X NEAPOLITAN BRAIN GROUP MEETING

BOOK OF ABSTRACTS

Comitato Organizzatore del Meeting:
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Neapolitan Brain Group

The NBG was born in 2015 from an idea of Professor Ennio del Giudice (University of Naples Federico II) to foster the interaction between basic and clinical researchers in the Neapolitan area and in Campania, interested in the study of physiology and pathologies of the nervous system.
The group wants to be an opportunity to meet, in an informal atmosphere, all fans of clinical and basic research in the field of Neuroscience who intend to improve mutual knowledge and, as far as possible, explore moments of fruitful collaboration with the purpose to create a cultural network among the Research centers and Universities located in Campania dedicated to the study of the physiology and pathology of the nervous system. The NBG is under the patronage of the University of Naples Federico II.
Recently the NBG has also established itself as a non-profit association with the aim of improving its cultural offer. The group is open to all those who are interested in the basic and translational themes of neuroscience and, in particular, to young people from the different Universities and Research Institutes in Campania.
Until today the NBG held the following meetings:

- 1st Meeting on 6/4/2015 at the Department of Biotechnology, University of Naples Federico II.
- 2nd Meeting on 2/4/2016 at the Department of Biotechnology, University of Naples Federico II.
- 3rd Meeting on 28/4/2016 at Department of Biotechnology, University of Naples Federico II.
- 4th Meeting "Molecular, physiopathological and clinical mechanisms in neuroprotection of neonatal hypoxia", on 9/6/2016 at the Department of Biotechnology, University of Naples Federico II.
- 5th Meeting on 15/12/2016 at CEINGE Naples.
- 6th Meeting on 12/14/2017 at Stazione Zoologica Anton Dohrn, Naples.
- 7th Meeting with ECM Course on "The diseases of the nervous system: pathogenetic bases and new therapeutic approaches", 31/5/2018 at CESTEV, University of Naples Federico II.
- 8th Meeting on 12/13/2018, at CESTEV, University of Naples Federico II.
- 9th Meeting on 12/12/2019 organized jointly with Tigem, at Tigem (Pozzuoli).
- 10th Meeting on 15/12/2022 held at at CESTEV, University of Naples Federico II.

At those meetings, more than 200 participants, including basic researchers, doctors, doctoral students, post-docs, post-graduates, trainees and undergraduates from the universities and research institutes of Campania presented their results.

The group's mailing list currently counts about 300 members.
The group has a dedicated web page (http://www.neapolitanbraingroup.it), a Facebook page (https://www.facebook.com/NBG2000/) and has been several times cited by the F2 Magazine UNINA:
https://www.unina.it/-/12300029-gruppo-di-confronto-nbg-neapolitan-brain-group

http://www.unina.it/-/13439443-5-meeting-del-neapolitan-brain-group

http://www.unina.it/-/18143154-viii-meeting-del-neapolitan-brain-group-

Letture Magistrali:

Prof. Giorgio Vallortigara (Università di Trento), "La rappresentazione cerebrale del numero"

Prof. Giuseppe Esposito (Università La Sapienza, Roma), "La glia enterica come elemento di unione dell'asse enterico-cerebrale"

Indice Sessione 1:

SINGLE-CELL TRANSCRIPTOMIC ANALYSIS REVEALS DEFECTIVE CORTICOGENESIS IN *Arx* MOUSE MODEL OF DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATY

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Developmental and Epileptic Encephalopathy (DEE) is a pediatric epilepsy characterized by abundant epileptiform activity resistant to traditional anti-epileptic therapies. A severe form of DEE affecting male children is caused by expanded runs of GCG repeats in the X-chromosome gene Aristaless-related homeobox (ARX), which encodes a homeotic transcription factor with a key role in mammalian corticogenesis. To ascertain cellular diversity and disease mechanisms associated to polyalanine elongations in ARX, we conducted single cell RNA sequencing (scRNA-seq) in the epileptogenic neocortex of the Arx polyalanine mouse (Arx (GCG)7/Y). This is a DEE model that spontaneously develops severe tonic-clonic seizures in a phenotype that recapitulates the chronic epilepsy detected in ARX male patients. We detailed cell-specific gene expression patterns unveiling how transcriptional changes in distinct cell subpopulations are associated with a lower proportion of neuronal precursors (NPs) and higher proportion of immature neurons (INs), and suggesting a defective cell composition of the Arx polyalanine neocortex. in vivo BrdU pulse-chase and immunofluorescence studies revealed that neurogenesis and cortical organization are both altered in the mutant embryos. Enrichment analysis in NPs identified consistently altered pathways implicated in cell cycle, chromatin remodelling and RNA metabolism; whereas functions involved in neuronal structure and synapse organization were widely damaged in INs. Finally, immunocytochemistry and morphometric analysis highlighted a defective neurite arborization and hypoconnectivity in the Arx (GCG)7/Y cortical neurons. These results provide new insights into the cell types and neuronal functions perturbed in the Arx polyalanine neocortex disclosening a defective brain development that could potentially underlie the DEE pathogenesis.

FRUCTOSE REMOVAL FROM THE DIET REVERSES INFLAMMATION, MITOCHONDRIAL DYSFUNCTION, AND OXIDATIVE STRESS IN HIPPOCAMPUS

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Young age is often characterized by high consumption of processed foods and fruit juices rich in fructose, which, besides inducing a tendency to become overweight, can promote alterations in brain function. The aim of this study was therefore to (a) clarify brain effects resulting from fructose consumption in juvenile age, a critical phase for brain development, and (b) verify whether these alterations can be rescued after removing fructose from the diet. Young rats were fed a fructose-rich or control diet for 3 weeks. Fructose-fed rats were then fed a control diet for a further 3 weeks. We evaluated mitochondrial bioenergetics by high-resolution respirometry in the hippocampus, a brain area that is critically involved in learning and memory. Glucose transporter-5, fructose and uric acid levels, oxidative status, and inflammatory and synaptic markers were investigated by Western blotting and spectrophotometric or enzyme-linked immunosorbent assays. A short-term fructose-rich diet induced mitochondrial dysfunction and oxidative stress, associated with an increased concentration of inflammatory markers and decreased Neurofilament-M and post-synaptic density protein 95. These alterations, except for increases in haptoglobin and nitrotyrosine, were recovered by returning to a control diet. Overall, our results point to the dangerous effects of excessive consumption of fructose in young age but also highlight the effect of partial recovery by switching back to a control diet.

BEHAVIOURAL MOTOR PROFILES OF INBRED AND OUTBRED MICE STRAINS

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The assessment of unconditioned motor activity, which can be recorded automatically, contribute to generate an effective mouse strain-specific profile, as well as to determine the effects of various experimental approaches, e.g. genetic manipulation, pharmacological intervention, etc. With this study, we in-depth characterize the circadian motor activity of three non-genetically altered mouse strains widely used in research, C57BL/6NCrl and BALB/cAnNCrl (inbred) and CRL:CD1(ICR) (outbred). The impact of light along with husbandry practices (i.e. cage-change) on spontaneous motor activity of group-housed mice was longitudinally measured from pre-puberty until early adulthood of animals by relying on a commercially available digital technology (DVC® Tecniplast). The analysis of different circadian metrics (i.e. day and night activity, diurnal activity, responses to lights-on and lights-off phases, acrophase and activity onset and regularity disruption index) were calculated to capture key behavioural circadian responses for each strain. Furthermore, the DVC technology enabled us to re-purpose our data to phenotyping the spatial pattern of spontaneous motor activity of the mice strains by calculating new metrics (i.e. activity along the cage walls to measure the thigmotaxis, frontality and Gini index). Our results clearly demonstrate significant differences i) in the circadian activity of the selected strains, when comparing inbred versus outbred as well as inbred strains (C57BL/6NCrl versus BALB/cAnNCrl); ii) in the space preference within the cage, with C57BL/6NCrl displaying higher variability in the behavioural phenotypes across lifetime compared to BALB/cAnNCrl, the least active and socially disaggregated, and CRL:CD1(ICR) highly active and socially aggregated.

IDENTIFICATION OF A NOVEL CLASS OF SMALL MOLECULES FOR THE TREATMENT OF TREM2- BASED DISEASES

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We recently reported the synthetic derivative Sulfavant A (SULF A) as a novel immunomodulatory molecule [1]. SULF A action is mediated by TREM2 receptor [2], an innate immune receptor expressed mainly in the resident microglia cells of the central nervous system and in dendritic cells (DCs) and macrophages. TREM2 is an emerging receptor in the field of immunomodulation study associated to mechanisms that avoid the onset of excessive inflammatory processes linked to acute syndromes, as in the case of COVID-19, or which can lead to chronic states such as in some neurodegenerative and autoimmune diseases. A number of TREM2 variants have been identified as risk factors for a wide array of neurodegenerative diseases (NDs), including Nasu-Hakola disease and Alzheimer's disease. SULF A stimulated the differentiation of DCs towards a novel homeostasis-determining phenotype (homeDCs). This response involves the SYK-NFAT axis and is compromised by blockade or gene silencing of TREM2. Activation by SULF A preserved the DC functions to excite the allogeneic T cell response, and increased interleukin-10 release after lipopolysaccharide treatment. Preliminary results suggested the ability of this molecule to activate also microglial cells towards an unconventional non inflammatory state, prompting the idea that the investigation of TREM2 pathway may lay the groundwork for the development of a new class of drugs with therapeutic potential in neurodegenerative diseases, chronic-inflammation and cancer.

DYSREGULATION OF ENDOSOMAL PATHWAY AND ITS IMPACT ON DOWN SYNDROME PATHOGENESIS

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Endosomal trafficking is essential for cellular homeostasis. At the crossroads of distinct intracellular pathways, the endolysosomal system is crucial hub to maintain critical functions and adapt to the environment. Alterations of endosomal compartments were observed in cells from adult individuals with Down syndrome (DS), suggesting that the dysfunction of the endosomal pathway may contribute to DS pathogenesis. However, the degree of impairment as well as the timing of onset remains elusive. By applying imaging and biochemical approaches, we show that the structure and dynamics of early endosomes (EEs) are altered in trisomy 21 fibroblasts, derived from foetuses at 18–20 gestational weeks. Furthermore, we found that recycling trafficking is markedly compromised in these cells. Remarkably, our results in foetal fibroblasts indicate that alterations in the endolysosomal pathway are already present early in development. Interestingly, preliminary analyses showed alterations of EE homeostasis and dynamics in neural precursor cells derived by differentiating trisomic and isogenic euploid iPSCs, further supporting that the dysfunction of this critical hub may be an early neuropathological mechanism of disease. Among chromosome 21 genes, SYNJ1 encodes the ubiquitous inositol-phosphatase synaptojanin 1 (Synj1), a key regulator of membrane trafficking. We demonstrate that overexpression of Synj1 recapitulates the alterations observed in DS cells, pointing out its critical role in DS pathogenesis. Overall, these data strengthen the link between endolysosomal pathway and DS, highlighting a dangerous liaison among Synj1, endosomal trafficking and DS.

THE ROLE OF SEX IN C. ELEGANS MODELS OF PARKINSON'S DISEASE

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Neurodegenerative diseases, including Parkinson (PD), are determined by genetic and environmental factors. PD, characterized by the loss of striatal dopaminergic neurons, affects men more often than women. Male predominance was not observed in Italian patients carrying G2019S mutation in LRRK2, one of the genes most frequently involved in PD (San Luciano et al., Ann. of Cl. and Tran. Neur. 2017; Cilia et al., Park. and Rel. Dis. 2014). This more equal distribution between sexes is not limited to LRRK2 and is shared by other genetic forms of PD. However, how this genetic variation is causative or can modulate PD-linked neurodegeneration remains unknown. To understand the molecular mechanisms causing the sex-specific differences in PD, I used two C. elegans PD models: the first overexpresses α-synuclein, a protein that can misfold and polymerize to form toxic fibrils coalescing into pathologic inclusions; the second overexpresses the G2019S mutated form of LRRK2. In both models, I demonstrated that sex can modulate the neurodegeneration in an age-dependent manner, with females showing higher neurodegeneration compared to males. Then, using LRRK2G2019S model, I investigated the genetic cues of sex-specific neurodegeneration and found that dafachronic acid, produced in the gonad, is involved in the LRRK2G2019S mediated sex-specific neurodegeneration. These results allowed us to identify the molecular mechanisms causing the sex-specific differences in a C.elegans model of PD and providing new insights on the molecular events triggering neurodegenerative deviations.

Indice sessione 2:

IDENTIFICATION OF NOVEL KNA1.1 CHANNEL BLOCKERS THROUGH IN SILICO AND IN VITRO SCREENING

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Sodium-activated potassium channels KNa1.1, encoded by the KCNT1 gene, play an important role in regulating neuronal excitability1.Pathogenic variants in KCNT1 cause a range of epileptic syndromes associated to intellectual disabilities and drug-resistant seizures2 . The largest majority of pathogenic KCNT1 variants increase KNa1.1 currents; therefore, quinidine, an antiarrhythmic drug also acting as a KNa1.1 blocker3 , has been used as a personalized treatment in these patients4,5 .However, quinidine is only partially effective in controlling seizures6 and severe side effects limit its clinical usefulness7To identify novel KNa1.1 channels blockers, in silico and in vitro screening techniques were used in this work. A human homology model built on the chicken KNa1.1 channel structure8 was used to virtual screen an in-house library of 945 compounds; docking energy scores and similarity to the conformation of quinidine bound to KNa1.19 were used as parameters for the in silico screening and led to the selection of a subset of 21 molecules. These compounds underwent in vitro screening using CHO cells stably expressing KNa1.1 subunits in a Thallium (Tl+)-based fluorescent assay. The KNa1.1 opener loxapine (10 μM) 10 was used to increase the fluorescent signal upon Tl+exposure and allow better quantification of the effects of blockers. In this assay, quinidine (0.3-1000 μM) dose-dependently reduced the maximal fluorescence, with an IC50 of 129.7±5.9 μM5. Among the previously selected molecules, tested at a 10 μM concentration in this model, five were found to be more effective than quinidine in reducing the fluorescent signal.

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ELECTRORETINOGRAPHIC EVALUATION OF TWO JUVENILE LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) OF MEDITERRANEAN REGION WITH CATARACTS

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The loggerhead sea turtle (*Caretta caretta*) is a globally threatened marine species (1,2). It has been demonstrated that sea turtles use vision more than smell to forage for food (3). Therefore, it is essential to ensure that vision of any turtles rescued is preserved to achieve a systematic protection and conservation effort for this species.
Electroretinography (ERG) is a noninvasive technique used to assess retinal function in clinical and research ophthalmology. There are several reports of ERG in various wild and exotic animal species. However, in loggerhead sea turtles few cases are reported. Furthermore, ERG has been mentioned in sea turtles with cataracts but without describing the methods of execution and the values of the measurements obtained (4).
This study describes the ERG technique and results of two juvenile loggerhead sea turtles with severe visual impairment due to bilateral cataracts.
Scotopic and photopic electroretinographic responses were recorded in turtles under sedation after dark and light adaption, respectively, using the device RETevet^{TM}. In scotopic ERG at 3.0 cd·s/m^2, median a-wave amplitude and peak time values were -35.7 (-33.5; -53.6) μV and 31.6 (29.6; 35.4) ms, respectively; median b-wave amplitude and peak time values were 142 (59.1; 154) μV and 97.3 (49.1; 103.4) ms, respectively. Although waves amplitudes and peak times values could not be recorded in all flash protocols tested, this report demonstrated that ERG is effective in loggerhead sea turtles with cataracts to evaluate visual prognosis before cataract surgery.

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AUTOPHAGY MODULATION THROUGH INHIBITION OF RPSA: IMPLICATIONS FOR NEURODEGENERATIVE DISEASES

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Autophagy is a highly conserved cellular catabolic process by which macromolecules or damaged organelles are targeted to lysosomes for degradation, thus representing an essential pathway for the maintenance of protein's homeostasis. Accordingly, growing evidence implicates autophagic dysfunction in several neurodegenerative disorders, including Alzheimer's disease (AD). Hence,modulation of autophagic pathway might represent a valuable tool in such pathologies. We have previously identified small molecules (inhibitor of the non-integrin laminin receptor RPSA), which control the generation of amyloid beta in primary cell culture of AD-affected patients. Beside these, searching for a compound that stimulates autophagic pathway, led us to identify RPSA inhibitor which induces the conversion of the cytosolic microtubule-associated LC3-I into the lapidated LC3-II. We found that LC3 lipidation occurs unconventionally on endosomal compartment of mouse neuronal cells. In addition, the effects of the inhibitor are independent from the upstream autophagic regulators ULK and BECN1; whereas they depend on ATG16L1 activity, and they cause upregulation of genes controlling endocytosis, vesicles fusion and autolysosomal maturation. Moreover, investigation of the inhibitor's mechanism of action in a pathological context, revealed that it controls the Akt and ERK1/2 kinases activity and as consequence mTOR pathway, in human fibroblasts from genetic AD-affected patients, thus strongly suggesting a downstream activation of autophagy. Collectively our findings suggest that RPSA might represent an appealing pharmacological target to modulate autophagy and its inhibition could be an interesting strategy that requires further consideration in diseases associated to autophagy defects.

LOSS OF HIPK2 RESEMBLES AN ALS-LIKE PHENOTYPE PROMOTING TDP-43 PROTEIN MISLOCALIZATION

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Homeodomain interacting protein kinase 2 (HIPK2) is a serine-threonine kinase which phosphorylates a wide range of transcriptional and chromatin regulators thus modulating crucial cellular processes, such as DNA damage response, proliferation and apoptosis. The relevant physiological role of HIPK2 also emerged from the phenotype of Hipk2 null mice (Hipk2-KO). These mice show several neuronal defects associated with several psychomotor behavioral abnormalities including dystonia, impaired coordination, reduced motility and clasping of posterior limbs, highly resembling a motor neuron disease overlapping Amyotrophic Lateral Sclerosis (ALS) pathology. One of the main proteins associated with ALS is the TAR DNA Binding Protein 43 kDa (TDP-43), whose cytoplasmic aggregates are a hallmark of both sporadic and familial ALS cases regardless of its mutational status. To investigate the possible role of HIPK2 in the pathogenesis of ALS, we analyzed the consequences of Hipk2 depletion in neuronal context by using in vivo and in vitro model systems. A strong mislocalization of TDP-43 protein from nucleus to cytoplasm was observed in spinal motor neurons from adult Hipk2-KO mice, and the same effect was observed upon Hipk2 silencing in human-derived neuroblastoma SH-SY5Y cells. Strikingly, the re-establishment of the expression of wild-type HIPK2, but not of its kinase-dead K221R mutant, was able to restore the nuclear TDP-43 localization, thus indicating that TDP-43 subcellular distribution may depend on HIPK2 expression level and activity. Altogether, these data unravel the relationship between HIPK2 and TDP-43 proteins that may have a role in the pathogenesis of ALS.

B-Raf/lin-45 IS A SIGNALING HUB FOR NEW GENES INVOLVED IN NEURODEGENERATION

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Spinal Muscular Atrophy (SMA) is a neurodegenerative disease caused by an insufficient level of the Survival of Motor Neuron (SMN) protein. Current treatments for SMA aming at restoring SMN protein levels and result in impressive but limited effects. To improve SMA therapeutic strategies and elucidate its molecular pathomechanisms, complementary SMN-independent approaches are needed. Using a phospho-array approach with SMA mice spinal cord and network-biology, we identified 38 dysregulated proteins involved in motoneuron degeneration, with B-Raf as major signaling hub. We characterized, in vivo, B-Raf/lin-45 role in neurodegeneration using C. elegans, demonstrating that lin-45 plays a cell-autonomous neuroprotective role when smn-1 is down-regulated. lin-45 protects from motoneurons (MNs) loss post-symptomatically rather than interfering with neurogenesis, and its neuroprotective effect is mediated by the MAPK/ERK pathway. These results strongly support a role of B-RAF in neurodegeneration. To elucidate the role played by the other 37 proteins identified in the phospho-array screening, we set up a new model of SMA in C. elegans that allows to rapidly identify modifier genes among candidates. We successfully obtained a new strain for RNA-interference mediated genetic screen, allowing to obtain an enhancement or a suppression of the neurodegeneration. We identified 9 suppressors and 9 enhancer genes among the 37 genes tested. Using cell-specific RNAi, we demonstrated that 11 play a role in neurons and 5 of those act specifically in MNs. These data unveil a neuroprotective role of a network of genes in MNs which are potential candidate targets for future therapies in SMA.

SEMAPHORIN 3A COMPROMISES AXONAL ELONGATION AND DENDRITIC ARBORIZATION IN HUMAN NEURAL PROGENITORS DURING DIFFERENTIATION

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Semaphorins (Sema) are the largest family of axon guidance molecules, crucial in regulating manyprocesses that shape the nervous system during development such as neuronal migration, axonal pathfinding, and synapse formation [1]. In particular, Sema3A belongs to the subclass-3 semaphorins, that are all secreted proteins thus exerting both autocrine and paracrine activity in mammals [2]. Notably, evidence highlight changes in Sema3A levels in brain from patients with schizophrenia (SZ) or autism [3]. Indeed, Sema 3A expression levels were found highly increased in the cerebellum and prefrontal cortex of patients with SZ [4] and mutations or polymorphisms in Sema3A or Sema 3A receptors (Npn1 and PlxnA2) have been described in autistic patients [5]. Considering these aspects, we investigated whether and how increased Sema3A levels affect human neural progenitors (HNPs) growth and survival during the early stages of neuronal differentiation. To this aim, we exposed HNPs to increased either endogenously or exogenously Sema 3A levels. In both the experimental paradigms, the increased Sema 3A levels resulted in axonal retraction and an aberrant dendritic arborization. In addition, inflammatory processes were activated upon Sema3A exposure in HNPs that ultimately die within 3 hours. Of note, all these events were prevented in HNPs in which Npn1 or PlxnA2 were silenced. In conclusion, alterations in Sema3A levels during the early stages of neuronal differentiation compromise axonal elongation and dendritic architecture and connections, thus affecting HNPs development. Future studies will be investigating whether increased Sema3A levels might represent one of the risk factors for neurodevelopmental disorders.

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SYNCRIP/HRPR-1 RESCUES NEURODEGENERATION VIA RTN/RET-1 IN A C.ELEGANS SMA MODEL.

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A correct splicing of mRNA is globally required in all cells, but neurons seem highly sensitive to perturbations with numerous neurological diseases caused by splicing defects, including spinal muscular atrophy (SMA). However, why neurons are more affected to splicing alterations in SMA is still debated. We are investigating the molecular mechanisms underlying the neurodegeneration caused by splicing defects in SMA. In our previous work, RNA-sequencing of induced pluripotent cell-derived motoneurons (iPSC-MNs) from SMA patients allowed the identification of differentially spliced genes, enriched in RNA motif 7. This motif is specifically bound by hnRNP Q/SYNCRIP, a spliceosomal component. To investigate in vivo SYNCRIP role
in SMN pathway, we used C. elegans. hrpr-1 and smn-1 are the C. elegans homologs of SYNCRIP and SMN. We demonstrated that hrpr-1 and smn-1 genetically interact in MNs, where the overexpression of hrpr-1 rescues the neurodegeneration caused by the MNs-specific silencing of smn-1. Since hrpr-1 is involved in regulating alternative splicing, we investigated the role in smn-1 pathway of a well-known hrpr-1 target, ret-1/RTN. We determined that in smn-1(KO) and hrpr-1(RNAi) animals the splicing pattern of ret-1 is similarly altered and that the rescue of MNs degeneration obtained after hrpr-1 overexpression in smn-1(MNs RNAi) animals, is mediated by ret-1. Interestingly, we observed that RTN transcription levels are altered in SMA mice and iPSC-MNs from SMA patients, suggesting a conserved role of ret-1 in SMA. These data support a
neuroprotective role of hrpr-1 and ret-1 in SMA and their possible involvement as potential new therapeutic targets.

LONG-LASTING IMPACT OF SUGAR INTAKE ON NEUROTROPHINS AND NEUROTRANSMITTERS IN RAT FRONTAL CORTEX

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The detrimental impact of fructose, a widely used sweetener in industrial foods, was previously evidenced on various brain regions. Although adolescents are among the highest consumers of sweet foods, whether brain alterations induced by the sugar intake during this age persist until young adulthood or are rescued returning to a healthy diet remains largely unexplored. To shed light on this issue, just weaned rats were fed with a fructose-rich or control diet for 3 weeks. At the end of the treatment, sugar-fed rats underwent a control diet until young adulthood phase, and compared with animals that received from the beginning the healthy control diet.
We focused on the consequences induced by the sugar on the main neurotrophins and neurotransmitters in the frontal cortex, as its maturation continues until late adolescence, thus being the last brain region to achieve a full maturity. We observed that fructose intake induces inflammation and oxidative stress, alteration of mitochondrial function, changes of brain derived neurotrophic factor (BDNF) and neurotrophin receptors, synaptic proteins, acetylcholine, dopamine and glutamate levels, as well as increased formation of the glycation end-products Nε-carboxymethyllysine (CML) and Nε-carboxyethyllysine (CEL). Importantly, many of these alterations (BDNF, CML, CEL, acetylcholinesterase activity, dysregulation of neurotransmitters levels) persisted after switching to the control diet, thus pointing out to the adolescence as a critical phase, in which extreme attention should be devoted to limit an excessive consumption of sweet foods that can impair brain health not only immediately but also in the long term.

Indice sessione 3:

PEA-OXA IMPROVES PSYCHOPHYSICAL AND METABOLIC ASPECTS ASSOCIATED WITH SOCIAL ISOLATION IN MALE MICE: AN IN VIVO AND IN VITRO STUDY

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Abstract

Chronic social isolation generates a persistent state of stress associated with obesity along with some neuro-endocrine disorders and central behavioral sequelae (eg anxiety, depression, aggression, and allodynia). In this study, we evaluated the effect of social isolation on weight gain, depressive- and anxious-aggressive-like behavior, as well as on phenotypic changes of adipocytes from visceral adipose tissue of control (group-housed) or socially isolated (single-housed) male mice. The effect of treatment with pentadecyl-2-oxazoline (PEA-OXA), a natural α-2 antagonist and histamine H3 protean partial agonist, on these pathological alterations was also evaluated. Behavioral sequelae were evaluated by different tests such as von Frey test for allodynia, tail suspension test for depression, hole bard test for anxiety and resident intruder test for aggressiveness. Furthermore, mesenchymal stromal cells (MSCs) from white adipose tissue of each group of mice were collected and we evaluated cell proliferation, senescence, apoptosis and ROS levels. Elisa tests were conducted for analyzing IL-17, IL-6, IL-1β, IL-10, and TNF-α levels. Single housed mice developed a weight gain, depression- and anxiety-like behavior, and aggressiveness. Single housed mice receiving PEA-OXA showed a general resolution of both, physical-metabolic and behavioral alterations associated with social isolation. This study confirms that persistent stress caused by social isolation predisposes to obesity and neuropsychiatric disorders. PEA-OXA, through its multi-target activity on α2 adrenoceptor and histamine H3 receptors, reduces weight gain, systemic pro-inflammatory state, allodynia, and affective disorders associated with social isolation.

Keywords: Social isolation, Weight gain, Anxious- and depressive-like behaviour, Aggressiveness, Allodynia, Adipocytes, PEA-OXA

IN-(SIGHT): A MORPHO-FUNCTIONAL ASSESSMENT OF AGE-RELATED RETINAL DEGENERATION

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Retina is a neuroectodermal tissue of highly differentiated layers of specialized cells and synapses. The outer nuclear layer contains photoreceptor cell (PRC) bodies, the inner nuclear layer contains the cell bodies of the bipolar, horizontal (HC) and amacrine cells while the ganglion cell layer contains the cell bodies of ganglion cells and displaced amacrine cells. In humans, the aging process causes a wide range of sensory impairments, including retinal degeneration leading to age-dependent visual defects. The high degree of evolutionary genetic relationship and functional similarities to the human eye make mouse a valuable model to study age-dependent vision impairment. In our work, we evaluated with a comprehensive approach of optical computed tomography (OCT) and immunofluorescence mouse retinas at four timepoints (2, 6, 12, and 18 months) to highlight and follow the onset of retinal decline during aging aiming to provide new insights inage-related neurodegeneration research. OCT provides structural information on thickness of each layer while immunofluorescence of 4 retinal-specific markers labels different key retinal structures: GNAT-2 marks PRCs, Synaptophysin highlights the synaptic region between photoreceptors and horizontal cells, PKCα is specific for rod bipolar cells (RBCs) while Calbindin for HCs. OCT analysis shows the tendency to reduce retinal thickness with age. Consistently, immunofluorescence highlights a significant age-dependent reduction of PRCs, HCs, RBSs as well as the area of synapses between PRCs and HCs. In conclusion, with this approach we described a model of retinal degeneration during aging, following the process with high temporal resolution.

FUNCTIONAL NEUROIMAGING BRAIN ANALYSIS OF DIFFERENT METABOLIC PATTERNS IN TREATMENT-RESISTANT COMPARED TO TREATMENT-RESPONSIVE SCHIZOPHRENIA PATIENTS AND CONTROLS

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Schizophrenia (SCZ) is a severe mental disorder characterized by positive, negative, and cognitive symptoms. Antipsychotics represent the first-line therapy, however, a third of patients suffer from a treatment-resistant schizophrenia (TRS) condition characterized by worse symptoms, functioning, and more frequent morphological central nervous system abnormalities. The present functional neuroimaging study aims at identifying significant differences in brain metabolism of TRS *vs.* treatment-responsive schizophrenia (nTRS) patients and controls (CTRL) to be used as metabolic biomarkers to address clinical response to antipsychotics in SCZ. The study enrolled 41 patients (31 male, 10 female, age 37 ±10, disease duration 15,5±8,6, chlorpromazine equivalents 465,6±368,8): 21 TRS, 20 nTRS patients and 12 CTRL. The images were acquired with [18F]Fluorodeoxyglucose positron emission tomography and analyzed with the voxel-per-voxel methodology. The visual analysis showed significant group differences in the frontal, fusiform, and occipital gyri bilaterally. The voxel-per-voxel SPM analysis revealed a bilateral hypometabolism in the superior frontal gyrus in TRS compared to nTRS. Compared to CTRL, only TRS showed a diffuse prefrontal hypometabolism with left prevalence, involving the left superior, middle, medial, and inferior frontal gyri, and the right superior and middle frontal gyri. A hypermetabolism was found in TRS compared to nTRS and CTRL in the right lingual/fusiform gyrus, right middle occipital gyrus, left fusiform gyrus, and cuneus ($p < 0.05$ corrected FWE). In conclusion the results suggest a progressive gradient of cerebral metabolism alterations in TRS compared to nTRS and CTRL that could be used as metabolic biomarker to address the response to treatment in SCZ.

THE ENDOCANNABINOID 2-AG CONTROLS DENTATE GYRUS EXCITABILITY IN THE PRESYNTOMATIC PHASE OF A GENETIC MOUSE MODEL OF EPILEPSY

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Idiopathic generalized epilepsy tends to appear during childhood or adolescence, but it may not be diagnosed until adulthood. The mechanism promoting transition from the pre-epileptic to epileptic state, a fundamental aspect of seizure pathophysiology, remains still poorly understood.

Synapsin II (SYN2), a member of the multigene synapsin family (SYN1/2/3) of synaptic vesicle phosphoproteins, fundamental in the control of neurotransmitter release, is mutated in epileptic patients. Mutant mice lacking SYN1/2 isoforms are all prone to develop epileptic seizures after 2-3 months of age.

Despite the role of the endocannabinoid system in regulating neuronal excitability, and the fact that deficit of 2-AG/CB1R signaling leads to hyperexcitability, seizure development and propagation, the involvement of this system in the excitatory/inhibitory balance of hippocampal networks of SynII KO mice has never been investigated before. Here, we sought to determine whether changes in endocannabinoid signaling might contribute to the development of seizures in SynII knockout mice. With this purpose, we performed electrophysiological, immunohistochemical, and biochemical studies of endocannabinoid signalling at CB1 receptors in the dentate gyrus (DG) of pre-epileptic (2 months old) and epileptic (6 months old) SynII KO mice.

We found an increment of endocannabinoid-mediated depolarization-induced suppression of excitation (DSE) in juvenile SynII mice compared to age-matched wild type mice while the endocannabinoid-mediated depolarization-induced suppression of inhibition (DSI) remained unchanged between the two genotypes at both ages. This alteration was accompanied by modification of CB1 receptor distribution in the DG. Our data suggest that 2-AG/CB1R signaling may contribute to the maintenance of network excitability in a mouse model of human idiopathic epilepsy.

THE MUTATIONS IN THE LYSOSOMAL POTASSIUM CHANNEL TMEM175 CONTRIBUTE TO PARKINSON'S DISEASE PATHOGENESIS.

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Parkinson's disease (PD) represents the most common neurodegenerative movement disorder. We recently identified 16 novel genes associated with PD.
In this study, we focused the attention on the common and rare variants identified in the lysosomal K+ channel TMEM175.
Our findings demonstrated that TMEM175 is highly expressed in dopaminergic neurons of the substantia nigra pars compacta and in microglia of the cerebral cortex in the human brain.
Clinical, genetic, and molecular analysis, performed on 420 patients and 300 healthy controls allowed identifying 4 variants associated with PD, including two novel variants, located in the Kozak consensus sequence and in the TM3II domain, respectively. We also disclosed 14 novel highly penetrant mutations. Importantly, our findings also suggested that detrimental mutations in the TMEM175 gene may be sufficient to cause the disease in about 6% of Italian patients, and the presence of polygenic mutations correlated with an earlier disease onset.
In vitro functional analyses of the mutant channels revealed a loss of the K+ conductance and a reduced binding affinity for AKT protein. Moreover, we observed an impaired autophagic-lysosomal flux and the activation of UPR markers in patient-derived fibroblasts.
Our results, highlight the importance of studying common and rare variants of PD genes to obtain a comprehensive genetic contribution of the disease.
In this frame, to further investigate the impact of multiple mutations on PD pathogenesis, in dopaminergic background, we generated hiPS cells of PD patients and healthy subjects carrying the most promising combination of mutations. The analysis is ongoing.

ASSESSMENT OF CENTRAL NERVOUS SYSTEM INVOLVEMENT IN FABRY DISEASE WITH DEEP LEARNING AND THE BRAIN-AGE PARADIGM

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Objectives: An accurate MRI assessment of CNS involvement in Fabry disease (FD) is hampered by the lack of quantitative imaging biomarkers. We aimed to assess FD-related brain damage using deep learning and the brain age paradigm.

Methods: A 3D Inception-ResNet-V2 network was trained to predict chronological age and evaluated on an external cohort of healthy subjects (HS) from 8 open-source datasets (2160subjects, M/F:1293/867, mean age:33[range=4-86]), split in training (64%=1382), validation (16%=346) and test (20%=432) sets. For hyperparameter optimization, models with different values were trained for 200 epochs using the ADAM optimizer, and accuracy was measured with mean absolute error (MAE). Lastly, age bias was statistically corrected, and the final model was applied to an internal cohort of 30 genetically confirmed FD patients with no prior cerebrovascular accidents and 78 age- and sex-comparable HS. The difference between predicted and chronological age (brain-predicted age difference, brain-PAD), considered as an index of structural brain health, was compared between the two groups using bootstrapped t-test.

Results: Following age bias correction, the final brain-age model had validation and testing MAEs of 3.527 (R^2=0.917) and 3.640 (R^2=0.917), respectively. FD patients and HS of the internal cohort were not significantly different in terms of age (t=0.85, p=0.40) or sex ratio (Chi-Square=1.66, p=0.28). When applying the brain-age model, FD patients showed significantly higher mean brain-PAD compared to HS (2.97 vs 0.49 years, Cohen's d=0.412, p=0.043).

Conclusions: FD patients show significantly higher than normal brain-predicted age, reflecting "accelerated" brain aging and possibly capturing FD-related brain damage

Indice sessione 4:

ASSESSING THE ROLE OF CHROMOSOME 21 GENES IN DOWN SYNDROME NEURONAL ALTERATIONS THROUGH SELECTIVE ATTENUATION OF THEIR EXPRESSION

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Down syndrome (DS) is the leading cause of intellectual disability, which is the consequence of alterations that occur during neuronal cell differentiation. DS is due to the presence of 3 copies of Hsa21 genes, which are globally overexpressed 1.5-fold due to gene dosage effect. By normalizing the overexpression of individual genes, it might be possible to find which of them is responsible for a specific neuronal alteration.
To this end, we identified candidate Hsa21 genes that are more likely to cause significant metabolic dysregulations, namely transcription factors, kinases and phosphatases, and ranked them according to parameters such as overexpression in neurons and high haploinsufficiency score. So far, we have modulated, in trisomic fibroblasts, the expression level of 3 Hsa21 genes, namely NRIP1, RUNX1 and SYNJ1, finding them responsible for mitochondrial, ECM and endosomal alterations, respectively. We have then set a model of trisomic iPSCs that we differentiated into neural precursor cells and neurons. We found that trisomic neuronal cells undergoing differentiation manifest defects in mitochondrial function, neurite number and length, and neuron/glia ratio. We are setting up rapid assays to detect these phenotypic alterations.
This model system may allow us to evaluate the phenotypic consequences of normalizing the expression of one candidate gene at a time, before and after differentiating trisomic iPSCs into neurons. The results of these experiments could lead to the discovery of new therapeutic targets.

LYSOSOMAL DYSFUNCTION IN GDP-MANNOSE PYROPHOSPHORYLASE B DEFICIENCY INDICATES A LINK BETWEEN CONGENITAL DISORDERS OF GLYCOSYLATION AND LYSOSOMAL STORAGE

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GDP-mannose pyrophosphorylase B (GMPPB) deficiency is a congenital disorder of glycosylation, due to mutations of the GMPPB gene. GMPPB catalyzes GDP-mannose synthesis, an early step in multiple glycosylation pathways, including N-glycosylation, O-mannosylation, C-mannosylation, and glycosylphosphatidylinositol-anchor formation. The phenotypic spectrum of GMPPB deficiency is broad and includes a progressive myopathy, brain and eye abnormalities, and intellectual disability. As lysosomal enzymes depend on glycosylation for their traffic to lysosomes, we speculated that GMPPB deficiency would result into lysosomal dysfunction and pathologic accumulation of storage material in cells and tissues from GMPPB patients.

In cultured fibroblasts and myoblasts from eight GMPPB-deficient patients and in the muscle biopsy from three of them we showed an expansion of the lysosomal compartment, with increased LAMP2 signal by immunofluorescence analysis and increased area occupied by lysosomes by high-content imaging, and presence of heterogeneous storage material with accumulation of glycogen, lipid droplets, multivesicular and multilamellar bodies by ultrastructural analysis. Acid alpha-glucosidase (GAA) showed the most evident reduction in GMPPB cells, compared to other lysosomal enzyme activities. GAA trafficking to lysosomes was impaired, with poor maturation (by western blot analysis) and targeting (co-localization with LAMP2 by immunofluorescence) to lysosomes. In contrast, human recombinant GAA (rhGAA), that is fully glycosylated, showed full correction of GAA activity, normal maturation and lysosomal trafficking, with complete clearance of glycogen storage. These results add information of the pathophysiology of congenital disorders of glycosylation and on the consequences of defective glycosylation on lysosomal function.

HALOPERIDOL ADMINISTRATION IN ANIMAL MODEL OF SCHIZOPHRENIA: SYNAPTIC PLASTICITY EVENTS AND HOMER1A-BASED NETWORK APPROACH

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Schizophrenia is a severe psychiatric disorder marked by the disruption of synaptic architecture and altered functional connectivity. The aim of our study was to compare *Homer1a* expression by *in situ* hybridization in brain animal models.Sprague-Dawley rats(n= 20) were divided into two groups(n=10 for each pre-treatment group) receiving a pre-treatment of VEH(NaCl 0.9%) or KET(Ketamine at 30 mg/kg), respectively.In the treatment step, animals were further randomly subdivided resulting in the following treatment: VEH+VEH, VEH+HAL0.8 mg/kg, KET+VEH, and KET+HAL0.8 mg/kg.All possible pairwise Pearson correlations were computed in each group and three networks were generated via Cytoscape3.8.2software.General topography and node attributes were compared among all networks. *Homer1a* expression was found to increase in the genu of corpus callosum in KET+VEH compared to VEH+VEH, and in caudate putamen in the KET+HAL group compared to KET+VEH. The KET+VEH network has shown disruption in cortical-subcortical connections.We demonstrated an important decrease in degree in cortical and subcortical nodes of the KET+VEH network compared to VEH+VEH, and a reduction and different modulation in cortical and subcortical regions compared to the KET+HAL group. Differently, an increase in degree and subsequent connections was found for lateral stripe of the striatum and septohippocampal nucleus in KET+VEH. These functional connectivity alterations appear to be absent in the vehicle and to be, in part, reversed or modified after haloperidol administration.After ketamine administration, we identified crucial abnormalities in functional properties and connectivity of regions involving perceptual, ideational, motor and memory functions with loss of cortical control on deep subcortical structures responsible for clinical symptoms.

MIR-218 PROMOTES DOPAMINERGIC DIFFERENTIATION AND CONTROLS NEURON EXCITABILITY AND NEUROTRANSMITTER RELEASE THROUGH THE REGULATION OF A SYNAPTIC RELATED GENES NETWORK

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The development of midbrain dopaminergic neurons (DAn) requires a fine temporal and spatial regulation of a very specific gene expression program. In these events of relevance is the role of microRNAs, a class of small non-coding single-strand RNA acting as post-transcriptional regulators. MiRNAs are potentially involved also in the etiopathogenesis of Parkinson's disease. Indeed, their specific depletion in the substantia nigra, by knocking out the key miRNA biosynthetic enzyme Dicer, results in motor learning impairment and progressive neuronal degeneration in mice.

By performing a microRNA-mRNA paired microarray screening, we identified the miR-218, a motor-neuron related miRNA, among the most upregulated miRNA during dopaminergic differentiation *in vitro*. Furthermore, it is expressed in the dopaminergic nuclei during the embryonal development of the mouse midbrain.

By combining different *in vitro* model systems, we showed that miR-218 promotes the differentiation of embryonic stem cells and the *trans*differentiation of fibroblasts into DAn.

Among the miR-218 targets, we found a significant enrichment in genes related to synaptic organization and function, suggesting the involvement of miR-218 in the modulation of neuronal activity. By creating unique conventional and conditional knock-outs mouse models, we observed that the miR-218 plays a role in the control of DAn excitability and neurotransmitter release, by regulating an unpredicted synaptic related gene network.

Thus, our data indicate the miR-218 as a key component in the maturation and activity of midbrain DAn, and highlight the potential of exploiting its role in enhancing the *in vitro* generation of functional DAn.

gH625-LIPOPACAP IN AN IN VITRO FLUID DYNAMIC MODEL OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is an aggressive neurodegenerative disease. The main characteristic is the death of dopaminergic neuron (DAn) in the substantia nigra due to the accumulation of protein aggregates (Lewi bodies). To date, the molecular mechanisms are not identified, and the current treatments used are mostly symptomatic. It is useful to develop an in vitro model to study the PD. In our previous work (Barra et al., 2022. gH625-liposomes deliver PACAP through a dynamic in vitro model of the blood–brain barrier) we recreated an in vitro fluid dynamic model of blood-brain barrier (BBB) composed by a millifluidic bioreactor (Livebox2, LB2) with an upper chamber and a lower chamber; in the former, murine endothelial brain cells (bEnd.3) were seeded on a porous membrane, and in the latter 3D neuroblastoma cells (SH-SY5Y) differentiated in DAn, were seeded. We evaluated the passage of neuroprotective Pituitary adenylate cyclase activating polypeptide (PACAP) delivered by a functionalized liposome (gH625-lipoPACAP) to ameliorate PACAP release through our endothelial monolayer. gH625 is a cell-penetrating peptide through the phospholipid double layer, deriving from glycoprotein H of the Herpes simplex virus type 1. PACAP is able to enhance DA synthesis. In this study we purpose to evaluate the PACAP neuroprotective effects through our in vitro fluid dynamic model of BBB. We treated our 3D DAn with 1-Methyl-4-phenylpyridinium (MPP+), a neurotoxin that induces parkinsonism. We evaluated through molecular investigation, how PACAP can acts against MPP+effects.

Posters:

TARGETING THE ENDOCANNABINOID AND MELATONERGIC SYSTEMS TO FOSTER NEUROPROTECTION AGAINST THE NEUROINFLAMMATORY DAMAGE

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Enhancing the endocannabinoid (EC) tone by inhibiting the catabolic enzyme *FAAH* is an attractive therapeutic perspective in several neuroinflammatory and neurodegenerative diseases. Melatonin is a hormone released by the pineal gland that is currently used as a dietary supplement for the short-term treatment of insomnia. Both endocannabinoids and melatonin exert physiological brain functions and are provided by immunomodulatory, antioxidant, and protective roles.
By employing a rodent organotypic explant model of inflammatory injury we reveal the effects of UCM1341, a novel dual-acting compound with FAAH inhibitory action and agonistic activity on melatonin receptors, against the neuroinflammatory damage. FAAH activity was measured by a radiometric assay and N-acylethanolamine levels were assessed by HPLC-MS/MS. FAAH distribution and evolution of inflammation were investigated by biochemical and confocal analyses.
UCM1341 attenuated demyelination, augmented the levels of AEA and OEA, and prevented the release of TNF□. The bivalent ligand exerted a greater dose-dependent neuroprotection against LPS+IFN-□-induced neuroinflammatory damage if compared to the reference compounds melatonin or URB597. During neuroinflammation, UCM1341 modulated the inflammatory response by contributing to microglia/macrophage polarization. The neuroprotective effects of UCM1341 were prevented by the PPAR□□and melatonin receptor antagonists.
Our findings suggest that enhancing the EC and melatoninergic tone with UCM1341 may represent a novel strategy to provide neuroprotection and modulate the microglia/macrophages response after a neuroinflammatory demyelinating insult.

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COMBINED DII AND ANTIBODY LABELING REVEALS COMPLEX DYSGENESIS OF HIPPOCAMPAL DENDRITIC SPINES IN A MOUSE MODEL OF FRAGILE X SYNDROME

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Morphological, functional, and molecular abnormalities in dendritic spines have been correlated with dysfunctions in neuronal networks in neurodevelopmental disorders. In order to have an advanced visualization of dendritic spine morphology and synaptic protein composition, we developed an optimized rapid and sensitive technique combining immunofluorescence with the use of the fluorescent dye DiIC18 (or DiI). Here, we successfully show the applicability of this approach by examining the properties of dendritic spines in the hippocampus of the Fragile X Syndrome mouse model (Fmr1 KO) at PND22. We find that the density of elongated thin spines, stubby spines, filopodia, and branched spines was higher in the Fmr1 KO than in WT. In contrast, mushroom spines were significantly less abundant in the mutant. We further find that mushroom spines expressing the actin-binding protein Synaptopodin -an essential component of spine apparatus- are more pervasive in mutant mice. Altogether, our data strongly suggest that these abnormalities of spines are associated with a deficiency in excitatory synapse formation/maintenance in Fmr1 KO mice. Overall, these findings reveal a novel aspect of dendritic spine dysgenesis in Fmr1 KO mice, taking advantage of the sensitivity of our novel technique.

MATERNAL TREATMENT WITH BUTYRATE PREVENTS THE BEHAVIORAL AND SYNAPTIC PLASTICITY DEFICITS IN AUTISM-LIKE MOUSE OFFSPRING

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The alterations in gut-microbiota play a critical role in brain dysfunctions such as autism spectrum disorders (ASD). The aim of our study was to evaluate whether the treatment with the microbial metabolite butyrate (BUT) at a very early stage of disease reduces the autism-like symptoms in the offspring of the ASD mouse model BTBR. For this purpose, we treated BTBR dams with BUT from mating to weaning and then we examined the effect of maternal treatment on the behavioral and synaptic plasticity deficits in juvenile and adult offspring. We showed that BUT treatment of BTBR dams prevents the social deficits and partially the repetitive behaviour in the offspring. Since cerebellar alterations have been observed in ASD in correlation with social and communication impairments, we also examined the effects of maternal BUT treatment on the structural and functional properties of the cerebellar cortex. Such analysis revealed that BUT treatment reduced the cerebellar cortical hypertrophy and rescued the alteration of Purkinje cell firing and long-term synaptic plasticity. These results suggest a new therapeutical strategy for early treatment of neurodevelopmental disorders.

DNA APTAMER-BASED APPROACH: AN EMERGING TOOL TO FIGHT HUNTINGTON'S DISEASE

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Aptamers, comprising single-stranded DNA or RNA, are molecular-recognition agents, predominantly emerged in the last years as valuable therapeutic candidates for several human diseases. Despite the exceptional advances made in Huntington's disease (HD) research, nowadays no definitive treatment is available for this invalidating progressive neurodegenerative disease, caused by elongation of CAG repeats in huntingtin (HTT) gene. Recently, four guanine-rich DNA-based aptamers to recognize mutant HTT with the expanded polyglutamine tract have been identified. We have performed a detailed physicochemical and biological characterization on the best aptamer in this series, MS3, and its two truncated variants (named MS3-33 and MS3-17) that we designed to reduce its intrinsic tendency to form aggregates. As determined by biophysical analyses, these aptamers adopt a stable, parallel G-quadruplex structure and show high resistance to nuclease digestion in serum. Confocal microscopy experiments showed a rapid, dose-dependent uptake of fluorescein-labelled apatmers in different cell types, demonstrating their effective internalization with no general cytotoxicity. In addition, they are stable over time as evidenced by the presence of fluorescent signal 72 hrs after their wash out of medium, further supporting the feasibility of their in vivo use. Remarkably, these aptamers are readily taken up and persist even in the widely used immortalized striatal cell line model of HD. Finally, a significant improvement in the motor neuronal function and lifespan of the well-established *Drosophila melanogaster* model for HD (Q128HD-FL) fed with these aptamers was observed, proving their in vivo efficacy.
Overall, our data open new valuable perspectives in the therapeutic approaches for HD.

FEATURING MORPHO-FUNCTIONAL AGE-RELATED DECLINE OF THE COCHLEA

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Aging is generally associated to sensory impairment and the age-dependent gradual deterioration in hearing function is almost considered an inexorable part of aging in humans. Age-related hearing loss as due to three main types of degenerations classified as sensory, strial and neural which affect sensory hair cells, stria vascularis and auditory nerve fibers respectively. Mouse is a major model organism for hearing research displaying morpho-functional analogies with human auditory system along with the high degree of evolutionary genetic relationship. In this work we featured the mouse cochlea over aging by analyzing four timepoints (2, 6, 12, and 18 months) to identify at which stage this decline occurs and track the degenerative process aiming to provide new insights in the identification of age-related neurodegeneration indicators. Over aging, the analysis of the histological sections showed a clear drop in the number of type 1 spiral ganglion neurons (SGNs), the first action potential generating neurons in the auditory pathway. These bipolar neurons receive almost the entire set of auditory inputs from the inner hair cells which codify the auditory message. To better feature the aging process, we marked hair cell components (i.e. cilia, cytoskeleton, etc.) playing a key role in signal transduction along with SGNs bodies and fibers to correlate synaptopathy with sensory hair cell loss. In addition, the translational impact of our study is enhanced by the parallel acquisition of functional data by measuring the brainstem auditory evoked potentials which reflect the central auditory path.

TWO GAIN-OF-FUNCTION PORE VARIANTS IN KCNQ2 AND KCNQ3 POTASSIUM CHANNELS CAUSE DEVELOPMENTAL AND EPILEPTIC ENCEPHALOPATHY

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Developmental and epileptic encephalopathies (DEEs) are a group of rare disorders where neurodevelopmental impairment may be due to both the underlying cause and the effect of uncontrolled seizure activity. DEEs have often a genetic aetiology and KCNQ2 or KCNQ3, encoding for Kv7.2 and Kv7.3 voltage-gated potassium channel subunits, respectively, are among the most frequently affected genes. These channels form heterotetrametric complexes that contributes to excitability control of several neuronal populations.

In the present work, the clinical and *in vitro* functional features of two heterozygous *de novo* KCNQ2 and KCNQ3 variants, affecting a residue in the pore region of Kv7.2 (A317T) and the corresponding residue in Kv7.3 (A356T) subunits, are described. The A317T variant in KCNQ2 was identified in a patient with DEE (followed by Dr. Ingrid Scheffer, at the University of Melbourne, Australia), whereas the corresponding variant in KCNQ3 (A356T) in a patient with isolated intellectual disability (DDD Study, Nature, 2017). Defining variant-specific pathogenesis mechanisms will likely led to personalized treatments in KCNQ-associated DEE.

The *in vitro* functional properties of Kv7.2 channels incorporating A317T variant revealed a gain-of-function (GoF) effect, both in homomeric and heteromeric configuration with Kv7.3 channel subunits. Surprisingly, homomeric Kv7.3 A356T channels were not functional, whereas co-expression of Kv7.3 A356T with Kv7.2 channels caused an increase of the maximal current. These results provide the first evidence for GoF effect associated to DEE-causing variant affecting a critical residue in Kv7.2 pore.

ALTERED SYNAPTIC PLASTICITY IN HUMAN MODEL OF EPM1

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Progressive myoclonic epilepsy 1 (EPM1) is a neurodegenerative disease characterized by biallelic partial loss-of-function mutations in Cystatin B (CSTB) gene. CSTB is an inhibitor of lysosomal cathepsins and of the chromatin-specific cathepsin L and it has been shown to be involved in the development of human brain cortex. We previously demonstrated that CSTB is present in synaptosomes isolated from rat and mouse brain cortex, and is also secreted and locally synthesized, indicating its role in synaptic plasticity. Our aim is to investigate if synaptic physiology is impaired by pathological low levels of functional CSTB in human Cerebral Organoids (hCOs) from EPM1 patients. By western blot analyses, we show that synaptosomal fractions from control hCOs are enriched in synaptic proteins and CSTB is present at all developmental stages analysed. Only at late maturation stages, synapse becomes physiologically active, i.e. particularly enriched with exocytosis-associated proteins. CSTB presence in the synaptic territories was confirmed by immunostaining on neurons *in vitro*. Moreover, extracellular vesicles are present and secreted from the synaptosomes. Interestingly, in synaptosomes from EPM1 hCOs, the expression profile of all these proteins is significantly changed. It is worth to notice the relevant decrease of the eukaryotic initiation factor EiF4G2 observed specifically in EPM1 synaptosomes and not in the total lysate, which suggested the impairment of synaptic system of protein synthesis in the pathology.
These data show alteration of synaptic plasticity in EPM1, opening new venues toward the understanding of molecular mechanisms underlying the physiopathology of the disease.

PHARMACOLOGICAL STIMULATION OF AUTOPHAGY TO RESCUE PROTEINOPATHY AND COGNITIVE DECLINE IN MUCOPOLYSACCHARIDOSIS-IIIA

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Mucopolysaccharidosis IIIA (MPS-IIIA) is a lysosomal storage disorder (LSD) characterized by the loss of function of the sulfamidase gene (SGSH), responsible for the degradation of the glycosaminoglycan (GAG) heparan sulfate (HS). Undegraded HS leads to the formation of primary and secondary storages responsible for neurodegeneration and dementia in children (1). Favouring the degradation of secondary storages is one of the most promising therapeutic strategies to prevent neurodegeneration. Genetic overexpression of the transcription factor EB (TFEB), through the control of genes involved in the autophagy/lysosomal degradation process, seems to promote the degradation of protein aggregates in animal models of neurodegeneration (2). However, there are still few synthetic drugs capable to stimulate TFEB and to cross the blood-brain barrier. We have identified a compound that stimulates TFEB translation, and this way promote autophagy and lysosomal biogenesis. In this project, using validated animal and cellular models of MPS-IIIA, we have tested it in in vitro and in vivo models of MPS-IIIA.

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POTENTIAL ROLE OF THE MICROBIOME-ENDOCANNABINOIDOME CONNECTION IN THE GUT-BRAIN AXIS AFTER TRAUMATIC BRAIN INJURY AND ITS ASSOCIATION WITH ALZHEIMER'S DISEASE.

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Traumatic brain injury (TBI) is the leading cause of death under the age 45 in the Western World and can lead, especially for veterans who have experienced multiple brain injuries, to long-term consequences such as increased prevalence of dementia and Alzheimer's disease (AD). The main challenge in this area is the development of new diagnostic and therapeutic approaches. In this context, both the microbiota gut-brain axis and the endocannabinoid (eCB) system, which is part of a wider signaling system known as the "endocannabinoidome" (eCBome), seem to play a decisive role in the pathogenesis of, and may represent the missing link to understand the association between, TBI and AD.
The objective of the project is to investigate the effects of a mild TBI on the subsequent development of AD-related neuropathology and cognitive impairments in APP/PS1 mice, using a multidisciplinary approach. To date, our data show significant alterations in the behavioral phenotype of these transgenic mice induced by mild TBI. Additionally, the levels of the □-amyloid(1-42) peptide increased significantly in the cortex of APP mice subjected to mTBI. Targeted lipidomics analysis by LC-MS-IT-TOF and MALDI-MS imaging showed significant alterations of the eCBome and neurotrasmitters in the brain and intestine of these mice, as well as in the metabolome of their fecal microbiome. For example, we have identified for the first time *N*-acylserotonins in the brains of mice that underwent to mTBI, which also led to a reduction of butyrate and acetate in feces, as assessed by NMR-based metabolomics. The possibility that these changes might underlie part of the phenotype of mTBI- APP/PS1 mice is currently under investigation.

CORRELATION BRTWEEN RETINAL VASCULARIZATION AND DISEASE AGGRESSIVENESS IN AMYOTROPHIC LATERAL SCLEROSIS

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Abstract

Abnormalities in retinal vascularization and neural density have been found in many neurodegenerative diseases, however conflicting results are described in Amyotrophic Lateral Sclerosis (ALS). The aim of the present study was, therefore, to analyse retinal layers and vascularization by means of structural and Optical Coherence Tomography angiography (OCT-A) in ALS patients classified according to disease aggressiveness.

We enrolled forty-eight ALS patients and 45 healthy controls. ALS patients were divided into three groups: slow progressors (n=10), intermediate progressors (n=24) and fast progressors (n=14) according to the disease progression rate. For structural-OCT we evaluated the Subfoveal Choroidal Thickness (SFCT), Ganglion Cell Complex (GCC), Retinal Nerve Fiber Layer (RNFL). Regarding the OCT-A we assessed the vessel density (VD) in Superficial and Deep Capillary Plexuses, Radial Peripapillary Capillary Plexus, Choriocapillary and the Foveal Avascular Zone (FAZ) area.

Structural-OCT exam did not show any significant differences in GCC and RNFL thicknesses between patients and controls, and among the three ALS groups. The SFCT was statistically greater in patients compared with controls (357.95 ± 55.15 μm vs 301.3 ± 55.80 μm, $p<0.001$), interestingly the SFCT was thicker in patients with slow and intermediate disease progression than in those with fast disease progression (394.45 ± 53.73 μm vs 393.09 ± 42.17 μm vs 267.71 ± 56.24 μm, $p<0.001$). OCT-A did not reveal any significant results. Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) and disease duration did not correlate with any of OCT parameters, except for SFCT with ALSFRS-R ($r=0.752$, $p=0.024$).

This study demonstrated the possible association between higher SFCT and disease activity in SLA, due to inflammatory vascular phenomena. OCT could be useful biomarker in management of this neurodegenerative disease.

ELECTROENCEPHALOGRAPHIC FINDINGS IN ATRX SYNDROME: A NEW CASE SERIES AND REVIEW OF LITERATURE

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Alpha-thalassemia X-linked intellectual disability syndrome (ATRX) is a rare genetic condition caused by mutations in the ATRX gene characterized by distinctive dysmorphic features, alpha-thalassemia, mild-to-profound intellectual disability, and epilepsy, reported in nearly 30% of the patients. To date, different types of seizures are reported in patients with ATRX syndrome including either clonic, tonic, myoclonic seizures or myoclonic absences. However, an accurate analysis of electroencephalographic features is lacking in literature. We report on the epileptic and electroencephalographic phenotype of seven male patients (age range: 3–23 years) with a clinical and molecular diagnosis of ATRX syndrome, caused by de novo pathogenic variants in ATRX gene, identified at 5 Italian Epilepsy Centers. To the best of our knowledge, this is the first attempt to describe the electroencephalographic phenotype in patients with ATRX syndrome. This syndrome could share a common EEG trait: we have highlighting, in our small cohort, the presence of a peculiar EEG pattern characterized by diffuse background slowing with superimposed low voltage fast activity.

NOVEL APPROACHES TO IMPROVE COMPULSIVE DISORDER SYMPTOMS IN DOGS: 2 CASE REPORTS

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Like in humans, compulsive disorder in dogs (CD) is characterized by repetitive and out of context behaviors, that severely compromise their everyday life activities. Dogs who suffer from such a psychosocial disorder can exhibit circling, barking, fly biting, chewing on toys, self-mutilation, and acral lick dermatitis. Therapeutic interventions aimed at counteracting CD in dogs mainly rely on the prescription of antidepressants, like fluoxetine, desipramine or memantine, which are not devoid of some relevant side effects, such as gastrointestinal pain, anxiety, fatigue, or drowsiness. Moreover, these drugs seem to be sometimes less effective in improving CD symptoms, thus implying a significant reduction of benefits, and making owners even more unwilling to treat their dogs with psychotropic drugs. Therefore, trying to find out alternative and more effective strategies to face this psychosocial disorder represents a clinical unmet need. Here, we reported a newly published case of a German Shepherd mixed breed dog, affected by tail chasing, who was given the decapeptide α-casozepine as add-on to fluoxetine, along with a tailored behavioral recovery program. Finally, we documented the encouraging results about the efficacy of a peculiar off-label therapy (cannabinoids plus melatonin) in a CD mongrel dog, resistant to previous conventional treatment, in parallel to the behavioral rehabilitation training. Collectively, our proof-of-concept data highlight the importance to better characterize the potential impact of natural bioactive compounds and rehabilitation program upon compulsive behaviors in companion animals.

IDENTIFICATION OF A NOVEL CLASS OF SMALL MOLECULES FOR THE MODULATION OF TREM2 IN INFLAMMATION AND NEURODEGERNERATION

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We recently reported the synthetic derivative Sulfavant A (SULF A) as a novel immunomodulatory molecule [1]. SULF A action is mediated by TREM2 receptor [2], an innate immune receptor expressed mainly in the resident microglia cells of the central nervous system and in dendritic cells (DCs) and macrophages. TREM2 is an emerging receptor in the field of immunomodulation study associated to mechanisms that avoid the onset of excessive inflammatory processes linked to acute syndromes, as in the case of COVID-19, or which can lead to chronic states such as in some neurodegenerative and autoimmune diseases. A number of TREM2 variants have been identified as risk factors for a wide array of neurodegenerative diseases (NDs), including Nasu-Hakola disease and Alzheimer's disease.
SULF A stimulated the differentiation of myeloid cells towards a novel homeostasis-determining phenotype (homeDCs). This response involves the SYK-NFAT axis and is compromised by blockade or gene silencing of TREM2. Activation by SULF A preserves immune functions, including phagocytosis and antigen presentation, but is devoid of any inflammatory signature. This seems to represent a shift paradigm in innate immune cell response and, in microglial cells, corresponds to the differentiation of an unconventional non-inflammatory phenotype. These results prompt the idea that the investigation of TREM2 pathway may pave the way for the development of a new class of drugs with therapeutic potential in neurodegenerative diseases, chronic-inflammation and cancer.

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QUANTITATIVE PROTEOMIC PROFILING OF FRUCTOSE DIET-INDUCED HYPOTHALAMIC CHANGES IN ADOLESCENT RATS

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The increased consumption of fructose as added sugar represents a major health concern. Fructose-rich diet induces hypothalamic inflammation, dysfunction of insulin signalling and oxidative stress. Our aim was to point out early protein expression changes associated to fructose feeding. To this aim, adolescent rats were fed a fructose-rich (F) or control diet (C) for 3 weeks.
Proteomic investigation revealed a reduced expression of proteins belonging to mitochondrial respiratory complexes I, IV and V in F rats ($p < 0.01$). The amount of VDAC-1, an outer mitochondrial membrane protein, was reduced in F rats ($p < 0.05$). Further, the amount of PGC-1α, a coregulator of mitochondrial biogenesis, was lower ($p < 0.001$) in F group, while the level of the mitophagy marker PINK-1 was higher in fructose-fed rats ($p < 0.01$). Fructose-associated inflammatory status was also observed, as enhanced NFkB activation, higher levels of the astrocytic marker GFAP, and the rise of TNF-α, IL-6 and Haptoglobin were evidenced in F rats ($p < 0.01$). Interestingly we found decreased amount of both BDNF and its high affinity receptor TrkB in sugar-fed rats ($p < 0.01$). Further, lower amounts of Synaptophysin, Synaptotagmin and post-synaptic protein PSD-95 ($p < 0.01$) were detected in F rats.
In conclusion, a fructose-rich diet impairs mitochondrial compartment and the level of specific markers of brain function and plasticity. Due to the central role of hypothalamus as regulator of whole physiology, the early identification of molecular signs of cerebral dysfunction may contribute to a timely implementation of nutritional interventions.

CHARACTERIZATION OF POLY(ADPR)RIBOSYLATION REACTION IN THE BRAIN OF ADULT ZEBRAFISH EXPOSED TO ALUMINUM

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One of the first molecular responses to DNA damage is the activation of poly(ADP)ribosylation, a covalent and reversible post-translational modification of proteins, catalyzed by a family of enzymes known as poly(ADP-ribose)polymerases (PARPs). Nuclear PARPs play important roles in various cellular processes, including modulation of chromatin structure, transcription, replication, recombination, and DNA repair. These enzymes are considered the "sensors" of DNA damage because they are activated following damage to genomic material.
Aluminum has toxic effects on a variety of organs, including the brain, and in light of our previous studies, we aimed to evaluate PARP expression and activity and poly(ADPR) content in homogenates of adult zebrafish brain, exposed to 11 mg/L of this metal for 10, 15, and 20 days.
Western blotting analysis with anti-PARP antibody showed the presence of different enzyme isoforms having various molecular weights, but no significant alteration in their expression was observed in brain samples exposed to metal compared with the control. On the contrary, the data on PARP activity was significantly higher in samples exposed to Aluminum for 10 and 15 days, while it decreases at longer exposure times (20 days). This trend was also followed by the production of poly(ADPR). Our preliminary data lead us to hypothesize that DNA damage with consequent PARP activation and poly(ADPR) production occurs at short exposure times. At longer times of exposure, the reduction of PARP activity is necessary because it preserves the intracellular energy necessary to guarantee the survival of the fish.

L-CARNITINE AS A NEW PHARMACOLOGICAL ALLOSTERIC CHAPERONE OF THE HUMAN LYSOSOMAL A-GLUCOSIDASE FOR POMPE DISEASE THERAPY

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Pompe disease is an inherited metabolic disorder due to the deficiency of the lysosomal acid a-glucosidase (GAA). At the moment the only approved treatment is enzyme replacement therapy (ERT) with the administration of a recombinant enzyme (rhGAA). But the ERT presents several limitations.
Not all patients respond equally well to treatment and not all clinical manifestations are corrected.
Several approaches have been proposed for Pompe disease, included gene therapy, and a very promising one, proposed as a complementary approach to ERT, is therapy with pharmacological chaperones, based on the stabilising effect induced by small molecules on the target enzyme. However, most known chaperones could be limited by their potential inhibitory effects on patient's enzymes. Here we report on the discovery of a novel chaperone for rhGAA, L-carnitine, that stabilises the enzyme at pH and temperature without inhibiting the activity and acted synergistically with active-site directed pharmacological chaperones. Remarkably, L-carnitine enhanced by 4-fold the acid a-glucosidase activity in fibroblasts from three Pompe patients with added rhGAA. This synergistic effect of L-carnitine and rhGAA had the potential to be translated into improved therapeutic efficacy of ERT in Pompe disease, in fact preliminary clinical data show important improvements in treated patients.

THE SHORT-CHAIN FATTY ACID ACETATE REDUCES OREXIN/HYPOCRETIN NEURONAL ACTIVITY : POSSIBLE IMPLICATIONS IN THE HYPOTHALAMIC REGULATION OF ENERGY HOMEOSTASIS AND APPETITE

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Obesity has been linked with the abnormal composition and function of the gut microbiota, a condition known as “dysbiosis”. Evidence is accumulating regarding the role of major products of gut microbial fermentation of dietary fibers, the SCFAs (acetate, propionate, and butyrate) in the fine-tuning of the gut-brain axis communication linking gastrointestinal to brain functions.
Several studies report discordant effects of acetate on the hypothalamic regulation of appetite via the gut-brain axis, such as: 1) hypophagia, via enhancement of POMC mRNA after acute acetate i.p. injection (Frost et al., 2014), or 2) hyperphagia, following chronic intragastric infusion in chow-fed rats (Perry et al 2016). Here, we extended these observations to orexin-A, a hypothalamic peptide acting as a master regulator of energy homeostasis, wakefulness and arousal (Forte et al., 2020 for review). In the present study, NMR spectroscopy analysis of SCFA brain levels revealed a selective reduction of acetate in obese leptin ko ob/ob mice compared to aged-matched wild-type lean littermates. Exogenous acetate administration in obese mice was able to reduce food intake, orexinergic neuron firing, orexin-A expression and release, as demonstrated by c-FOS immunoreactivity and patch-clamp recording of orexin-A-eGFP neurons and prepro-orexin mRNA quantification. Confocal microscopy revealed induction of GPR43-immunoreactivity in the hypothalamus of obese compared to lean mice.
In summary, our data provide novel insights into the mechanism of decreased energy intake and body weight under chronic supplementation of acetate or fermentable complex carbohydrates with the diet, effects that might be partly attributable to the reduction of orexin-A production and inhibition of orexinergic neuron firing.

GENETIC AND MOLECULAR ANALYSIS OF A DE NOVO FOXP1 TRUNCATING MUTATION DETECTED IN A PATIENT AFFECTED BY AUTISM SPECTRUM DISORDERS (ASD)

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Forkhead box protein 1 (FOXP1) syndrome is a rare neurodevelopmental disorder characterized by global developmental delay, intellectual disability, and language delay, with or without autistic features. Located on chromosome 3p13, FOXP1 gene is a member of the FOX transcription factor family with a crucial role in language development.
Here we present a young female patient, now thirteen years old, with speech impairment carrying a novel de novo heterozygote truncating FOXP1 mutation, identified by WES analysis. She is the only child of unrelated parents. The family history is positive about neurological or neurobehavioral disorders in the two branches of parental derivation. She presented a delay in the acquisition of the stages of psychomotor development and began a diagnostic process by carrying out standard karyotype (46,XX) and array-CGH analysis, which did not show significant rearrangements. At our last clinical examination, she presented occipitofrontal circumference 54.5 cm (50th-75th centile) vs weight 45,5 kg (25th centile) and height 154 cm (25th centile); cubitus valgus was presents.
In lymphoblastoid cell lines (LCLs), derived from the heterozygous ASD patient and individual controls, the FOXP1 expression levels were measured. A dramatic reduction in FOXP1 mRNA and protein expression was detected in the proband compared with the controls, suggesting that this mutation leads to FOXP1 haploinsufficiency. We examined the effects of FOXP1 haploinsufficiency establishing a transcriptional impairment on direct target genes involved in language development. This study extends the phenotypic and allelic spectra of the FOXP1 syndrome and open new studies toward the molecular correction of FOXP1 dosage.

OREXIN IMPAIRS EPISODIC MEMORY IN OBESE MICE BY PROMOTING TAU PHOSPHORYLATION IN CA1-CA3 HIPPOCAMPAL PYRAMIDAL NEURONS VIA ENDOCANNABINOID-DERIVED LYSOPHOSPHATIDIC ACID

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The finding that obesity is accompanied by chronic low-grade multi-organ inflammation, dysbiosis, gliosis, leaky gut and leaky brain has fundamentally changed our approach to understanding the early causes of neurodegenerative disturbances like non-genetic Alzheimer's Disease (AD) [1]. The High-Fat composition of the Western Diet (HDF) represents a critical risk factor for neurodegeneration [2]. Orexin-A (OX-A) is an hypothalamic neuropeptide regulating appetite, energy homeostasis, and sleep-wake cycle. We and others demonstrated that OxA is a strong inducer of endocannabinoid [2-arachidonoylglycerol (2-AG)] biosynthesis in the brain of obese mice. We recently demonstrated that HFD-induced obesity is associated with enhancement of OX-A signaling, which triggers synaptic loss and impairment of adult neurogenesis and cognitive memory tasks [3]. Here we used behavioural tasks and integrative morphofunctional approaches, from multi-organ to single-cell imaging (patch-clamp cell recording, time-lapse cell imaging, confocal microscopy, CLEM and TEM) to dissect, in the CA1-CA3 fields of the hippocampus, a new OX-A-dependent molecular pathway that, by recruiting the endocannabinoid 2-AG-derived 2-arachidonoyl-sn-glycerol-3-phosphate (2-AGP), a bioactive lipid belonging to the class of lysophosphatidic acids (LPAs) [4], promotes TAU phosphorylation in obesity. A positive correlation between OX-A and 2-AGP levels in the serum of obese or AD human subjects was also observed.

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ANALYSIS OF THE HIF1□-KDM5C AXIS AND IDENTIFICATION OF A NEW PATHOGENIC PATHWAY IN GLIOBLASTOMA MULTIFORME (GBM)

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Glioblastoma multiforme (GBM) is the most aggressive brain tumour without an effective pharmacological treatment. We analyzed the levels of the cancer driver gene Lysine (K)-specific demethylase 5C (KDM5C) in a pilot series of GBM tissues. *KDM5C* belongs to the Jumonji C domain-containing histone demethylase family involved in various types of cancers. This chromatin enzyme catalyzes the removal of the methyl groups from di- and tri-methylated lysine 4 on histone H3 in a Fe (II)- and α-ketoglutarate-dependent manner. By using real-time quantitative PCR and Western blotting analysis, we found an altered abundance of KDM5C transcript and protein in GBM samples identifying patients with higher ($KDM5C^{High}$) and lower ($KDM5C^{Low}$) levels compared to control samples. By exploring the impact of the defective KDM5C quantity, a positive and negative relationship respectively with hypoxia-inducible transcription factor-1α (HIF-1α) and BDNF levels were found in $KDM5C^{High}$ patients. KDM5C overexpression and hypoxic studies performed in glioblastoma cell line (T98G) suggest that the stimulation of KDM5C expression is preceded by the induction of HIF-1α. High levels of HIF1α-KDM5C axis was also found associated with high levels of NANOG, SOX2 and NESTIN in GBM tissues isolated from conventional and 5-aminoleveulinic acid (5-ALA) fluorescence-guided surgery (FGS). A pro-inflammatory condition was also detected in 5-ALA FGS highlighting differences across the GBM microenvironment. Taken together, our study reveals for the first time a correlation between the HIF-1α-KDM5C axis and GBM opening a new field of investigation to validate KDM5C as a new GBM biomarker.

SYSTEMIC INFLAMMATION AFFECTS GROWTH AND SHAPE OF MICROGLIA IN ALZHEIMER'S DISEASE PATIENTS

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Alzheimer's disease (AD) is a progressive neurodegenerative disorder associated with ageing [1]. Emerging evidence suggests that it is not restricted to the neuronal compartment but also includes other brain populations such as astrocytes and microglia [2]. In addition, systemic peripheral inflammation has been suggested as risk factor in AD [3]. Interestingly, monocytes change phenotype and function with age and produce more pro-inflammatory cytokines, such as IL6 and TNFα. However, whether this contributes to AD onset and/or progression is still controversial [4]. Particularly, increased levels of IL6 and TNFα correlate with risk of classical "diseases of age" such as dementia, cardiovascular disease as well as frailty and all-cause mortality [5-6]. Herein, we aim to investigate whether alterations in the levels of peripheral inflammatory factors might predispose to the risk of developing AD.
Blood samples were collected from 130 subjects: 33 healthy volunteers, 30 patients with subjective cognitive impairment (SCI), 30 with mild cognitive impairment (MCI), 37 with AD.
ELISA analysis showed that Aβ42 and TNFα levels were both increased in the plasma of AD patients when compared to SCI, MCI and healthy individuals. Moreover, healthy microglia exposed to plasma from 4 healthy subjects, 4 patients with MCI, 4 SCI and 4 AD changed morphology and shape. Plasma from AD patients activated proinflammatory signals in healthy microglia and triggered the amyloidogenic APP processing to produce Aβ42.
All together, these findings advise that systemic inflammatory pathways may activate an AD like phenotype in healthy microglia and trigger neurodegenerative processes.

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THE FIRST TWO YEARS AFTER THE REWARMING: EXPLORING THE RELATIONSHIP BETWEEN NEONATAL MRI AND THE DEVLOPMENTAL PATHWAYS OF INFANTS WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY TREATED WITH THERAPEUTIC HYPOTHERMIA

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Introduction: Hypoxic-Ischemic Encephalopathy (HIE) affects around 1-to-9 per 1000 live births and it is an important cause of neurodevelopmental abnormalities. Magnetic Resonance Imaging (MRI) is the gold standard for evaluate brain injury. However, an optimal MRI scoring system is still missing. This study aims to explore the relationship between neonatal MRI findings rated using Okereafor scoring system and neurodevelopmental outcomes at 12-24 months of age in HIE infants treated with therapeutic hypothermia (TH).
Methods: Retrospective observational single-center study. Thirty-one infants born ≥36 GA with signs of moderate-to-severe HIE treated with TH between January 2017 and December 2021. MRI scan was performed within the first postnatal week after rewarming. MRI scans were evaluated through Okereafor score by two raters blinded to infant outcomes. Infants were assessed by means of Griffiths Mental Development Scale (GMDS) at 12-24 months. Spearman's rank correlation was run among MRI scores and GMDS outcomes at 12-24 months.
Results: At 12 months, MRI score significantly correlated with Socio-emotional skills, Language, Eye-hand coordination abilities, Performance subscale and the general Developmental Quotient (DQ). At 24 months, MRI score was associated with Motor impairments, Socio-emotional skills, Language and the DQ.
Conclusion: This study confirmed the wide association among neonatal MRI findings and neurodevelopmental outcomes as soon as 12 months in HIE infants. This association appears stable during the first two years of life. Finally, this study found an association among Okereafor score and specific developmental domains. This could lead to implement early focused interventions in order to promote infant development.