinosinic-polyribocytidylic acid (Poly I:C), a synthetic double-stranded RNA, that triggers an innate immune response. Starting 7 days before treatment, and for 19 total days, mice were administered a diet enriched with the PPAR-α agonist fenofibrate (0.2% w/w). At 15 months, we performed *in vivo* single-unit recordings in anesthetized mice in the mPFC.

Results

Putative mPFC pyramidal neurons from Poly I:C-treated mice showed a reduced firing rate as compared with the vehicle-treated group. Interestingly, treatment with fenofibrate reverted this effect (two-way ANOVA, $F_{(1,54)}$=10.32; p=0.0022). No differences were detected in firing pattern, expressed as coefficient of variation. Moreover, Poly I:C-treated mice displayed a higher number of spontaneously active cells, an effect that was mitigated by fenofibrate (two-way ANOVA, $F_{(1,6)}$=6.45; p=0.0441).
No difference was detected in the firing activity or the number of putative GABAergic neurons following Poly I:C treatment.

Conclusions

Our results show that inflammation induced an alteration of the electrical activity in the mPFC of 3xtg-AD mice. Moreover, our preliminary data support modulation of PPAR-α as a possible pharmacological approach and suggest fenofibrate as potentially effective in preventing functional impairments.

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CHARACTERIZATION OF NASCO GRAPE POMACE-LOADED NUTRIOSOMES AND THEIR ANTI-INFLAMMATORY EFFECTS IN THE MPTP MOUSE MODEL OF PARKINSON'S DISEASE

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Grape pomaces, a waste by-product of wine production, have received great attention for their richness in polyphenols, compounds known to exert anti-inflammatory and antioxidant effects in animal models of neurodegenerative diseases. These pomaces, however, have low brain bioavailability upon oral administration due to their extensive degradation in the gastrointestinal tract. To overcome the above-mentioned problems, Nasco pomace extract was incorporated into a novel nanovesicle system, nutriosomes, composed of phospholipid (S75) and maltodextrin (Nutriose® FM06) and their biocompatibility was assessed on intestinal epithelial cells (Caco-2). Of note, the anti-inflammatory effect of Nasco pomaces, using Nasco nutriosomes or Nasco suspension, was investigated in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of Parkinson's Disease (PD). Nasco nutriosomes or Nasco suspension were administered intragastrically and their anti-inflammatory effects were evaluated by immunohistochemical evaluation of glial fibrillary acidic protein (GFAP), a marker of astroglia and ionized calcium-binding adaptor molecule 1 (IBA1), a marker of microglia in the both caudate- putamen (CPu) and substantia nigra pars compacta (SNc). Additionally, we co-localized the pro- inflammatory interleukin, IL-1β with IBA1, to gain more understanding about the microglia phenotype. The obtained nutriosomes were highly biocompatible towards Caco-2 cells. Nasco pomaces extract resulted rich in polyphenols, i.e., gallic acid, (+)-catechin, (-)-epicatechin, procyanidin-B2, and quercetin. Immunohistochemical analysis revealed that Nasco nutriosomes but not Nasco suspension significantly contrasted the MPTP-induced astrogliosis both in the CPu and SNc, and the microgliosis in the CPu. Additionally, administration of Nasco nutriosome effectively reduced the production of IL-1β in the IBA1- positive cells. Taken together, these results highlight the promising therapeutic effects of Nasco pomace extracts when administered in a nutriosome formulation in the MPTP-mouse model of PD and validate the effectiveness of this nutriosome preparation over suspension as an innovative nano-drug delivery system.

INVESTIGATING THE MOLECULAR DIVERSITY OF COPII-DEPENDENT TRANSPORT IN CORTICAL NEURONS

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Neurons are the most highly compartmentalized and morphologically complex cells of the body. In particular, synapses are dynamic nanomachines composed by a unique repertoire of molecules arranged in multimolecular complexes. For this reason, their correct development and function require sophisticated mechanisms to target proteins and lipids to their site of action in the right amount at the right time. However, how this daunting task is achieved is largely unknown. Here, we focus on the role of the first stations of the secretory pathway, namely the Endoplasmic Reticulum (ER) and the Golgi Apparatus (GA), in the transport of newly synthetized neuronal proteins to their final destination. In particular, we investigate the role of the molecular diversity of SEC24, a component of the inner coat of COPII vesicles involved in cargo selection. To this aim, we developed a proteomic screen based on proximity-dependent biotinylation to identify the protein interaction networks of distinct SEC24 isoforms. Preliminary data obtained from heterologous cells and primary cultures of cortical neurons indicate that SEC24 proteins fused to the biotin ligase are enriched at the ER exit sites, interact with protein cargoes and, upon biotin administration, efficiently biotinylate proteins in an isoform-specific manner. Second, we are also investigating the subcellular localization of SEC24 isoforms in neuronal compartments to assess their potential role in directing cargoes to specific transport pathways. Together, our approaches may shed the light on the contribution of the early secretory pathway to neurodevelopment and unravel novel mechanisms underlying the formation and function of synaptic connections.

MICROGLIA-DERIVED EXTRACELLULAR VESICLES ARE INVOLVED IN SYNAPTIC PRUNING IN VITRO

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During development, microglia is responsible for beneficial synaptic pruning by phagocytosis of aberrant synapses tagged by complement factors. Moreover, excessive complement-mediated synaptic pruning is activated during neurodegeneration, causing pathological loss of synapses. However, how complement factors are delivered to synapses is not yet completely clear.
Extracellular Vesicles (EVs) released by microglia carry multiple signals implicated in synaptic pruning and scan the neuron surface before establishing a stable contact with dendrites, thus representing ideal vehicles to tag synapses with molecules guiding microglial removal.
To investigate the role of microglial EVs in synaptic pruning we cocultured mature hippocampal neurons with wild type (wt) microglia or C9orf72 knock out (ko) microglia, which produce a double amount of EVs and more complement factors (C1q/C3) associated to EVs compared to wt cells.
While wt microglia induced a decrease in the density of Shank-2-positive (postsynaptic) but not Bassoon (presynaptic) puncta, C9orf72 ko microglia reduced the density of both Bassoon and Shank-2-positive puncta in neurons. Parallel quantification of synaptic puncta in wt and C9orf72 ko microglia revealed increased uptake of pre-synaptic markers in C9orf72 ko microglia compared to wt cells.
Importantly, pretreatment of C9orf72 ko microglia with GW4869, an inhibitor of EV biogenesis that reduces EV production by 50%, restored normal pre-synaptic density in microglia-neuron cocultures, while addition of microglial EVs to neurons before co-culturing with wt microglia induced a selective decrease in Bassoon-positive puncta, leaving the density of post-synaptic puncta unchanged.
Our results indicate that microglial EVs promote *pre-synapses* engulfment, thus possibly influencing the synaptic density during developmental critical periods as well in brain pathologies.
Analysis of synaptic density in hippocampi of C9orf72 are ongoing to validate *in vivo* microglial EVs-mediated pre-synaptic pruning.

ROLE OF PENTRAXIN 3 IN NEURODEVELOPMENTAL DISORDERS

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Control of synapse number and function is critical to the formation of neural circuits. We recently demonstrated that the innate immune molecule, pentraxin3 (PTX3), released by astrocytes, promotes the formation of functional excitatory synapses. PTX3 interacts with thrombospondin1 (TSP1), an astrocyte-derived factor which controls the formation of silent synapses. PTX3:TSP1 interaction exerts a negative regulation of PTX3 itself suggesting that the relative amount of these proteins and their complex are crucial to set the balance between synaptic growth and synapse maturation.

Maternal immune activation (MIA) and prenatal inflammation are recognized risk factors for neurodevelopmental diseases (NDD). Preclinical studies established a causal link between MIA and the disruption of the proper neurodevelopmental trajectory in the offspring.

Elucidating whether PTX3, which is strongly stimulated by inflammatory insults, might be involved in this process is the objective of our research.

Our data indicate that prenatal inflammation affects the developmental pattern of expression of PTX3 in the brain resulting in a significant alteration of the PTX3:TSP1 ratio in the cerebral cortex during the postnatal period of synaptogenesis. The involvement of glial cells in this process is investigated by using both the mouse model and iPSC derived-glial cells.

IMPLEMENTING NOVEL GENE THERAPY STRATEGIES FOR RETT SYNDROME

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Rett syndrome (RTT) is a severe neurodevelopmental disorder caused by loss-of-function mutations in the gene encoding for methyl-CpG binding-protein 2 (MeCP2). RTT patients suffer from many motor and psychiatric symptoms and there are no cures available to treat this terrible disorder. In this regards, the main therapeutic opportunity is the gene therapy, providing a functional copy of the gene through viral administration. Several groups, including ours, have already demonstrated that this approach can revert most of the pathological symptoms of RTT mice. However, these promising strategies need to be further optimized in order to increase their safety and efficiency. Indeed, *MECP2* gene regulation is crucial for brain homeostasis, since also its overexpression can lead to dramatic neurological alterations. Moreover, the expression profile of MeCP2 in the brain is highly heterogeneous with neurons presenting much higher MeCP2 levels than glia. To overcome these limitations that have been ignored by the previous gene therapy strategies, we developed two different approaches. On the one hand, we have been optimizing our original gene therapy transgene cassette (iMecp2) previously published (Luoni et al., 2020) to better regulate *Mecp2* expression in the brain. In sum, we introduced within the 3' UTR domain four miRNA binding sites for a miRNA exclusively expressed in astrocytes in order to downregulate the exogenous transgene expression exclusively in these cells. With this strategy, we confirmed by immunofluorescence the difference in Mecp2 abundance between neurons and astrocytes, that better recapitulated its endogenous profile. Currently, we are testing this novel cassette (herein called iMecp2 2.0) in RTT mice, in order to evaluate the efficiency and safety in comparison with the original version.
On the other side, we have been optimizing a novel CRISPR-based strategy aiming to correct the mutant *Mecp2* gene. In brief, we exploited the Adenine Base Editors (ABEs) technology to revert the RTT-causing mutation R106W (G to A transition) in order to restore *Mecp2* expression under the control of the endogenous regulative elements. To date, we have already demonstrated that the correction of the mutant gene is feasible in *Mecp2*^{R106W} mouse primary neuronal cultures achieving physiological levels of Mecp2 after ABE treatment.
In summary, both of these approaches are promising in order to improve the safety and specificity of RTT gene therapy helping to fill the gap between the research studies and the clinical application.

AN ENGINEERED EPIGENETIC SILENCER FACTOR REPRESSES THE GLIOBLASTOMA GENETIC PROGRAM AND RESTRAINS TUMOR DEVELOPMENT

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Glioblastoma multiforme (GBM) is the most common and lethal primary brain cancer with around 250,000 patients diagnosed every year. Despite the current research advances in the field and the multimodal treatment available, GBM remains largely incurable due to its aggressiveness and the limited efficacy of therapies. Evidences indicate that after resection of the primary tumor, the remaining cancer stem cells (CSCs) with tumor-initiating potential infiltrate the parenchymal tissue and become resistant to adjuvant treatments, supporting recurrence of the disease. Many efforts are focused on inactivating genes fundamental for CSCs survival, however the silencing of a single oncogene can be easily bypassed through rearrangements of their genetic program. Therefore, we believe that by silencing the entire transcriptional network of a gene involved in GBM progression, rather than a single transcription factor, would suggest a more efficient approach to treat these brain tumors.

In order to do so, by rational engineering of the transcription factor SOX2, a key promoter of GBM malignancy, we generated a synthetic epigenetic repressor of SOX2 (named SES). Our transcription factor together with KRAB and DNMT3A/L catalytic domains has the ability to bind and permanently switch off the downstream oncogenic gene network by inducing stable epigenetic modifications. Therefore, leading to an inhibition of proliferation in both glioma cell lines and patient-derived CSCs in vitro and in vivo. In fact, by RNA-seq and ATAC-seq we have seen that SES-treated glioma cells exhibit massive transcriptional changes with significant upregulation of apoptosis-related genes and silencing of genes encoding proliferative and cancer-promoting factors. Moreover, SOX2 gene targets were found epigenetically downregulated with increased methylation of DNA as inferred by CHIP-seq and MEDIP-seq. In overall, SES expression through local viral delivery in mouse xenograft have demonstrated a strong regression of human tumors and significant survival rescue. In addition, SES is not harmful to neurons and glia, thanks to a minimal promoter that restricts its expression in mitotically active cells, rarely present in the brain parenchyma. Collectively, these findings indicate that SES is capable of a significant silencing of a large fraction of the SOX2 transcriptional network, achieving high levels of efficacy in repressing aggressive brain tumors. Moreover, this approach could be applied to several more tumor-involved transcription factors, combining their core DNA binding domain with the epigenetic repressor domains. In fact, we generated epigenetic repressors with selective and efficient repression of the TEAD and MYC transcriptional pathways. This could suggest an attractive system to be used not only in aggressive brain tumors but as therapy for many others. However, even though we have extensive data on human GBM cell lines, patient derived cancer stem cells and their engraftment in immunosuppressed animals, no data have been so far collected on the immune system behavior. Therefore, a new project is being developed to assess the contribution of the immune system in our proposed treatment. To do so, a murine immunocompetent model of GBM is being generated to test our approach based on synthetic factors on both the tumor growth and the immune environment.

CONCOMITANT INHIBITION OF α2 AND D2 RECEPTOR POTENTIATES THE DOPAMINE SYSTEM: IMPLICATIONS FOR PHARMACOTHERAPY IN SCHIZOPHRENIA

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Impairments in catecholamine systems, i.e. the noradrenergic Locus Coeruleus (LC) and the dopaminergic Ventral Tegmental Area (VTA), have been associated with neuropsychiatric conditions, such as schizophrenia. Accordingly, combined antagonism of dopamine receptor type 2 (D2) and noradrenergic alpha 2 receptor (α2-adrenoceptor) has proven potentiation of the antipsychotic effect as compared with first generation drugs, which solely address D2 (Lehman, Lieberman et al. 2004). Advantageous effect of the add-on pharmacological strategy has been attributed to possible rescue of typical dopamine hypoactivity in the prefrontal cortex (PFC) (Masana, Bortolozzi et al. 2011). Despite clinical evidence, molecular mechanisms underlying the LC-VTA-PFC circuit and mutual regulation of neurotransmitter release are currently under debate (Hecht and Landy 2012, Vollbrecht, 2010). Here we investigated the mechanisms by which α2-adrenoceptor antagonism elicits enhancement of dopamine output in the PFC.

In vivo single unit recordings were performed from LC and VTA of anesthetized male Sprague Dawley rats. The selective α2 antagonist atipamezole (0.25 or 0.50 mg/kg in LC and in VTA, respectively), the α1 antagonist prazosin (1 mg/kg) and the D2-antagonist raclopride (0.025 mg/kg) were administered intravenously (i.v.). Brain microdialysis was performed in the mPFC of freely moving rats following intraperitonal (i.p.) atipamezole (3 mg/kg) or raclopride (0.5 mg/kg), or both drugs.

The selective inhibition of α2-adrenoceptor by atipamezole increased the firing rate of noradrenergic neurons in the LC and, consistently, extracellular noradrenaline in the mPFC (by 140%). In the VTA, atipamezole-induced α2 inhibition did not increase dopamine neuron firing activity, as it would be expected by α1-adrenoceptor activation following enhanced noradrenaline. This result could be ascribed to co-release of dopamine from noradrenergic terminals, which is confirmed by increased dopamine levels in the mPFC (by 160%), in turn reducing dopamine cell depolarization through D2 autoreceptor activation. Accordingly, we found that selective α2 antagonism markedly increased both the stimulation of dopamine neuron by D2 inhibition, and the extracellular output of noradrenaline and dopamine in the mPFC (by 220% and 240%, respectively). We hypothesized that in this condition noradrenaline stimulates α1-adrenoceptor to increase dopamine firing. Indeed, α1 blockade by prazosin reversed the potentiation induced by combined α2/D2 inhibition.

Our results help elucidate the mechanisms underlying pharmacotherapy based on cathecolamine modulation in several neuropsychiatric disorders, and in particular the beneficial effect of combined D2 and α2 antagonism to counteract mesocorticolimbic dopamine imbalance in schizophrenia.

HYPERPHOSPHORYLATION CDK1 DEPENDENT MODULATES RIP3 DURING MITOSIS IN HUMAN CELLS

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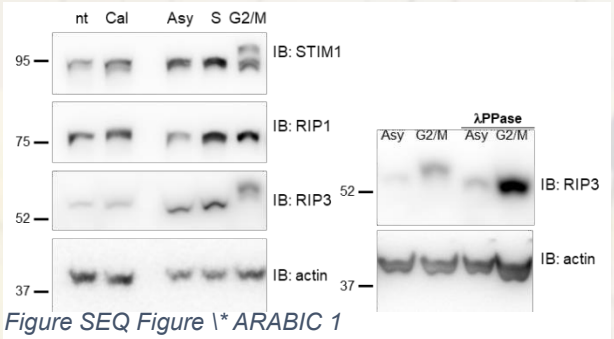
Deregulation of the balance between cell death and survival is a causal event in a number of human diseases from cancer (reduced cell death) to neurodegenerative disorders and HF (increased cell death). The old dichotomy of programmed (apoptosis) *versus* non-regulated (necrosis), was challenged by the discovery of necroptosis, a novel type of regulated cell death that has necrosis-like morphological characteristics but depends by a specific molecular machinery. Necroptosis is closely associated with the pathogenesis of different kinds of neurodegenerative disease Alzheimer's and PD. A crucial effector of necroptosis is the Receptor Interacting Protein Kinase 3 (RIP3); activation of RIP3 can lead to cell death, however several studies have attributed to this protein additional functions showcasing its multifunctional nature and raising the question of how RIP3 can be efficiently and safely exploited by the cells.



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In this study, we found that RIP3 display a mitosis-specific slow migration pattern on SDS-gels in several human cell lines. Deeper analyses in HT29 cells further indicated that the slow migration pattern of RIP3 is due to phosphorylation, as it was abolished after treatment with λ-phosphatase [*Figure 1*]. In a time course experiment prometaphase arrested nocodazole-treated cells showed a strong shift in RIP3 migration that disappeared 5h post-release, while the slow migration band returned to a fast migration pattern 2h post-release, suggesting that RIP3 phosphorylation occurs before prometaphase and terminates before cells exit mitosis. To make sure that the hyperphosphorylation of RIP3 was specific for G2/M arrest, we tested the effect of other drugs that are known to synchronize cells in G2/M, showing that all treatments induced a comparable slow pattern of migration.

Interestingly, the slow migrating band of RIP3 was observed in different tested human cell lines but not in mouse cells, neither in human cells overexpressing the mouse protein, indicating that RIP3 post-translational modification is ubiquitous and independent of human cell origin, but does not occur in murine cells. Furthermore, we show that RIP3 was phosphorylated on several amino acids residues, localized as clusters in the C-terminal by the cyclin dependent kinase 1 (Cdk1), an enzyme known to play crucial roles during mitosis. The electrophoretic shift was not caused by phosphorylation from any of the other known kinases, as inhibition of the corresponding kinase had no effect on the upper band. Introduction of phospho-mimetic or phospho-null mutations in RIP3 did not impair its RIP1-binding capacity or cell proliferation, suggesting that RIP3 phosphorylation in mitosis is not likely to be crucial for cell cycle progression.

Taking together, our results highlight for the first time the fact that the necroptosis modulator is finely regulated in a cell-stage-dependant manner, nevertheless the meaning of this post-translational modification is still elusive.

IN VIVO CHARACTERIZATION OF INTRACORTICAL CONNECTIVITY IN A MOUSE MODEL FOR STROKE

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Stroke is a neurological injury caused by the occlusion or rupture of cerebral blood vessels, and represents one of the major causes of adult disability worldwide. During a "critical period" after stroke, neural circuits in the peri-infarct zone undergo plastic changes to allow a spontaneous restoration of the neurological function. However, this recovery is highly variable among patients and only partially depends on structural and functional factors related to the damage, such as the lesion location and volume and the topology of the neural connectivity within the peri-infarct zone. Great is the need for a deeper understanding of the plasticity mechanisms allowing the identification of novel biomarkers that could represent predictive signals of recovery. Therefore, we propose to investigate the plastic rearrangements underlying post-stroke impairment and recovery.

To address this aim, we used a well-established mouse model of stroke (middle cerebral artery occlusion, MCAo) and investigated the related motor impairments, at both behavioral and functional level. To define the degree of motor deficits, we performed the commonly used Gridwalk, rotarod and grip strength tests at different days (D) after surgery (D2, D9, D16, D23 and D30) in MCAo- and sham-mice. We found that only the Gridwalk test was sensitive to unveil a statistically significant difference between the two groups, showing a higher motor impairment in MCAo-mice compared to controls. To validate the neuronal changes of inter- and intra-hemispheric connectivity post-stroke, we performed in vivo electrophysiological recordings of local field potentials (LFPs) from the peri-infarct zone, i.e. the forelimb primary motor cortex (caudal forelimb area, CFA), in the subacute (D9) and chronic (D30) phases of stroke. In particular, to investigate both the spontaneous and the evoked activities, electrophysiological measurements were carried out in Thy1-ChR2 transgenic mice expressing the channelrhodopsin ChR2 mainly in layer V corticospinal neurons. The analysis of the electrophysiological data, while optogenetically stimulating the ipsi- or contralateral hemisphere, will be needed to reveal the expected imbalance of the neuronal connectivity in MCAo-mice compared to controls. Moreover, to follow the neuronal reorganization from the onset of the stroke to the chronic phase, we performed preliminary single-photon calcium imaging experiments in freely moving mice (i.e. during the behavioral motor tests), from neurons expressing the genetically encoded calcium indicator GCaMP6f in the peri-infarct area.

Further experiments, together with the evaluation of the anatomical assessment of the volume of the lesion correlated to each of the MCAo-animals, will be essential for a deeper understanding of the high variability in motor-recovery outcome after stroke.

THE PERCEPTION OF EMOTIONAL BODY POSTURES IS INFLUENCED BY THE DYNAMIC EXPERIENCE OF THE SURROUNDING ENVIRONMENT: EVIDENCE FROM AN EYE-TRACKING STUDY IN VIRTUAL REALITY

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To comprehend other's affective states our judgement relies on body cues. Several studies demonstrated that the context influence the way we perceive emotional body posture [1]. Considering the significant amount of time and social interactions taking place within the built environment, we investigate how enclosed virtual environments influence the affective judgement and oculomotor patterns related to the observation of emotional body postures. Hence, we designed an adaption aftereffect paradigm in virtual reality to demonstrate that the dynamic experience of arousing environment influences the eye gaze pattern during the observation of avatar's body postures, and the subsequent judgment of perceived arousal.

Virtual reality was used to provide an immersive and dynamic experience of the environments. These were conceived as a progressive manipulation of architectural Forms (Low, High arousing) to generate low and high arousing states [2], and provided with two colors (Cold, Warm). Virtual avatars assumed different body postures to convey three possible levels of arousal (Low, Middle, High) [3]. Thirty-two participants experienced a virtual promenade within the architectures (adapting stimulus) and were asked to rate the arousal level of the avatar body posture (target stimulus) using a visual analogue scale. Subjective ratings were analyzed by means of repeated measure ANOVA, with within factors Avatar, Form and Color. Fixation durations were analyzed over 4 ROIs (head, trunk, arms, and legs) by means of a rmANOVA with within factors ROI, Form and Avatar. Furthermore, a cluster-based analysis (Montecarlo method) was performed to compare the spatial distribution and time course of eye fixation across experimental conditions.

Considering the arousal subjective ratings, the main effect Form was significant ($F(1,28) = 5.864$, $p = .022$), revealing that avatar's bodily arousal was judged as high arousing after the dynamic experience of low arousing environment. The main effect Form on fixation durations revealed that participants spent significantly more time looking at the avatar's body in the low arousing architecture ($F(1,27) = 4.170$, $p = .038$). Specifically, within this architecture, the cluster-based analysis showed that participants spent more time on the avatar's head ($p = .002$) between 600 and 1300 ms from the avatar appearance.

Overall, this study demonstrates that the arousal level generated by the dynamic experience of different environment induced different gaze patterns during the observation of the avatar's body posture, biasing the subsequent judgement of perceived arousal. These findings show that the environment influences the way we perceive the other's affective states, hence possibly affecting social interactions. This increasing knowledge will contribute to foster a design approach considering the individual's affective states as fundamental for the creation of new environments matching the changing needs of the human being.

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MAPPING PERCEPTUAL AWARENESS WITH MULTIPLE SENSORY STIMULATIONS, NO-REPORT PARADIGM AND INTRACEREBRAL RECORDING IN HUMANS

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BACKGROUND. What are the neuronal correlates of conscious perception (NCCP)? This is one of the most debated questions in the neuroscience of consciousness, bearing theoretical, empirical and clinical implications. According to the original definition by Crick and Koch[1] - the neural correlates of consciousness are "*the minimal neural mechanisms that are together necessary and sufficient for experiencing any conscious percept*". Typically, this question is addressed by a contrastive approach whereby the neural activity evoked by unperceived stimuli is subtracted from that evoked by sensory stimuli that are reported by as consciously perceived. However, such contrastive approach based on subjective reports comes with the price of the potential confound of additional cortical activations related to post-perceptual processes[2,3].

AIM: Here we propose an alternative approach that allows identifying the set of cortical areas that are necessary and sufficient (albeit not minimal) for the conscious perception of different stimuli. This novel strategy involves multiple sensory stimulation in a no-report condition with an intensity that is well beyond the perceptual threshold, combined with intracerebral recordings in a large population, covering all cortical areas.

METHODS. In 120 epileptic patients implanted for presurgical evaluation we separately delivered (1) auditory stimulation, by playing clicks via headphones at 85dB, (2) somatosensory stimulation, by administering median nerve electrical stimulation test at motor threshold (3-6mA), and
(3) visual stimulation, by providing light flashes with wearable goggles (3cd/m2). For each stimulation modality and for all the patients we collected the intracerebral evoked potentials and we identified the intracerebral leads showing significant broadband Event Related Potentials (ERP) and gamma power (50- to 150-Hz) increases - with respect to baseline (t-test, $p<0.001$, Bonferroni corrected). We then computed the topographical representation across subjects of gamma power and ERP responsiveness (in % of responsive leads) and their differences, for both hemispheres and all three modalities. Finally, we measured the amplitude and the latency of Local Field Potentials (LFPs) of all the responding leads (both gamma and ERP alone) and their connectivity with the other implanted areas, using Cortico Cortical Evoked Potentials performed in the same patients during the presurgical evaluation.

RESULTS.
1) Gamma power responsiveness maps clearly showed well-defined clusters of activation that did not overlap - with a few exceptions - and were mainly limited to occipital, temporal and parietal lobes. Conversely, ERPs were more widespread with respect to gamma activations, percolating within areas pertaining to cingulate and frontal cortices.
2) The areas showing significant gamma power modulations were associated with LFPs that were higher in amplitude and more prolonged with respect to those recoded in the areas showing significant ERP alone (i.e. without underpinning significant gamma modulation)
3) Areas showing ERP alone were monosinaptically connected with those responding to sensory stimuli with significant gamma power modulations, as assessed by CCEP.

CONCLUSIONS. Neuronal activity (high intracranial gamma[4]) associated to the processing of sensory stimuli that are clearly perceived but not task relevant do not converge into a unique "hub"; rather they are limited and segregated to local circuits mainly pertaining to the posterior portion of the brain. The neuronal activity generated by the peripheral stimuli in these areas induces - through feed- forward monosynaptic connections - local post-synaptic potentials in the distant areas, including cingulate and frontal areas. These results are in line with information integration theory and with recurrent processing theory claiming that the neural correlates of conscious perception are located in more posterior, sensory regions of the brain[5]. Importantly, our results also suggest that intracerebral recording is important to distinguish between "real" NCCP and their echoes resonating in distant areas, possibly preparing the brain for the consequences of conscious processing of sensory stimuli.

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