Abstract book

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Biotechnology and Nanomedicine

P23 - The Extent of Human Apolipoprotein A-I Lipidation Strongly Affects the beta-Amyloid Efflux Across the Blood-Brain Barrier in vitro

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Object. Failure in the clearance of beta-amyloid peptide from the brain is considered a risk factor in Alzheimer's disease (AD). Many studies show that the levels of high-density lipoproteins (HDL) and their main apoprotein, apoA-I, decrease in the plasma and cerebrospinal fluid of AD patients, respect to age-matched healthy controls. This suggests a protective role of HDL in AD pathology. The biogenesis of nascent HDL derives from a first lipidation of apoA-I, which is synthesized by the liver and intestine but not in the brain, in a process mediated by ABCA1. The maturation of nascent HDL in mature spherical HDL is due to a subsequent lipidation step, LCAT-mediated cholesterol esterification, and the change of apoA-I conformation. Therefore, different subclasses of apoA-I-HDL simultaneously exist in the blood circulation.

The present in vitro study aims to investigate if and how the lipidation state affects the ability of apoA-I-HDL to target and modulate the cerebral beta-amyloid content from the periphery, that is thus far unclear.

Methods The total HDL pool, spherical HDL and lipid-free apoA-I were purified from the human plasma of healthy donors. Discoidal HDL were obtained by reconstructing apoA-I in phosphatidylcholine/cholesterol (1:1 M/M) matrix. The ability of the different subclasses to cross an in vitro model of blood-brain barrier (BBB), made of hCMEC/D3 cells seeded on transwell inserts, and to enhance the Aβ efflux from the "brain"-side of the transwell was assessed by biochemical assays. The lipoproteins effect on Aβ disaggregation was evaluated by atomic force microscopy.

Results The results showed that discoidal HDL displayed a superior capability to promote beta-amyloid efflux in vitro (9×10^{-5} cm/min), when compared to apoA-I in other lipidation states. In particular, no effect on beta-amyloid efflux was detected when apoA-I was in mature spherical HDL, suggesting that apoA-I conformation, and lipidation could play a role in beta-amyloid clearance from the brain. Finally, when apoA-I folded its structure in discoidal HDL, rather than in spherical ones, it was able to cross the BBB in vitro and strongly destabilize the conformation of beta-amyloid fibrils by decreasing the order of the fibril structure (-24%) and the beta-sheet content (-14%).

Conclusions Our findings suggest that the extent of apoA-I lipidation, and consequently its conformation, may represent crucial features that could exert their protective role in AD pathogenesis.

P24 - Radiotherapy and Adjuvant Drug-Loaded Liposomes target Glioblastoma Stem Cells and Trigger Immune Response

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Object Main prognostic factor for GBM survival is the extent of surgical resection. However, due to the infiltrative capacity of GBM cells complete eradication is most of the time impossible to achieve. Therefore, to control tumor growth, and ultimately cure patients, it is essential to develop treatment strategies to kill therapy refractory cells and to mount robust immunosurveillance to prevent disease recurrence. The radio- and chemo- resistance of GSCs together with their innate tumor-initiating aptitude, make this cell population a crucial target for effective therapies. However targeting GSCs is hardly difficult and complex, due to the presence of the BBB and for GSCs infiltrative nature arousing their dispersion within the brain parenchyma.

Methods To enable BBB crossing, selective GSCs targeting and anti-tumor immune response activation, doxorubicin as paradigm of cytotoxic drug triggering ICD, was encapsulated into LIPs surface-functionalized with an ApoE-derived peptide (mApoE). Results Our results indicate that

encapsulation into mApoE-LIPs prevents DOXO toxicity on BBB cells and enhances its accumulation within mouse brain in vivo. mApoE confers GSC specificity through the engagement of the Low-Density Lipoprotein Receptor. When administered to patient-derived GSC NOD/SCID mouse xenograft mApoE-DOXO-LIPs, but not DOXO-LIPs, triggered GSC apoptosis resulting in a remarkable reduction of tumor growth and invasion of the contralateral hemisphere through commissural fibers. Apoptotic GSCs prompted microglia/macrophage phagocytic activity coupled to the activation of the antigen-presenting machinery propaedeutic to T cell priming. Importantly, the concomitant administration of radiation (2Gy) enhanced the anti-tumor effects by altering BBB permeability and promoting the expression of LDLR on both BBB and GSCs.
Conclusions Our results advocate for RT and adjuvant administration of drug-loaded targeted nanovector as an effective strategy to deliver cytotoxic molecules, ICD inducers particularly, circumventing BBB hurdles and targeting GSCs at the tumor burden, the forefront of GBM recurrence. The proposed combined approach responds to the need of selective targeting of the GBM stem cells remaining after surgery within the irradiated field while preserving healthy brain by harmful side effects.

P25 and SELECTED ORAL COMMUNICATION - Differential exchange of multifunctional liposomes between glioblastoma cells and healthy astrocytes via tunnelling nanotubes

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Object Despite advances in cancer therapies, nanomedicine approaches included, the treatment of glioblastoma (GBM), the most common, aggressive brain tumour, remains inefficient. These failures are likely attributable to the complex, and not yet completely know, biology of this tumour, which is responsible for its strong invasiveness, high degree of metastasis, high proliferation potential and resistance to radiation and chemotherapy. The intimate connection through which the cells communicate between them plays an important role in these biological processes. In this scenario, tunneling nanotubes (TnTs) are recently gaining importance as a key features in tumor progression and in particular in the re-growth of GBM after surgery.
Methods: In this context, we firstly identified structural differences of TnTs formed by U87-MG cells, as model of GBM cells, in comparison to those formed by normal human astrocytes (NHA), used as a model of healthy cells. Successively, we have studied the possibility to exploit U87-MG TnTs as drug-delivery channels in cancer therapy, using liposomes composed of cholesterol/sphingomyelin and surface functionalized with mApoE and chlorotoxin peptides (Mf-LIP) as nanovehicle model.
Results: The results showed that U87-MG cells formed almost exclusively thick and long protrusions, while NHA formed more thin and short TnTs. Considering that only thick TnTs are really active in transport of vesicles and organelles, we showed that fluorescent-labelled Mf-LIP can be transported via TnTs between U87-MG cells, but not through the protrusions formed by NHA cells. Moreover, Mf-LIP boost the formation of thick TnTs in U87-MG cells, where they localized, but not in NHA cells, probably due to the interaction between ClTx and Annexin A2, which is involved in the formation of cell protrusions and in the high plasticity of GBM cells.
Conclusions: Our results demonstrate that nanotubes are potentially useful as drug-delivery channels for cancer therapy, facilitating the intercellular redistribution of this drug in close and far away cells, thus reaching isolated tumour niches that are hardly targeted by simple drug diffusion in the brain parenchyma. Moreover, the differences identified in TnTs formed by GBM and NHA cells can be exploited to increase treatments precision and specificity.

Clinical Neuroscience

P26 - Repetition deficits in the different primary progressive aphasia variants

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Object The present study aimed to investigate single-word repetition ability in three variants of primary progressive aphasias (PPA): non-fluent PPA (nfPPA), semantic dementia (SD) and logopenic variant PPA (lvPPA). Based on prior literature, and in reference to Patterson's two-way model of repetition, we expected that repetition deficits observed in lvPPA would be due to impairment of the phonemic buffer unit, while nfPPA would show difficulties at the

articulatory planning level. In SD patients, repetition should be spared.
Materials Thirty-three patients (8 nfPPA, 15 SD, 10 lvPPA) and 10 healthy elderly controls underwent a word/non-word repetition task, including words from four psycholinguistic categories: frequent concrete nouns, rare concrete nouns, frequent abstract nouns and function words. In addition, a verbal short-term memory test (Digit span) was administered to all participants.
Methods Comparisons of repetition performance and digit span between patient groups and controls was carried out both at a group- and at a single-case level. Performance on the Digit span was evaluated in terms of order errors and item errors.
Results Patients with lvPPA were mildly impaired on the repetition task and moderately impaired on the short-term memory task, producing predominantly item errors. Patients with nfPPA showed major deficits in repeating non-words and rare and abstract nouns than frequent concrete nouns and a mild impairment of digit span, with slightly more item errors. SD patients had preserved span and relatively preserved repetition, however they showed more difficulties in repeating frequent concrete nouns than rare or abstract nouns.
Discussion Our findings in lvPPA agree with prior evidence showing that these patients 1. are only minimally impaired on repetition of single words, 2. show significant short-term memory deficits (that underpin their characteristic inability to repete sentences). In the nfPPA group, relative sparing of repetition of frequent nouns might be due to the fact that their articulation might require less voluntary control than articulation of the other categories. As to the paradoxical effect found in SD, we hypothesize that it might follow from strong activation of the non-lexical pathway for rare and abstract nouns, whose meaning is compromised by the semantic deficit, coupled with weak activation of the same pathway for frequent and concrete nouns, whose intact meaning tends to automatically activate the dysfunctional lexical pathway.
Conclusions Repetition and verbal short-term memory are variably affected in different forms of PPA and should be assessed for an accurate discrimination of patients with nfPPA, SD or lvPPA.

P27 - Anti-TDP-43 autoantibodies are increased in ALS serum

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Object: Amyotrophic lateral sclerosis (ALS) is a relentless neurodegenerative disorder preferentially affecting motoneurons. TDP-43 inclusions are typically found in affected neurons of ALS patients. Remarkably, the presence of these inclusions has been shown also in peripheral mononuclear cells (PBMC), raising suspicion on a potential functional derangement, in a disorder for which the contribution of neuroinflammation is beyond all doubt. Presumably, the adaptive immune response against TDP-43 includes the lymphocyte production of specific naturally-occurring auto-antibodies (NAb) that have never been previously demonstrated.
Aim of this work consisted in testing the hypothesis that TDP-43 NAb could be measured in human serum samples and assessing a putative difference between ALS patients and controls.
Materials & Methods: n=70 ALS outpatients diagnosed according to El Escorial criteria were recruited. n=40 sex- and age-comparable heathy controls (CTRL) and n=20 typical Alzheimer's disease (AD) patients were recruited as well according to IWG-2 criteria. A specific custom-made ELISA test was used for assessing in serum samples TDP-43 Nab level, while a commercial ELISA (LSBio) was used for assessing TDP-43 protein levels.
Results: Total serum anti-TDP-43 NAb levels were doubled with respect to both CTRL and AD ($p<0.001$); this result did not change when considering the ratio with total IgG, which were not different in ALS patients versus control groups. Serum TDP-43 soluble levels were also about fourfold increased on average in ALS patients with respect to CTRL ($p<0.001$) but not to AD that displayed a more dispersed pattern. No correlation was found between TDP-43 and cognate NAb serum levels ($p=0.14$). A correlation was found between TDP-43 serum levels and the DPI ($r=0.41$ $p=0.001$). No correlations were found with all the other collected variables.
Discussion: In this work, we report for the first time the presence of anti-TDP-43 NAb in human serum samples, demonstrating a significant increase of this NAb titer in ALS patients. The specificity of this finding was highlighted by including a group of patients affected by AD, a neurodegenerative condition that should not entail altered TDP-43 proteostasis. This increase is apparently unrelated to clinical and

demographic parameters and does not correlate with the serum levels of the cognate protein as well.
Conclusions: anti-TDP-43 Nab are increased in ALS serum; future work will clarify the significance of this increase, potentially playing a pathogenic role in ALS establishment and progression.

P28 - Innovative protocol targeting cognitive dysfunction in multiple sclerosis: HD-tDCS to enhance cognitive training in a randomized, double-blind, controlled, exploratory pilot study

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Objective: to test the efficacy for relapsing-remitting MS (RRMS) patients of our innovative cognitive remediation protocol, characterized by a non-conventional focal high-definition transcranial direct current stimulation (HD-tDCS) on the left dorsolateral prefrontal cortex (DLPFC) to support and boost the effects of a computer-based cognitive training (CCT) in improving frontal-executive abilities, in a randomized, double-blind, sham-controlled exploratory pilot study. We also propose to: a) assess protocol feasibility and safety; b) evaluate its medium/long-lasting effect; c) estimate the extent of changes in cognitive abilities and verify any widespread effect; d) improve the understanding of the mechanisms of neuroplasticity and brain recruitment through any neurophysiological changes; e) indagate any related changes in other clinic-behavioural measures.
Matherials: Fourty-four RRMS patients with predominant deficits in information processing and working memory will be selected. They will be randomised and equally divided to: 1) real HD-tDCS + CCT (experimental group); 2) sham HD-tDCS + CCT (control group).
Methods: Study treatment will last 40 minutes/day, 5 day/week for 2 weeks. CCT will focus on improving fronto-executive skills. HD-tDCS will be administered on the left DLPFC with a 4x1 ring electrode placement and at an intensity of 2mA (real stimulation) for the first 20 minutes of the protocol. Pre- and post-treatment and at 3- and 6-months follow-ups, participants will undergo neuropsychological, neurological and neurophysiological measurements. To assess changes over time, a repeated-measures analysis of variance will be applied. Functional and effective cerebral network connectivity will be calculated using phase-based metrics and Granger causality analysis. Statistical significance will be set at $p < 0.05$. The relationship between clinico-demographical measures and cognitive/behavioural/physiological measures will be assessed using the correlation coefficient. Descriptive analyses will be provided for feasibility (overall compliance) and for safety (any tDCS-related discomfort/side effect or adverse event).
Results: We expect an improvement in cognitive performance in both groups, boosted in our experimental arm and not confined to general frontal-cognitive abilities; potential changes would be reflected also by neurophysiological measures and in QoL.
Discussion: Our hope is to provide additional treatment tools for RRMS subjects, with a medium-long term efficacy and an extensive effect. This exploratory pilot study will help us to set the rationale for future studies, providing preliminary data useful for selecting the best primary outcome and for calculating a better sample size.
Conclusion: If proven effective, this combination of non-invasive, non-drug-based cognitive treatment approaches would be a promising therapeutic alternative for MS population.

P29 - DBI serum levels are increased in delirium and in Alzheimer's dementia: may neuroinflammation drive agitation?

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Object: An increasing amount of data confirms the presence of a mutual relationship between Alzheimer's dementia (AD) and delirium, suggesting that these two different conditions share common pathogenic mechanisms. Neuroinflammation, in particular, represents the plausible candidate link, since playing a major pathogenic role in both. A special role in neuroinflammation is played by peripheral monocytes that are chemoattracted into the brain fueling the damaging process. The Diazepam Binding Inhibitor (DBI) is the ligand of the TSPO, a receptor expressed by both activated

microglia and peripheral monocytes. For this reason, DBI may play a major role in transversely modulating the neuroinflammatory process between the CNS and the periphery. Aim of this preliminary work consisted in assessing DBI serum levels in delirium and AD patients with respect to healthy controls.
Materials & Methods: n=20 delirium patients were screened by the 4AT scale and recruited from the Neurology wards, together with n=20 matched healthy controls (CTRL) and n=110 AD outpatients. Serum DBI was assessed by commercial ELISA (AB Frontier).
Results: DBI serum levels were more than fourfold higher in delirium patients and twofold higher in AD with respect to CTRL (ANOVA $p<0.0001$, followed by Newman-Keuls post hoc test $p<0.001$ CTRL vs. AD and delirium, $p<0.001$ AD vs. delirium).
Interestingly, DBI serum levels correlated to the Neuropsychiatric Inventory (NPI) total score ($r=0.21$ $p<0.05$) and with the Agitation/Aggression NPI subscore ($r=0.29$ $p<0.01$). Finally, cognate CSF samples were available n=23 AD patients: DBI CSF levels correlated to serum ones ($r=0.50$ $p<0.05$) and to total-Tau CSF levels ($r=0.57$ $p<0.01$).
Discussion: Increased DBI serum levels in delirium and AD marks the shared neuroinflammatory landscape both in periphery and the CNS. Notably, DBI levels seems to be increased in particular in agitated patients.
Conclusions: Further studies may disclose if serum DBI may be useful as an agitation marker in dementia, offering new perspectives for effective treatments.

P30 and SELECTED ORAL COMMUNICATION - Effects of Direct Current Stimulation: in vitro study on human neuroblastoma SH-SY5Y cells.

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Object: to elucidate the mechanisms of action, both at cellular and molecular level, of transcranial Direct Current Stimulation (tDCS), especially focusing on its possible on-line and off-line effects on the expression, aggregation and degradation of alpha-synuclein (asyn), the pathogenic protein that accumulates in the brain of patients with Parkinson's disease (PD).
Materials: human neuroblastoma SH-SY5Y cells were used both under basal conditions and after exposure to rotenone (400 nM, 24 hours), a PD-related toxic able to inhibit mitochondrial complex I.
Methods: cells underwent direct current stimulation (1 mA, 20 mins) applied using a battery-driven constant stimulator and a pair of electrodes in two saline-soaked synthetic sponges (3x3 cm area). At the end of stimulation (recovery 0, R0) and after 1 (R1) and 17 hours (R17), cellular morphology was assessed under optic microscope, cell pellets collected and stored for the evaluation of gene and protein expression by real-time PCR and Western blot, respectively, of asyn and its main autophagic catabolic pathways macroautophagy and chaperone-mediated autophagy (CMA).
Results: in standard culture conditions, no morphological alterations and no cytotoxicity were observed after exposure of cells to electric field; electric field induced an increase of monomeric asyn at R1 and R17, with a reduction of asyn oligomeric forms at all time points. The macroautophagy markers beclin-1 and LC3 was reduced at R17, with an increase in the substrate p62. An increase of CMA substrate MEF2D was also found after exposure to electric field. Moreover, the electric field was able to reduce the increase of asyn induced by rotenone, with no direct effect on asyn mRNA levels. In rotenone-treated cells, electric field reduced p62 levels and induced a mild increase in LC3II expression. Similarly, electric field decreased MEF2D expression and induced an increase in the CMA receptor LAMP2A.
Discussion: these results indicate that the exposure of cells to electric field is able to exert on-line and off-line effects on the expression and aggregation status of asyn; furthermore, in presence of a synucleinopathy (as obtained after rotenone exposure), electric field counteracts asyn accumulation, not through a modulation of its gene transcription, but likely potentiating asyn autophagic degradation.
Conclusions: electric field shows a therapeutic potential in modulating the toxicity related to aggregation of asyn and, possibly, of other aggregation-prone proteins associated to neurodegenerative diseases.

P31 and SELECTED ORAL COMMUNICATION - Risk of ALS in Professional Soccer Players

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Background: Since the observation of several deaths from ALS among Italian professional soccer players, an association between ALS and soccer has been postulated. Repeated injuries are reported more frequently by ALS patients than the general population. An association between exercise and ALS has also been suggested, but results are conflicting.
Research hypothesis
No studies verified the incidence of ALS in former soccer players with a long follow up period. In addition, published reports have methodological limitations. For these reasons, the relationship between ALS and soccer is still unknown. On this background, we carried out this study with the aim to quantify the risk of ALS in a wider well-structured cohort of former professional soccer players with a long-term follow up.
Design/Methods: All professional soccer players who practiced in the period 1959-2000 were identified through the archives of the Panini soccer cards, the major Italian football cards publisher. These individuals represent the exposed cohort. For each player, date and place of birth, playing role and team history were recorded. Each player was followed since the year of start of professional activity. Incident ALS cases among the exposed were all soccer players first diagnosed with ALS during the period 1959-2016. The expected incidence rate was the number of cases/100,000 person-years expected in the cohort using a well-defined Italian population for reference. Standardized incidence ratio (SIR) was the ratio between observed and expected incidence rate.
Results: The study cohort (23,875 players) was followed for 1,012,337 person-years. 33 individuals in the cohort developed ALS. The number of expected cases was 17.6. The SIR was 1.8 (95% CI 1.3-2.6) in the entire sample and 4.9 (95% CI 2.8-7.8) in subjects aged less than 45 years at diagnosis. The median age at diagnosis was 43.3 yr. Compared to the median age of onset of ALS in the general population (65.2 yr; range 56.0-72.2 yr), the disease in former soccer players occurred 21.9 years earlier.
Conclusions: Soccer players are at higher risk of developing ALS than the general population. The disease develops at an earlier than expected age. Soccer players with ALS might be susceptible individuals who develop the disease in response to combinations of environmental factors.
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P32 - Good- or bad-responders? A 'novel' single-case statistical method to evaluate pharmacological treatment effectiveness through neuropsychological changes in amnesic mild cognitive impairment

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Object Neuropsychological changes are thought to reflect trends in disease progression when monitoring the effectiveness of pharmacological treatment with acetylcholinesterase inhibitors (AChEI) in patients with amnesic mild cognitive impairment (a-MCI).
Due to high inter-individual variability in the response to AChEI treatment, psychometric instruments usually administered by clinicians might fail to detect quantitatively subtle but clinically significant modifications in neuropsychological functioning when group analyses are conducted. Single-case approaches can increase statistical validity of longitudinal assessments. The aim of this study is to provide a proof of concept for a practical single-case statistical method to evaluate AChEI treatment effectiveness in patients with a-MCI in clinical settings.
Materials Three consecutive a-MCI patients undergoing AChEI treatment were recruited according to Petersen's criteria (2004). Treatment effectiveness was assessed by evaluating anterograde long-term episodic memory. Ad hoc use frequency-, length in syllables- and semantic category-balanced word lists were created and used as verbal learning test material.
Methods The C statistic for single-case time series analyses [Neumann, 1941; Young, 1941] was adopted. C tests the null hypothesis that a time series/time series aggregations follows a random

trend. Eight observations are sufficient for its test statistic z to approximately be normally distributed (with a critic z = 1.64 at α = .05 for a bidirectional hypothesis [Young, 1941]). Eight different 15-words lists were thus administered at 3 time points according to an A-B-A' repeated measures design: before the beginning of treatment (A; baseline), after 8 weeks of treatment (B; intervention) and after 2 wash-out weeks from treatment suspension occurred at time B (A'; inversion). The outcome variable was the number of words immediately recalled. Patients were classified as good- or bad-responders to AChEI treatment depending upon the significance of AB and BA' time series aggregations trends.
Results One patient was classified as a good-responder (AB and BA' $p < .01$; AA' $p > .05$). Two patients were classified as bad-reponders (no significant trends).
Discussion It has been provided a proof of concept that C statistic can discriminate between a-MCI patients who do and do not benefit from AChEI treatment. This method could be extended to other clinical scenarios requiring single-patient-level analyses due to high inter-individual treatment response variability.
Conclusions Single-case approaches are encouraged when evaluating effectiveness of AChEI treatment in a-MCI. The C statistic subserves this aim and is suitable for clinical settings since it requires a small number of observations.

P33 - Does all music feel the same? Musical anhedonia after right brain-damage: an hemispheric difference

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Musical anhedonia is a condition characterized by an impairment in experiencing emotion and expressing aesthetical judgments while listening to music. This study aimed at investigating the putative presence of a defective processing of the aesthetical and emotional components of music appreciation in brain-damaged patients, and at testing the validity of an instrument, devised to assess liking and emotions in music. Left- (N=15, 6 females, mean age=70, SD=9.8, years of schooling=10.9, SD=4.9) and right- (N=15, 7 females , mean age=67.8, SD=9.2, years of schooling=12.1, SD=4.6) brain-damaged cerebrovascular patients and healthy control participants (N=30, 16 females, mean age=67.7, SD=8.6, years of schooling=11.7, SD=2.8) were given a behavioural task requiring listening to 45 musical excerpts, and expressing two types of appraisals: i) "aesthetical" or "liking", ii) "emotional" judgments. Right-brain damaged patients showed a flat pattern in both tasks, while patients with left-brain damage proved to be able to distinguish among different categories as much as healthy participants. In all groups the two judgements were related. The present results suggest that "liking" and "emotional" judgments in music are related, and impaired after right-brain damage. The impairment is unrelated to presence of unilateral spatial neglect, suggesting that the two disorders are independent.

Cognitive and Behavioural Neuroscience

P34 - Hebbian associative plasticity drives the emergence of motor resonance: a novel paired associative stimulation protocol

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Objectives. Hebbian learning, and spike-timing-dependent plasticity (STDP), its neurophysiological basis, has been implied in the formation of the association between sensory and motor representations of actions in the Mirror Neuron System (MNS). At date, such inductor role of STDP still needs empirical support[1]. To address this issue, we have assessed whether a paired associative stimulation (PAS) protocol, a protocol known to activate STDP[2], can induce the formation of atypical (i.e., absent in normal conditions), visuo-motor associations, in turn reshaping motor resonance.
Methods & Materials. Twenty healthy participants underwent our novel mirror-PAS (m-PAS) protocol during which they were exposed to 180 repeated pairings of transcranial magnetic stimulation (TMS) pulses, applied over the right primary motor cortex (M1), time-locked with the view of index-finger movements of the right (ipsilateral) hand at a frequency of 0.2 Hz. In two different sessions, the

inter-stimulus interval (ISI) between the onset of the visual action stimulus and TMS pulse was varied following the chronometry of motor control (25 ms) or that of MNS activation (250 ms). Before and after each m-PAS session, motor resonance was assessed by recording Motor Evoked Potentials (MEPs) induced by single-pulse TMS applied to the right M1, during the observation of both contralateral (left) and ipsilateral (right) index-finger movements or static hands.

Results. As expected from literature[3], before m-PAS, a facilitation of cortico-spinal excitability (i.e., MEPs) occurred only during the view of left, contralateral (with respect to the TMS side) index-finger movements. Importantly, the m-PAS successfully induced new ipsilateral motor resonance responses, indexed by an atypical facilitation of cortico-spinal excitability by the view of ipsilateral (i.e., right) hand movements. Crucially, this effect occurred only if the associative stimulation followed the chronometry of motor control (ISI of 25 ms).

Discussion & Conclusions. The present findings provide empirical evidence that STDP, and thus Hebbian learning, shapes the visuo-motor matching properties of the MNS, which could be modulable by PAS. The m-PAS represents a promising non-invasive protocol to shed light on the neurofunctional bases of the human MNS.

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P35 and SELECTED ORAL COMMUNICATION - Do 'mirror neurons' get old? A functional MRI study on action prediction in young elderly participants.

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Object. The ability to predict the unfolding of another person's action (action prediction) and to adapt accordingly is crucial for successful interpersonal coordination. Age-related changes in this domain have remained unexplored so far. Here we evaluated whether the neurophysiological and cognitive plasticity that occurs in healthy aging also determines age-related changes in the ability to anticipate the conclusion of action observed in others.

Materials and methods. We characterized the behavioral performance and the neural correlates of action prediction in a group of younger and older healthy volunteers. During functional MRI (fMRI), the participants were required to observe sequences of pictures where an implied-motion posture (depicting a pointing or grasping action at mid-flight) was followed by an action conclusion (a hand touching an object by grasping vs. pointing to it). Participants had to judge whether the action conclusion matched the preceding implied-motion posture. As a control task, participants performed a color-discrimination task.

Results. Although older adults showed a general slowdown in response times, this was not specific for the action prediction task, and no group difference in accuracy was found. fMRI results revealed that this was associated with the recruitment, in older participants, of alternative neural resources to solve the action prediction task. Younger participants showed significantly higher recruitment of premotor and parietal areas, typically involved in the motor simulation of observed actions, during the observation of the implied-motion posture. On the contrary, older participants showed hyperactivation of higher-order visual areas when responding on whether the action conclusion was matching the mid-flight posture observed previously. We interpret this as an indication of greater reliance on post hoc visual comparison strategies.

Discussion and Conclusions. Altogether, these results suggest that aging entails reduced efficiency in the sensorimotor processes involved in reading on-line the movements of others, which are compensated by alternative strategies supporting high levels of performance levels in healthy older observers.

P36 - Clustering applied on behavioral data reveals right hemisphere dominance for reading in a group of left-handers.

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Neuropsychological observations, together with recent experimental evidence on right-handers have suggested that the Right Visual Field (RVF) advantage over the Left Visual Field (LVF) for lateralized reading might mirror the existence of a proficient orthographic lexicon in the left hemisphere and a

more rough lexical store in the right hemisphere. In this work, we wanted to explore to what extent such functional architecture could be valid also for left-handers, who are known to display a mixture of left-hemisphere-dominant, ambilateral, and right-hemisphere-dominant functional lateralization patterns (Branch, Milner & Rasmussen, 1964, Mazoyer et al., 2014).
For this reason, we used an eye-tracking-controlled lateralized lexical decision task to compare the performance of 60 right-handers with that of 60 left-handers. These latter were either considered as a group (hand-preference-based analyses), or with reference to their behavioral laterality patterns (cluster-based analyses), after a clustering procedure was applied on a laterality index.
The former set of analyses revealed an RVF advantage in right-handers as well as in left-handers, at least when considered as a group. The second set of analyses revealed a subgroup of left-handers who show the same RVF advantage as right-handers, a subgroup showing no consistent hemispheric dominance and a subgroup showing an advantage for the LVF over the RVF. The same procedure applied to right-handers did not yield any cluster of right-hemisphere-dominant subjects. The left-handers' subgroup showing an LVF advantage provided evidence for a completely reversed organization of orthographic lexical knowledge with respect to subjects showing left-hemisphere dominance. Indeed, contrary to right-handers and the subgroup of left-handers with a RVF advantage, in left-handers with a LVF advantage a chance-level performance emerged in the RVF (and not in the LVF) for low-frequency words.
Overall, data provide support for a functional architecture of the orthographic knowledge involving separate lexical stores for each hemisphere. Furthermore, we highlight that the advantage of the left-lateralized orthographic lexicon over the right one, which is often taken for granted in the reading literature, is not valid for the whole population.

P37 - Neurolinguistic investigation of noun-verb dissociation in motor neuron disease

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Object Selective deficit of verbs (V) vs. nouns (N) processing has been consistently reported in the motor neuron disease – frontotemporal dementia (MND-FTD) complex. (MND-FTD). Prerolandic regions atrophy has been traditionally thought to account for N-V dissociation in the MND-FTD complex via selective action semantics impairment (Embodied Cognition Theory, ECT). Nonetheless, explanations pertaining to both sensitivity of those regions to morpho-phonological structure of Vs and executive functioning (EF) contribution have not been endorsed. The aims of this study was to both assess neurocognitive mechanisms underlying N-V dissociation in MND patients and validate its cognitive diagnostic role in this population.
Materials Thirty consecutive MND patients and 29 healthy controls were recruited. Patients' clinical features (disease duration; site of onset; bulbar signs) and both neuropsychological (Edinburgh Cognitive and Behavioral ALS Screen, ECAS) and functional outcomes were considered.
Methods The two groups were compared on tasks evaluating N and V lexical retrieval, object- and action-semantics, and V lexical-morphosyntactic implementation, while controlling for EF measures. Effects of motor feature degree (actionality) and lexical-morphosyntactic complexity of items on lexical retrieval were assessed via logistic linear mixed models, and by between-groups comparisons via overdispersed linear models. Clinical relevance of N-V dissociation was investigated by assessing its association with disease-related variables.
Results Both groups performed worse in V than in N naming. Patients performed worse than controls in V naming and both object- and action-semantic tasks. Both groups were comparable on remaining linguistic measures. Low-actionality and transitive Vs were the most demanding for patients. EF measures did not discriminate patients from controls but were mostly related to Vs naming in patients. V deficit was related to patients' ECAS scores but not to other disease-related variables.
Discussion Impaired action semantics cannot account for N-V dissociation in MND patients, which would rather reflect a magnification of a differential EF-related processing demand for Vs vs. Ns intrinsic to the neurocognitive system. Nonetheless, since N-V dissociation in MND patients is augmented by psycholinguistic variables, it might imply prerolandic involvement in V lexical processing. Furthermore, V deficit has been shown to be predictive towards patients' neuropsychological outcome.
Conclusions ECT-framed explanations for V deficit in the MND patients are not valid. On the contrary, interplays of linguistic and extra-linguistic

explanations should be considered. Deficit in V naming can be found in MND patients and should be considered as a sensitive marker of cognitive impairment in those patients.

P38 - Implicit mechanisms of body image alterations: interoception restrains the covert attention exposure effect.

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Object: Body image is a multidimensional construct that refers to our mental picture of diverse aspects of our body, such as the perception of body size. Visual exposure to extreme-sized bodies elicits explicit self-body size perception variations. Several features of such modulation remain to be clarified. In this study we explored whether this effect: (i) acts on implicit mechanisms in modifying one's body size perception, (ii) is body-exposure specific also at the implicit level, and (iii) is modulated by interoceptive sensibility.
Materials: One hundred healthy females (age range=19–56, M=24.02 years, SD=5.87 years; education M=16.36 years, SD=2.13 years) participated in the study. A measure of Body Mass Index (BMI) was collected using a self-report assessment (BMI range=18–30, M=20.62, SD=2.48). All participants were native Italian speakers, had a normal or corrected-to-normal vision, and had no previous history of mental or neurological illness. None of the participants reported a previous history of eating disorders, and at the time of testing, none was on a restrictive diet, as assessed by a self-reported questionnaire.
Methods: We assigned a covert attention task to the participants, exposing them to extreme-sized bodies (thin and fat) or extreme-sized objects (thin and fat bottles). They were randomly assigned to one experimental condition only (body fat, body thin, bottle fat, bottle thin). Before and after the attentional exposure, we tested the association between the 'self/others' and 'thin/fat' concepts using an Implicit Association Test. We also collected a measure of interoceptive sensibility by means of a self-report questionnaire.
Results: Results showed that participants exposed to fat bodies implicitly presented a stronger association between the 'self' and 'thin' concepts. This association was significantly weaker in the group exposed to thin bodies. This effect was absent after exposure to thin and fat bottles. Notably, participants with a higher tolerance of negative bodily interoceptive signals were less susceptible to the malleability of body image exerted by the exposure attentional task.
Discussion: In line with previous findings, this study found body image modifications after attentional exposure to bodies. Additionally, we found that this effect: i) also relies on implicit mechanisms, ii) is body-shape specific also at the implicit level, and (iii) is modulated by interoceptive sensibility.
Conclusions: In conclusion, our findings shed new light on the relationship between the perception of internal (e.g. visceral) and external (e.g. visual) signals in the representation of our body.

P39 - Primary somatosensory cortex and short-term retention of body- and non body-related visual information: preliminary results from a repetitive TMS study

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Background & object. The primary somatosensory cortex (S1), once thought to be only modality-specific, is involved in higher level functions like emotion recognition[4] and motor learning by observation[3]. S1 functions may extend even to memory: some theories speculate that sensorimotor areas may be involved not only in the perception of intrinsic features of the percept but also in the retention of that information. In a recent EEG study, it has been suggested that the neural responses of somatosensory cortices to visually perceived body-related information probably reflect their involvement in short-term memory (STM)[2]. The present experiment looks for causal evidence that S1 is implicated in the retention of visual information that may be salient for this cortical area[1]. To this purpose we interfered with S1 activity by means of repetitive Transcranial Magnetic Stimulation (rTMS) during a STM task where body-related information had to be retained.
Methods & materials. Eighteen healthy volunteers took part in a three sessions within-subjects experiment. The STM task (i.e., delayed match-to-sample task) consisted in the rapid presentation of two lateralized arrays, each one depicting three body-related stimuli (i.e., pictures of hands in different position, taken from previous literature[2]). In the task, half of the trials were identical and half were different

for only one stimulus. In each session, every subject performed the task with and without rTMS, with the latter condition serving as a baseline. The rTMS protocol consisted in a train of 3 TMS-pulses (at 10 Hz frequency, fixed intensity of 60% of the maximum stimulator output) delivered after 200 ms from the offset of the first array (i.e. during the retention phase). As control sites for rTMS effects, we stimulated two other cortical areas: the dorsolateral prefrontal cortex (dlPFC; an area commonly activated during WM tasks) and the lateral occipital cortex (lOC; an area activated in feature-based analysis of visual stimuli). Only the right hemisphere was targeted.
Results. We analyzed the sensitivity detection index (d') in the STM task through a 2 X 3 repetitive-measures ANOVA with the within-subjects factors "Condition" (baseline vs. rTMS) and "Area" (S1, dlPFC, lOC). We found a statistically significant interaction between these two factors ($p=0.036$). Planned comparisons revealed an improvement of subjects' performance in the STM task only when rTMS is delivered over S1 (vs. baseline, $p<0.001$).
Discussion & conclusions. Our results demonstrate that rTMS over S1 applied during a visual STM task improves performance, suggesting that S1 may be involved in visual STM when body-related stimuli had to be retained. These results shed light on the crossmodal involvement of primary sensory cortices in the retention of information in memory[2], showing that their recruitment is driven by the intrinsic features of the percept rather than by the sensory modality in which objects are presented.
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P40 - Measuring implicit mental representations related to ethnic stereotypes with ERPs

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Object. Event-related potentials (ERPs) are a valid measure to detect implicit mental associations between concepts, circumventing the problem of self-report and behavioural methods. The present investigation used ERPs to detect the activation of implicit stereotypical representations associated to different ethnic groups, using a completely implicit paradigm.
Materials. 285 sentences were presented to 19 Italian Caucasian participants. Sentences could either violate (Incongruent condition), non-violate (Congruent condition) or be neutral (Neutral condition) with regard to stereotypical concepts concerning different ethnic groups (e.g. Asians, Africans, Arabians). In addition, were presented 24 target sentences ending with an animal name. At the end of the experiment, a revised version of the Subtle and Blatant Prejudice Scale assessing the degree of ethnic prejudice toward immigrants was administered to participants.
Methods. EEG signals were recorded from 128 scalp sites while participants read sentences that were visually presented on a PC screen. No awareness or judgment about stereotypes was required. Participants were instructed to press a key in response to animal words, while ignoring the overall study's purpose. ERPs were time-locked to the terminal words.
Results. Terminal words violating ethnic stereotypes elicited a greater anterior N400 response, whereas sentences that confirmed stereotypes evoked a greater anterior P300. The individual amplitude values of the N400 evoked by Incongruent sentences showed a direct linear correlation with the individual scores obtained at the implicit prejudice scale. According to the swLORETA inverse solution, the neural representation of ethnic stereotypes mostly involved the Inferior Temporal Gyrus and the Middle Frontal Gyrus. An anterior Late Positivity was found in response to sentences concerning other ethnic groups (Congruent and Incongruent sentences) than the one of the participants (Neutral sentences regarding Italian characters).
Discussion. The N400 response reflected a difficulty in integrating the information contained in the Incongruent sentences with a pre-existing stereotypical knowledge. Intra-cortical sources explaining the N400 involved areas in the social cognition network supporting processing of information about other people and impression formation. Moreover, an LP response was sensitive to the differentiation between the representation of one's own ethnicity and ethnicities other than the Caucasian (Other-Race Effect, ORE).
Conclusions. The study investigated the timing and neural mechanisms underpinning the implicit representation of ethnic prejudice in an effective manner. The paradigm used allowed to access implicitly to complex contextual and semantic representations, avoiding the activation of mental processes (and related brain areas) related to

prejudice suppression and inhibition caused by social desirability pressure.

P41 - Multisensory integration effects in sweet functional foods: expectations and gustatory effects

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Object Functional foods are dietary items that might modulate one or more targeted functions in the body. Their use might be related to health-promotion or disease prevention. However, such food, beside their possible effectiveness, need also to be appealing to consumers' brains. Here, we tried to understand the role of the kind of information provided, taste, colour, texture, and kind of flour used in the food item in order to generate preference choices by young and over 65 consumers.

Materials In our experiments we used eight cookies prototypes. Four pictures of the same foods were also adopted.

Methods We evaluated the role of the visual and gustatory aspects of the food items by means of Visual Analogue Scale. The expectations based on the visual appearance of the food (experiment 1) and actual taste of the items (experiment 2) were tested. Participants evaluated the food using the following scales Pleasant, Price Range, Quality, Purchase Desire, Healthy, Friable, Bouba-Kiki, Light, heavy, and Sweet.

Results Experiment 1: visual aspect of food

We found that different kinds of visual information regarding the food (e.g., colour and shape), affect the participants evaluation on several perceptual dimensions (e.g. Pleasant, Bouba-Kiki, Sweet).

Experiment 2 Our results showed that over 65yo participants preferred on several evaluation scales (e.g., Pleasant and Quality) the cookies filled with apple, as compared to those filled with orange. Moreover, the cookies prepared with white flour and cocoa grain in the dough are perceived as more sweet, as compared to those prepared with wholemeal flour and cocoa grain in the middle.

Discussion In Experiment 1, the cookies colour and shape differentially affect the potential consumer's expectations on several dimensions. This result might suggest the importance of the different visual presentation of the same food to the consumer in order to affect his/her choice. In Experiment 2, taste and texture interacted in affecting functional food evaluation of over 65yo participants.

Conclusions We found that both in young and elderly people, the expectations and the gustatory perception of sweet functional foods can be significantly modulated by complex cognitive and multisensory interactive effects occurring in several brain areas.

P42 and SELECTED ORAL COMMUNICATION - Many neglect patients do not have neglect

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Object Classical neglect tests involve a set of targets presented in different positions which need to be responded to by the patient. On this tasks, not only neglect, but also other deficits can yield a decrease in hit rate. Recent methodological work has shown that such non-neglect deficits can lead to massive inflation of false positive rates for neglect when one is using the classical normality cut-offs method. A recently proposed measure, the Mean Position of Hits (MPH) can theoretically allow for correction of such inflation. The purpose of the present work is to estimate the False Positive and False Negative rates on a large sample of right hemisphere patients performing a letter cancellation task, both using the classical normality cut-offs, and the adjusted cut-off that takes into account the effects by deficits other than neglect.

Materials Archive-data from 129 right hemisphere patients and 51 neurologically intact subjects who performed a variant of Diller & Weinberg's letter cancellation test were collected.

Methods MPH scores and their instability scores SE(MPH) were derived for each subject. A mixture model was implemented in Stan to estimate the proportions of patients who behave like neurologically-intact subjects (no-deficit, ND), those who showed a lateral deviation indicative of genuine neglect (N+), and those who showed some form of deficit without lateral components (N-). Cut-offs from the classical diagnostic methods and from the adjusted diagnostic method were derived.

Results Patients were classified as 6% ND, 44% as N-, 50% N+. As for neglect diagnosis, with nominal one-tailed 0.02 alpha value, the classical method showed a false positive rate of 0.36, and a sensitivity of 0.92, while the adjusted method gave 0.07 and 0.83 figures. Nominal 0.02 false positive rates can be achieved via empirical (rather than theoretical) derivation.

Discussion The predicted inflation of false positive rates by classical diagnostic procedure was confirmed, and found to be very large (0.36); the

adjusted scores proved able to strongly reduce that rate (0.07) at little expense in sensitivity.
Conclusions The vast majority of neglect tests produce sizeable inflation of false positive rates which can be reduced or nullified by using the suggested correction to MPH. Software for analysing data for both clinical and experimental scenario is available.

P43 - Increased sensitivity to the rewarding effects of delta9-tetrahydrocannabinol (THC) and MDMA after exposure to nicotine in mice and zebrafish

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

P44 and SELECTED ORAL COMMUNICATION - Artificial Intelligence for Food Recognition

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Object. Automatic food recognition is an important task for automatic or semi-automatic daily dietary monitoring. This document briefly describes some results of the Imaging and Vision Laboratory in the food recognition field. Specifically, the activities of the IVL here described are mainly related to two tasks: Food Recognition in a controlled environment (i.e. canteens and restaurants), and Food Recognition in an uncontrolled environment (i.e. in-the-wild).
Materials. In order to perform food recognition, different datasets have been created by IVL. For food recognition in a controlled environment a set of about 1,000 images has been acquired at the canteen of the University of Milano-Bicocca. Another set of images has been acquired at the "Marchesino" restaurant with the aim of recognizing the compliance of the plates with respect to reference models. For the food recognition in an uncontrolled environment, a set of more than 247,000 food images of 475 different

categories as well as a more than 160,000 images of 200 types of fruits and vegetables have been collected from different sources.
Methods. We designed and implemented algorithms based on computer vision and advanced machine learning techniques. Specifically, we exploited Deep Learning (DL), and Convolutional Neural Networks (CNN) for robust feature learning and classification in the context of the food domain. Different neural network architectures coupled with domain adaptation strategies have been investigated and evaluated for both recognition tasks.
Results. The developed algorithms can recognize the 475 food in an uncontrolled environment with an accuracy of 82.5%. They can correctly assess the compliance of the food delivered by the Marchesino's kitchen with an accuracy of more than 90%. We can also estimated the quantity of the food in a plate, before and after the meal with an error of about 8.5%. Fruits and vegetables are recognized with an accuracy of 84.5%.
Discussion. The algorithms have been integrated into a smartphone application that allows users to use it in everyday life and in different contexts. Through the application, the user can acquire the dish with his smartphone. The application analyzes the dish and if it recognizes it with a high degree of confidence, automatically proceeds with the estimation of its quantity and updates the user's food diary.
Conclusions. The above activities have been performed within the project FooDesArt: Food Design Art - The Art of Wellness, CUP E48I16000350009 - Call "Smart Fashion and Design", co-financed by POR FESR 2014-2020 Regione Lombardia.

P45 - Analyzing and Modeling Cooking Procedures

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Object Cooking recipes provide ingredients and step by step instructions for making a dish, and thousands of recipes are available. Discovering usage patterns of cooking actions and ingredients is important to understand how to prepare a healthy and safe meal. The aim of this project is to design a system for automatically analyzing and modeling cooking procedures and to allow users to browse and navigate the recipes, to avoid food intolerance and to learn how to cook a balanced and healthy meal.
Materials CookIT (http://arm.mi.imati.cnr.it/cookIT), a web portal on Traditional Italian Recipes with the aim of preserving, safeguarding and disseminating them, is used here as a source of textual recipes with ingredients and detailed cooking procedures. Different NLP tools able to elaborate Italian texts have been identified, and tested.
Methods The first step is to analyze and interpret the recipes with the aim of identifying terms able to describe ingredients, actions, and order of actions. The information extracted with the aid of NLP tools is structured in a XML file, the rules of which are defined by a DTD. This file is then translated in a Petri net, a graphical and mathematical tool suitable to represent concurrency.
Results For each recipe, ingredients, tools, actions, with their duration and order, have been identified. We defined a DTD to formally represent a recipe procedure. We implemented a translator for the description with Petri nets. The recipes can be visualized and executed in the net. We defined a distance between nets representing recipes that take into account both the names of places and transitions and their relations.
Discussion Preliminary results are quite satisfactory for the automatic identification of ingredients, tools, and duration, while the identification of actions and their order in the execution of the recipe requires further refinement, especially with regard to actions started and then resumed after some time. The DTD proved itself able to catch most of the relations between actions and ingredients. The petri nets are suitable for modeling recipes.
Conclusions In future, we will continue working on the comparison between nets, defining new distances taking into account different properties. In addition, we will use the structure of the net to automatically gain information about some features of the recipes that could be interesting for possible users. A graphical interface for recipe procedures visualization, navigation, and step-by-step execution is in development.

P46 - The highs and the lows of a Neural Code Theory: theoretical frameworks and clinical phenomena

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The search of a proper Theoretical Framework for a theory of the neural code may be like the quest for a maternal environment , whereas a reasonable Conceptual Framework might be thought as the

corresponding concerned womb, in the light of associated metaphorical degrees of freedom and constraints. A motherly intellectual environment, for the purpose of this poster, might be found in Subject-oriented Approach to Knowledge (S.O.A.) as stated by Arne Kjelmann, with the addendum that academic human knowledge as it stands today cannot be explicated otherwise than by a code we do not still have. The epistemic status of "feeling" and therefore moreover "Clinical Neurological Feeling" is offered as a step to share from grassroots the perception of common and regular phenomena (although neuropathological) of the human body. The addendum about the Code as a tool of explication, on the other side of the spectrum, might find its conceptual framework in the definition by Marcello Barbieri that a code is a set of rules that establish a correspondence, or a mapping, between the objects of two independent worlds . In this perspective my project, as stemming from grassroots of the neuroclinical feeling, obviously bypasses and deliberately neglects, the frequently debated problem of consciousness and moreover such things like "reading the mind". The neuroclinical feeling of an epileptic seizure or of a sudden hemiparesis, take advantage of a phenomenological shared status , with a by far minor degree of absolute variability in respect of individual consciousness phenomena (if that can be said), even the simplest one as sharing the feeling of a precise colour. The observation of regular natural phenomena ad example the hyposthenic flaccidity of a previously healthy limb or the EEG brainwaves, already benefits of a shared, although frequently empirical, framework. As a neurologist the choice of such a main road in the mapping of the theoretical framework for the search of the Neural Code, is an optimal one, in front of the difficulty of a task which is the quintessence of the multidiciplinarity, with objective risk to be the quintessential setting to deploy the incommunicability of single-minded, although perfect, teories of everything. Indeed if I were a Physicist I'd aim toward a Theory Of Everything but, beeing a Neurologist, I'll aim toward a Theory Of Everybody's Brain, so that probably, differently from atoms They may argue as well.

Molecular and Cellular Neuroscience

P47 and SELECTED ORAL COMMUNICATION - Circulatory miR-223-3p Discriminates Between Parkinson's and Alzheimer's Patients

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Object: miR-223-3p is involved in the regulation of a broad range of cellular processes and in many types of pathological processes as cancer, autoimmune and inflammatory diseases. MiR-223-3p has been indicated as negative regulator of NLRP3 protein, a key protein of inflammasome. The chronic inflammasome activation, an underlying feature of neurodegenerative disorders, is induced by misfolded protein aggregates, including amyloid-beta and alpha-synuclein, resulting in pro-inflammatory cytokines secretion and propagating of neuroinflammation. The aim of the study was to analyze whether circulatory miR-223-3p could be used as biomarker in neurodegeneration and to clarify its possible relationship with inflammasome activation.
Material and Methods; miR-223-3p concentration was evaluated in serum of Alzheimer's (AD) and Parkinson's disease (PD) or mild cognitive impairment (MCI) patients and healthy controls (HC).
Results: Compared to HC, miR-223-3p serum concentration was reduced in MCI and AD, but up-regulated in PD ($p<0.0001$), and it decreased progressively from MCI to moderate ($p<0.0001$) to severe AD ($p=0.0016$). Receiver operating characteristic analysis showed that miR-223-3p concentration discriminate between AD, PD and MCI vs. HC, as well as between AD and PD.
Conclusion: miR-223-3p serum concentration discriminates between AD/MCI and PD, suggesting that this molecule could be a potential non-invasive biomarker for differential diagnosis and prognosis of these neurodegenerative conditions.

P48 and SELECTED ORAL COMMUNICATION - Nerve excitability testing as the link between anatomy and physiology: axonal excitability testing to test in vivo mechanisms of peripheral nerve damage

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Object we have introduced a refined neurophysiological technique, Nerve Excitability Testing (NET), to test our consolidated animal models of peripheral nerve damage. This allowed us to test precocious axonal functional alterations, that

precede anatomical damage in order to obtain insights on pathogenetic mechanisms. The final objective is to better characterize peripheral nerve damage in in vivo models to drive future drug discovery.
Materials A cohort of female Wistar rats was divided into 3 groups (n=8, each): control, Oxaliplatin (OHP) and Paclitaxel (PTX) group.
Methods Animals were tested with standard neurophysiology (sensory and motor recordings) and dynamic test at base line and at end of treatment. NET was assessed at 24, 48, 72 hours after the 1st administration and at end of treatment to characterize axonal properties. At end of treatment harvesting of caudal and sciatic nerves was also performed.
Results NET findings after the 1st injection showed in OHP group alterations compatible only with a transient alteration of kinetics in sodium-voltage operated channels; PTX group, instead, showed precocious signs of actual axonal damage. At end of treatment, standard neurophysiology showed a mild sensory neuropathy in OHP animals and a relevant sensory-motor neuropathy in PTX animals. Both groups showed a painful behaviour. Neuropathological analysis confirmed the pattern and severity of neuropathy seen at nerve conduction studies.
Discussion Our NET recordings, after the 1st administration, showed a different pattern in PTX and OHP animals. PTX animals showed signs of a rather precocious axonal damage, whereas OHP animals showed signs of a functional axonal hyperexcitability. This mirrors a different pattern observed at the end of treatment: OHP animals showed a mild sensory neuropathy, while PTX animals developed a relevant sensory-motor polyneuropathy.
Conclusions NET monitoring gave insights on the different pattern of alterations in the OHP and PTX animals, giving precious information concerning pathogenesis and time course of nerve damage. In OHP treated animals a functional alteration of sodium voltage-operated channel was observed, thus anti-epileptic drugs can be proposed as potential neuroprotectant agents. In PTX group we identified a pattern predictive for relevant axonal damage since the first administration; this is relevant to drive future drug discovery: new neuroprotectant agents against PTX neuropathy should demonstrate the ability to prevent these precocious alterations seen at NET, in order to select a molecule able to act against first events of axonal damage.

P49 - A novel HCN2 mutation associated with progressive epileptic encephalopathy

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A novel mutation was found in HCN2 gene encoding for the hyperpolarization-activated cyclic nucleotide-gated channel 2. The proband, now aged 7 years old, presents a congenital encephalopathy characterized by drug resistant epilepsy, severe developmental delay, ataxia, dystonia and cerebral visual impairment. The onset of epilepsy was marked by a convulsive status epilepticus at 5 months of age; since then, the patient presented recurrence of fever-triggered generalized seizures, in many cases featuring status epilepticus. The mutation affected an aminoacid located in the transmembrane S6 helix (p.Gly460Asp) and is carried in heterozygosis. To describe the functional consequences of the mutant channel, whole-cell patch-clamp experiments were performed in HEK293 cells expressing HCN2 wild type (wt) or mutant channel. Heterozygotic condition was mimic through a coexpression of the same amount of plasmid encoding for the wt or the mutant form of the channel. A complete abolishment of the current density was observed considering the mutant compared to the wt channel (-9.9±1.2 pA/pF n=20 vs -31.2±8.4 pA/pF n=44 respectively; $p<0.05$). A significant reduction of the current density was also found when heterozygotic condition was tested compared to the wt one (-20.1±5.1 pA/pF n=35; $p<0.05$). Both wt and heterozygotic channels share overlapping activation curves ($V_{1/2}$ and k -92.9±0.3 mV and 5.6±0.3 n=29 vs -91.6±0.2 mV and 5.6±0.2 n=16 respectively). No significant differences were present in the kinetics of both activation and deactivation of

the wt and of the heterozygotic channel. In conclusion, the mutation seems to act as a loss-of-function that could potentially affect the control of neuronal excitability and therefore be linked to the proband pathological condition.

P50 - Assessment of oxaliplatin-induced peripheral neurotoxicity with different treatment schedules

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Object One of the most neurotoxic antineoplastic drug is oxaliplatin (OHP), a platinum-based agent widely used for the treatment of metastatic colorectal cancer. Despite extensive investigation on the symptoms and characteristics of OHP-induced peripheral neuropathy (OIPN), the pathogenesis is still largely unknown. In literature several preclinical in vivo studies different each other in schedules of OHP treatment are described but the characterization of peripheral neurotoxicity was limited. In particular, we compared three different schedules (Jiang et al., 2016, Sakamoto et al., 2016, Ta et al., 2009) with OHP animal model used in our laboratory.

Materials Adult mice were treated with OHP, with the four different treatment schedules: 1) OHP 10 mg/Kg, d0 e d2, ip; 2) OHP 3 mg/Kg, single ip; 3) OHP 3 mg/Kg, q1dx5, 2 cycles with 5 days rest, ip; 4) OHP 5 mg/Kg, 2qwx4, iv.

Methods To assess acute and chronic syndromes of OIPN a multimodal approach was used. Behavioural tests were performed to evaluate peripheral neuropathic pain while neurophysiological examination, neuropathological analysis were conducted to evaluate to the effects of OHP on peripheral nervous system.

Results Mechanical allodynia and neurophysiological alterations were observed only in schedule 4. Alterations in cold sensitivity were present in all schedules, except for schedule 2.

Discussion The study that better mimic the OIPN features is our laboratory schedule (OHP 5 mg/Kg, 2qwx4, iv)

Conclusions In literature different animal models of OIPN are proposed. However not all of them are solid since they are not studied with a multimodal approach; thereby they reproduce only painful features and not the whole OIPN clinical spectrum. Our model instead is also able to induce neurotoxicity symptoms and signs other than pain.

P51 - Insights on Mesenchymal Stem Cell mediated neuroprotection

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Object Mesenchymal Stem Cells (MSCs) showed to be a promising tool for treatment of different kind cells traumatic or ischemic injuries. Moreover, during the last two decades, Mesenchymal Stem Cells have been proposed as a possible treatment of several neurological diseases, such as Alzheimer or Parkinson's Disease, initially with the aim to replace the damaged neuronal cells, and later to cure, rather than to replace the neuronal cells. In particular, previous studies demonstrated that MSCs directly co-cultured with sensory neurons were able to increase strongly the neuronal survival, and to protect them from different toxic stimuli, thus theoretically being efficient to change the course of all diseases affecting sensory neurons. However beneficial effect of MSCs on nervous tissue repair has not yet been completely understood and it is crucial to examine mechanisms involved in such interaction. Due to it, aim of this work is to investigate the different interaction manners, and to identify molecules used by MSCs and neurons to communicate.

Materials Immunofluorescence and Electron microscopy techniques were applied for analyzing cellular structures.

Methods In particular, we observed the formation of gap junctions and tunneling nanotubes, cellular structures potentially allowing the flow of vesicles, organelles and small molecules. In addition, with the diffusible fluorescent dye Calcein, we demonstrated the flux direction from MSCs to neurons. Furthermore, we analyzed the nature of the exchanged materials.

Results As result, we observed an involvement of exosome and more in general vesicular structures, and even subcellular components as mitochondria. All these molecules and structures can be used by MSCs to improve neuronal survival.

Discussion Our observations brought some understanding on neuroprotective effects of MSCs. Additionally, as a proof of concept, we will expose neurons to the putative protective MSC-derived

molecules, to determine if they are sufficient to achieve a positive effect.
Conclusions Based on identified interactions and the pivotal molecules exchanged, it will be possible to enhance the MSC protective effect on neurons by exploiting the identified key molecules.

P52 and SELECTED ORAL COMMUNICATION - Beta-amyloid promotes monocytes chemotaxis in Alzheimer's disease: the role of TSPO

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Object: Neuroinflammation is a key event in the pathogenesis of Alzheimer's disease (AD) and is sustained by both chronical activated microglial resident cells and blood derived monocytes (BDM), with two different spectra of actions. Specifically, peripheral monocytes, could potentially promote the inflammatory damage. BDM can penetrate into the CNS attracted by microglia-derived chemokines and help microglia in Abeta phagocytosis. TSPO receptor is present on both activated microglia and BDM, and plays a role in monocyte chemotaxis. The aim of this work is to evaluate if Abeta1-42, the principal protein accumulated in AD CNS, is able to chemoattract BDM, and if this event is mediated by the TSPO receptor.
Materials & Methods: Chemotaxis experiment were performed on both THP-1 cells and BDM from AD patients and controls. As chemoattractants MCP-1 and oligomeric Abeta1-42 were used; furthermore, different TSPO ligands were tested. Time-lapse microscopy was performed in Ibidi µ-slide chambers. Boyden chambers with pores of 8 µm (for THP-1) or 5 µm (for BDM) were left at 37 °C for 6 h (THP-1) or 3 h (BDM). Subsequently, filters were fixed and stained. 10 random fields photos per filter were taken for calculating the mean number of migrated cells. The chemotactic index was expressed normalizing each condition to vehicle.
Results: Abeta 125 pM resulted able to significantly induce THP-1 cell chemotaxis in both Ibidi µ-slide ($p<0.01$) and Boyden chambers ($p<0.01$). Abeta 125pM was also able to significantly induce chemotaxis in monocytes from healthy subjects ($p<0.05$). Moreover, Abeta induced chemotaxis of monocytes obtained from AD patients: this process was two-fold increased ($p<0.01$) with respect to controls. To evaluate the involvement of TSPO in chemotaxis we evaluate the influence of different TSPO ligands. The inhibitor, PK11195, was able to partially revert Abeta-induced chemotaxis in both cell types in a concentration dependent manner ($p<0.05$). As regard the two agonists, Emapunil and Ro5-4864, they alone increased chemotaxis respect to vehicle ($p<0.05$).
Discussion: These results demonstrate the ability of oligomeric Abeta1-42 to attract BDM. Moreover, BDM from AD patients resulted more prone to chemotaxis. We may hypothesize that BDM from AD, could have been already primed by the chronically exposure to Abeta. We confirmed the involvement of TSPO in chemotaxis, since its antagonist reduces Abeta-induced chemotaxis.
Conclusions: Further study will clarify the role of TSPO in chemotaxis. The possibility of modulating monocytes chemotaxis represents a putative pharmacological target aimed at reducing the CNS inflammatory burden in AD.

P53 - Stavudine protects against amyloid cytotoxicity inhibiting NLRP3 activation in Alzheimer's Disease

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Object: Stavudine (d4T), a nucleoside reverse transcriptase inhibitor, modulates Abeta-mediated NLRP3-inflammasome activation, but its molecular mechanisms are unknown. NLRP3 is a neuroinflammatory complex activated in microglial cells from Alzheimer's disease (AD). In THP-1-derived macrophages, stavudine reduces TREM2 expression, a receptor promoting Abeta phagocytosis, but it induces autophagy, supporting the idea that d4T promotes this Abeta-catabolic pathway.
Materials: Peripheral blood mononuclear cells (PBMC) from 10 AD patients and 10 age-matched

controls were treated with Abeta (10microg/ml, 1h) after Lipopolysaccharide (1microg/ml, 23h)-priming in the presence/absence of D4T (50microM, 22h). As PBMC PMA-differentiated THP-1 cells were treated to analyse mRNA.
Methods: p38- and pERK1/2-MAPKinase, PI3K-Akt, p70 S6K, Beclin-1, LAMP2A (autophagy targets), pCREB, Bax and TREM2 expression was evaluated with Western-blot. By RT-PCR, NLRP3, beta-arrestin1 and EAAT1 mRNA was detected.
Results: In Abeta-treated PBMC, stavudine regulates Ras/MAPK and GSK-3beta/PI3K/Akt pathways, through reduction of p38 phosphorylation (in controls, $p<0.05$ and in AD, $p<0.001$) and induction of pERK1/2 and pAkt (in controls and AD, $p<0.05$). D4T decreases p-p38 in AD PBMC compared to untreated cells. D4t modulates Beclin-1, p70 S6K and LAMP2A expression, too. Moreover, stavudine induces the transcription factor CREB phosphorylation and the pro-apoptotic Bax protein expression ($p<0.05$ vs Abeta-treatment), but reduces TREM2 expression ($p=0.025$ in AD-treated PBMC), the promoting phagocytosis receptor. In THP-1-derived-macrophages, mRNA transcription was modulated by stavudine, as shown in NLRP3 and beta-arrestin1, contrasting Abeta toxicity ($p<0.05$); no modification was detected in EAAT1 mRNA.
Discussion: MAPKs and Akt modulation remarks the key role of Ras and PI3K/Akt signaling pathways induced by stavudine. The regulation of their downstream targets strengthens this hypothesis, suggesting that d4T has effects also on autophagy, tau phosphorylation and protein synthesis because of its regulation of beclin-1, p70 S6K and LAMP2A. pCREB increase suggests that stavudine may modulate EAAT1 and antinflammatory cytochines transcription, and memory process, while its inducing effect on Bax suggests an its involvement in apoptosis. Abeta-phagocytosis-TREM2-dependent was absent in PBMC, too. Results in THP-1- support the idea that stavudine down-regulates NLRP3 decreasing beta-arrestin1 transcription, which is involved in NLRP3 activation, if overexpressed.
Conclusion: In our experimental conditions, stavudine modulates Ras/MAPK and GSK-3beta/PI3K/Akt signaling pathways, through which it prevents inflammasome-activation. It will be interesting to verify if inflammasome shutdown due to stavudine could counteract AD progression linked to Abeta cytotoxicity.

P54 - MV1035: a new molecule targeting cell migration and invasiveness of glioblastoma cells

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Glioblastoma (GBM) is one of the most aggressive brain neoplasm and patients with GBM show a median survival of only 15 months after diagnosis. To date, the effectiveness of chemotherapy and radiotherapy for GBM are limited by chemoresistence and by the difficulty to pass the blood-brain barrier[1]. Among the various strategies that could limit the aggressiveness of GBM, sodium channels could be a suitable approach. Our hypothesis was based on the fact that several studies reported the role of Voltage-Gated Sodium Channels in cancer cells and their association with cell migration and invasiveness[2,3].
Therefore, we evaluated several new NaV channels inhibitors against a glioblastoma cell line, U87-MG. Among the compounds tested, the imidazobenzoxazin-5-thione MV1035 reduced cell migration and invasion, but, unexpectedly, the activity seemed not related to NaV channels blockade and it was probably due to an effect on a different pathway. Using an in silico approach we evaluated alternative targets of MV1035. The SPILLO potential binding site searcher (SPILLO-SBSS) software performed a wide screening of the human structural proteome available in the RCSB protein data bank[4].
SPILLO found several alternative targets and among top-ranked proteins we focused on the RNA demethylase ALKBH5 (□-ketoglutarate-dependent dioxygenase alkB homolog 5), which catalyses the conversion of N6-methyladenosine to adenosine in mRNA. This protein is often overexpressed in glioblastoma and RNA methylation is crucial for modulating tumor initiation and progression[5,6]. Moreover, Ecto-5'-nucleotidase (NT5E or CD73) RNA is one of the targets of ALKBH5 and the protein is involved in cell adhesion, invasiveness and metastatic properties of cancer cells[7]. In vitro experiments of RNA demethylation and CD73 protein expression demonstrated ALKBH5 inhibition by MV1035.
Here we demonstrated, integrating cellular, molecular and in silico techniques, that MV1035 is able to reduce U87-MG cell migration and invasion, targeting the SPILLO-PBSS recognized ALKBH5 activity

and ultimately inducing a decrease in CD73 expression.

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P55 - Mitochondrial proteins in human peripheral blood mononuclear cells (PBMC) as potential peripheral biomarkers of Parkinson's disease

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Introduction Diagnosis of Parkinson's disease (PD), Alzheimer (AD) and other neurodegenerative pathologies occurs at late stages of diseases. Early diagnosis of neurodegenerative disorders would be fundamental for effective therapeutic intervention. Based on the well-known role of neuroinflammation and mitochondrial dysfunction in PD and AD, we aimed to identify potential biomarkers of pathology in peripheral tissues, such as blood. To this end, we assessed levels of proteins regulating neuroinflammation, mitophagy and redox homeostasis in peripheral blood mononuclear cells (PBMC) from PD, MCI-AD and healthy control subjects.

Methods A total of 51 subjects was analyzed in this study, including 23 PD patients, 6 MCI-AD (3 each) and 22 healthy controls (age and sex-matched). Total proteins prepared from PBMC samples were analyzed by western blot analysis for proteins regulating: inflammation (IL6ST), oxidative stress-sensor (DJ-1), mitochondrial fission-fusion proteins (P-Drp-1, Opa-1 and Mfn-2), mitophagy (PINK-1 and Parkin), mitochondrial biogenesis (mtTFAM), and redox homeostasis (POR, GPX7, etc), and many others.

Results PBMC from PD patients show higher levels of Interleukin-6 receptor subunit beta Precursor (IL6ST), Catechol-O-methyltransferase domain-containing protein 1 (COMTD1) and Proton-coupled folate transporter (SLC46A1), while displaying lower content of NADPH-cytochrome P450 reductase (POR) and Glutathione peroxidase 7 Precursor (GPX7), which are indicative of inflammation and reduced redox homeostasis. Protein levels are partially restored in PD patients under conventional treatment. In addition, we find decreased levels of Opa-1 and Mfn-2, suggesting a decrease of mitochondrial fusion and dynamics, which might underlie decreased mitochondrial activity. Different expression patterns of these proteins are also found in MCI-AD samples, compared to healthy subjects.

Discussion Our data support the hypothesis that differential levels of proteins regulating inflammation and mitochondrial function might be considered as markers of specific neurodegenerative disease. Further analyses on extended number of patients are in progress to substantiate current results and potential development of inflammation or mitochondrial protein changes as peripheral biomarker of PD/AD.

P56 - Identification and functional characterization of Sox2-target genes involved in the self-renewal and differentiation of neural stem cells cultured from the mouse brain

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The Sox2 gene encodes a transcription factor active in neural stem/progenitor cells (NSCs) in the developing vertebrate central nervous system (CNS). Heterozygous Sox2 mutations in humans cause a characteristic spectrum of CNS abnormalities. To understand the role of Sox2 in neural development, we previously generated Sox2 conditional KO mutations in mouse, that allowed us to observe an important function for Sox2 in the maintenance of NSCs self-renewal, in long-term in vitro cultures derived from P0 mouse forebrain, as well as in vivo (e.g. in hippocampus). In in vitro cultures, Sox2-ablated NSCs self-renew for several passages like the wild-type ones, but then undergo progressive exhaustion. We found that, upon differentiation, they also generate reduced numbers of neurons, with reduced arborization. Sox2 can regulate its targets by controlling long-range interactions between genes and distal enhancers, which regulate gene expression; indeed, many of these interactions are lost in Sox2-mutant cells.

By RNAseq, we identified genes that are downregulated following Sox2 ablation. To test their role as mediators of Sox2 functions, we re-introduced some of them into Sox2-deleted cells via lentiviral vectors, to test if they can rescue long-term self-renewal, and neuronal differentiation. The most downregulated gene in mutant, compared to wild-type cells, is Socs3 (Suppressor Of Cytokine Signalling 3). Its overexpression rescues the ability of mutant cells to grow long-term, and may partially rescue the neuronal differentiation defect. Other genes downregulated in Sox2-mutant NSCs include key regulators of cell proliferation, like c-Fos, Jun and Egr2. We found that c-Fos is the most important to rescue the ability of Sox2-mutant cells to grow long-term.

Having found that Fos acts downstream to Sox2 in the maintenance of NSCs self-renewal, we conducted further functional analysis to understand if Fos itself is required to maintain NSCs, in the presence of wild-type Sox2. We thus mutated the endogenous Fos gene, using the CRISPR/Cas9 system. We tested the ability of NSCs to self-renew generating stem cells capable of giving rise to clonal progeny. A small number of clones growing from mutagenized cells could be progressively expanded and continued growing, while the majority stopped growing and died out at previous stages, compared to clones growing from cells transduced using a control virus. These data indicate that mutation of the endogenous Fos gene progressively reduces the rate of cell growth. This is in agreement with a functional role for Fos in brain-derived NSCs maintenance.

P57 - Role of the transcription factor Sox2 and its target genes in the maintenance of glioma tumor stem cells

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Cancer Stem Cells (CSCs) are a tumor cell sub-population with stemness features, i.e. self-renewal (ability to re-form a tumor of the same type) and the ability to “differentiate” into cells constituting the tumor bulk. These hallmarks make them responsible for events such as tumor relapse, metastasis and drug resistance. For this reason it is very important to understand which are the «factors» fundamental for their maintenance.

We previously demonstrated an essential role of transcription factor Sox2 for the maintenance of neural CSCs, using a Sox2 conditional deletion mutant in a mouse model of PDGF-induced high-grade oligodendroglioma (pHGG). Transplanting wild-type (wt) pHGG cells into mouse brain generated lethal tumors, but mice transplanted with Sox2-deleted cells remained tumor-free. Cultured Sox2-deleted pHGG cells show decreased growth-rate, activation of glial differentiation, and increased cell death compared to pHGG cells that express Sox2. We think that among upregulated genes following Sox2 loss (individuated via microarray) we can find important tumor suppressors, that normally are repressed by Sox2. To test this hypothesis we overexpressed the upregulated Sox2 downstream target genes in Sox2-WT oligodendroglioma cells in order to see if this is sufficient to recapitulate the anti oncogenic effects of Sox2 loss. And indeed the

overexpression of four downstream Sox2 target genes encoding known tumor suppressors in Sox2 wt pHGG cells (Zfp423, Ebf1, Hey2 and Cdkn2B), though not of others (Hopx, Wif1, Sdc4, Cryab, Rgs2), reproduces, to varying degrees, the tumor suppressive effects of the Sox2 deletion both ex vivo and in vivo. Their important tumor suppressive role downstream Sox2 is confirmed by the fact that their mutation in Sox2-deleted cells rescue, at least in part, the proliferation of these cells. Since one of these Sox2 targets in a well known effector of Notch signalling pathway (Hey2), we are testing the hypothesis that the repression Notch signalling pathway by Sox2 is essential in CSCs maintenance. For this reason we treated pHGG cells with a Notch pathway inhibitor (DAPT) in Sox2-deleted cells. This was sufficient to rescue proliferation, at least in part, in Sox2-deleted pHGG cells. This result means that part of Sox2 role in maintaining cancer stem cells in our model is due to the repression of Notch pathway. Interestingly these four factors decrease also the clonogenicity in human primary glioblastoma cells from patients.

These data make stronger the idea that we identify four tumor suppressor genes that normally are repressed by Sox2, and whose repression is essential in maintenance of CSCs in our murine model, and open the possibility that this regulation is conserved also in human gliomas.

P58 - Solving the puzzle of protocadherin-19 mosaicism to understand the pathophysiology of pcdh19 female epilepsy

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Object Mutations in the X-linked gene PCDH19 cause early infantile epileptic encephalopathy-9 (EIEE9), a neurodevelopmental disorder characterized by clusters of seizures, intellectual disability and autistic features (Dibbens et al. 2008; Kolc et al., 2018). The peculiarity of EIEE9 is that it affects females with PCDH19 heterozygous mutations while it spares males, with the exception of somatic mosaic males (de Lange et al. 2017). Therefore, a cell-interference model has been proposed (Depienne et al. 2009), according to which the coexistence of PCDH19-positive and PCDH19-negative neurons would scramble neurons communication.

Materials & Methods To validate this hypothesis and investigate EIEE9 underpinning mechanisms, we exploited two models of PCDH19 mosaicism by using the PCDH19 floxed mouse generated in the laboratory: the Cre recombinase was delivered either locally in the cortex of postnatal mice, by injection of adeno-associated vectors (AAVs), or in the whole brain, by crossing PCDH19 floxed mice with hSyn1-Cre mice to prevent PCDH19 expression from the embryonic development.

Results In vivo electrophysiological studies in anesthetized mice show that a PCDH19 mosaic patch in the visual cortex is associated with the disruption of slow wave activity (SWA) and causes transient episodes of hyperexcitability. Combined local field potential (LFP) recordings and two-photon calcium imaging suggest that mosaic mice display an increased calcium activity. The mosaic expression of PCDH19 in the progeny of PCDH19 floxed X hSyn1-Cre is visible not only in heterozygous females, as expected, but also in hemizygous males, most likely as a result of the incomplete enzymatic action of the Cre recombinase, and this will allow the comparison of PCDH19 mosaic effects between genders. Interestingly, mosaic KO females display a transient growth delay between the 3rd and 4th postnatal week, suggesting a potential critical time-window in which to reconfirm brain hyperexcitability in this model. With this regard, the surface expression of the alpha1 subunit of $GABA_A$ receptor, which mediates fast inhibitory transmission, is reduced in the brain of KO mosaic mice. Furthermore, preliminary data on hippocampal slices from adult mosaic KO females indicate that long-term potentiation (LTP) is impaired.

Discussion & Conclusions PCDH19 mosaic mice appear to be a promising model able to recapitulate the main features of PCDH19-related syndrome. Ongoing and future experiments aim at elucidating both the causes of the hyperexcitability in PCDH19 mosaic models and the consequences of this phenotype on circuit functioning and mice behavior.

P59 - Effect of donepezil on the expression and responsiveness to LPS of CHRNA7 and CHRFAM7A in macrophages: a possible link to the cholinergic anti-inflammatory pathway

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Object: The α7 nicotinic acetylcholine receptor (CHRNA7) plays a role in the modulation of the inflammatory response through the activation of the "cholinergic anti-inflammatory pathway". In humans, a recombination event involving the exon 5 to 10 of CHRNA7 gene, fused to four novel exons A, B, C and D (FAM7A), gave rise to the CHRFAM7A gene. This hybrid gene, located on chromosome 15q13-q14, 1.6 Mb apart from CHRNA7, is highly expressed in inflammatory cells, where it can regulate the anti-inflammatory effects of α7 activation, and in the CNS. Acute treatment of macrophages with LPS down-regulates CHRFAM7A by a mechanism driven by NF-κB, paralleled by CHRNA7 up-regulation. As studies are emerging, which identify CHRFAM7A expression alteration in inflammatory or infective pathologies, the regulation of its expression may become a key step in the modulation of inflammation. CHRNA7 and CHRFAM7A may become suitable targets for the pharmacological treatment of disorders characterised by systemic inflammation supported by alterations in their expression and function. Recent studies indicated that acetylcholine esterase (AChE) inhibitors, widely used for the symptomatic treatment of Alzheimer's disease and other dementias, have neuroprotective properties mediated via α7 nAChRs and also significantly modulate innate immunity possibly as a result of the increased availability of acetylcholine activating the CAIP. We thus explored the link between the "Cholinergic Anti-Inflammatory Pathway" and the AChEI Donepezil, by focusing on the regulation of CHRFAM7A and CHRNA7 expression in immune cell models and defined a human restricted mechanism modulating the inflammatory response.

Materials and methods: Human primary macrophages and the THP-1 monocytic-like cell line have been treated with donepezil in the presence or absence of LPS and CHRNA7 and CHRFAM7A expression level detected by means of qPCR and western blotting experiments.

Results: We provide a detailed analysis of the CHRFAM7A gene regulatory region and its pro-inflammatory stimuli responsiveness. Furthermore, given the anti-inflammatory potential of the acetylcholinesterase inhibitor donepezil, we investigated the CHRFAM7A expression profile in macrophages treated with donepezil, showing an unexpected up-regulation of both CHRFAM7A and CHRNA7 gene.

Discussion and conclusions: The unexpected up-regulation of both CHRFAM7A and CHRNA7 genes by donepezil suggests that the immunomodulatory potential of the drug may be exerted by regulating the activation of CAIP through the modulation of the expression of α7 nAChR and CHRFAM7A at transcriptional level. Given the great therapeutic potential of donepezil, we consider that the results provided could contribute to a better characterization of its pharmacological activity.

P60 - Novel therapeutic agents for neurological and psychiatric disorders targeting the oxytocin receptors

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Object The neurohormone oxytocin (OT) is a key regulator of diverse social behaviors and has emerged as one of the most promising treatment for a variety of neurodevelopmental and neuropsychiatric disorders, including autism spectrum disorders (ASD), schizophrenia, anxiety and depression. However, the use of OT in humans presents a number of open issues, including the knowledge of its specific molecular and cellular targets in the CNS, its gender and age specific indications, its side-effects, way of administration and dosage. In addition, we need to overcome some OT limitations: its short half-life, its poor selectivity and scarce capability to cross the blood brain barrier (BBB).

We previously developed "functional selective" ligands active on specific G-proteins downstream the OTR that let to explore the cellular and behavioral effects of OTR-Gi/o and Gq signaling (Reversi et al. J.Biol Chem 2005; Busnelli et al J.Biol Chem 2012); one of these analogs, atosiban, allowed us to identify a new population of OT-neurons involved in pain and showed a potent analgesic effect (Eliava et al. Neuron 2016). We also generated a new class of OT homobivalent ligands specific for OTR homodimers, that act as superagonist and boost social interactions in a mouse model of ASD and in zebrafish (Busnelli et al. J.Med Chem 2016).

Materials and Methods To clarify the multiple OT actions in the brain and to generate more effective drugs targeting the OT system, we are now studying in vitro and ex vivo the interactions between OTRs and other GPCRs relevant for neuropsychiatric

disorders, such as dopamine D2R, and we are designing OT analogues able to pass the BBB and to produce long-acting effect.
Results We are mapping the distribution of OTR monomer and heterodimers in the brain using i) our newly developed fluorescent OT analogues; ii) a "DNA nanoruler", a new and original method based on OT-DNA complexes whose development is ongoing in our lab; iii) heterobivalent ligands specific for OTR/D2 heterodimers that we specifically designed.
In the pipeline we have OT molecules fused to nanoparticles able to cross the BBB and OT peptidic analogs modified to resist to degradation.
Conclusions Our studies will contribute to define the role of OT on specific molecular cellular targets and processes in the brain, with the ultimate goal to pave the way for the development of advanced therapies for neurological and psychiatric disorders.

P61 - Role of rare missense variants of the human β4 subunit in the expression and surface exposure of α3β4 nicotinic receptors

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Object 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. 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 β4 variants 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.
The aim of this work was to investigate the role of the subunit present in fifth position in the α3β4 nAChRs expression at the cell surface. Moreover we wanted to analyse whether the presence of the β4 variants in the fifth position could alter the nAChRs localization.
Materials and Methods We set up a system to express single population of pentameric receptors with fixed stoichiometry in coltured cells. We could choose the subunit present in fifth position by transfecting cells with a plasmid coding for a β4 subunit followed by an α3 subunit (dimer) plus a plasmid coding for the subunit (monomer) we want to be present as "accessory subunit".
Results 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 (Crespi et al., 2018). Moreover we found that β4 variants present in fifth position alter the exposure to the surface of α3β4 nAChRs.
Discussion This study demonstrates a novel function of the accessory subunit in the α3β4 receptor that may be relevant also for other pentameric receptors. The different exposure at the cell surface of nAChRs with the β4 variants in fifth position could explain the functional data obtained expressing these variants in habenula and in hippocampal neurons (Slimak et al, 2014).

P62 - Extracellular Vesicles from Mesenchymal Stem Cells reduce Aβ plaque burden in early stages of Alzheimer's disease

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Object Bone marrow mesenchymal stem cells (MSC), due to their strong protective and anti-inflammatory abilities, are widely investigated in the context of several diseases for their possible therapeutic role, based on the release of a highly proactive secretome composed of soluble factors and Extracellular Vesicles (EVs). MSC-EVs, in particular, convey many of the beneficial features of parental cells, including direct and indirect β-amyloid degrading-activities, immunoregulatory and neurotrophic abilities. Therefore EVs represent an extremely attractive tool for therapeutic purposes in neurodegenerative diseases, including Alzheimer's disease (AD). We examined the therapeutic potential of intracerebrally injected MSC-EVs into the neocortex of APP/PS1 mice at 3 and 5 months of age, a time window in which the cognitive behavioral phenotype is not yet detectable or just starts to appear.
Materials Primary cultures of Bone Marrow-derived MSC (up to P12 passage), APPswe/PS1dE9 (APP/PS1) AD mice.
Methods MSC were stimulated by serum-deprivation for 3 hrs and supernatant was submitted to differential ultracentrifugation to collect EVs (including exosomes and microvesicles pools), which were characterized by Nanoparticle tracking, cryo-EM, flow cytometry and Western blot analysis.
APP/PS1 mice, 3 and 5 months old, were injected intracortically with 4 uL of BM-MSC-derived EV suspension, corresponding to 22.4 ug of total proteins. Brain sections of mice treated or not with EVs were immunohistochemically stained for $Abeta_{1-42}$ peptide (6-E10 antibody). Smi31 and 32 antibodies recognizing Neurofilaments were used for staining dystrophic neurites around $Abeta_{1-42}$ plaques stained by Thioflavin-T.
Results Intracerebral injection of MSC-EVs into the neocortex of APP/PS1 mice at 3 and 5 months of age reduced $Abeta_{1-42}$ plaques burden one month later compared to same-age untreated mice. At 3 months, when plaques have just started to form, treatment conferred a preventive significance. In addition, following treatment with MSC-EVs, a reduction in dystrophic neurites could been measured. This decrease resulted significantly different in 6 month-old AD mice. Neprilysin, a metal-membrane endopeptidase able to degrade $Abeta_{1-42}$, was detected on MSC-derived EV lysates.
Discussion We demonstrate that MSC-EVs are effective in reducing the Aβ plaque burden and the amount of dystrophic neurites, in both cortex and hippocampus. The presence of Neprilysin on MSC-EVs opens the possibility of a direct β-amyloid degrading-action as a possible mechanisms of action.
Conclusions Our results indicate a potential role for MSC-EVs already at early stages of AD, suggesting the possibility to intervene before overt clinical manifestations.

P63 - Molecular targets of oxytocin signalling in neurodevelopment

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Object Oxytocin (OT) is a hypothalamic neuropeptide that, when given in the perinatal period, exerts a striking rescue effect on impaired social behaviour that persists until adulthood (Meziane et al., BiolPsy, 2015; Penagarikano et al., SciTranslMed, 2015), suggesting that OT is involved in crucial steps of perinatal neurodevelopment. Our studies aim at unravelling the molecular targets and pathways involved in this key OT modulatory role in neurodevelopment. We previously focused on the excitatory/inhibitory (E/I) balance in the brain, which is often altered in neurodevelopmental disorders. In particular, we investigated the timing of the switch of GABA from excitatory to inhibitory and unveiled a molecular signalling pathway through which OT modulates the Cl^--transporter KCC2 in hippocampal neurons (Leonzino et al., CellReports, 2016). Because IGF-1 and IGF-1 receptor (IGF-1R) have also been shown to modulate KCC2 and restore a correct E/I balance in the $MeCP2^{-/-}$ mouse model of Rett Syndrome (Banerjee et al., PNAS, 2016), we are now studying the role of OT/IGF-1 cross talk.
Materials and Methods We are using autoradiography and infrared immunostaining to map and quantify OTR, IGF-1 receptor and KCC2 expression in brain slices of adult $MeCP2^{m+/p-}$ hemizigous mice. Hippocampal cultures and Ca^{2+}-imaging will be used to study the cooperative effects

of OT and IGF-1 in the modulation of the E/I balance in neuronal development.
Results We found region-specific alterations of OTR and IGF-1R levels in young adult male MeCP2$^{m+/p-}$ mice; similarly, treatment with hrIGF-1 or OT restored OTR and IGF-1R levels in a region-specific manner.
Discussion Pharmacological manipulation of the OTergic and/or IGF-1 systems may be possible therapeutical strategies in neurodevelopmental disorders that warrants further investigation. In addition, as the immune system is crucial in neurodevelopment, and because OTR expression has been demonstrated in T-cells and microglia (Yuan et al., J.Neuroinfl, 2016), we plan to investigate the modulatory role of OT on the immune system. For this work we will employ the LgDel/+ mouse model of the 22q11.2 Deletion Syndrome, one of the major genetic vulnerability factor for psychiatric disorders in humans (Telethon grant GGP19103), in which important alterations in the immune system have been reported.

P64 - A novel positive allosteric modulator of mGlu5 receptor rescues behavioral and synaptic defects in SHANK3 knock-out mice.

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Object Shank proteins are large scaffold proteins located at post-synaptic density (PSD) of excitatory synapses which have a crucial role in formation, maturation and function of synapses. It is known that haploinsufficiency of SHANK3 is the major cause of neurological symptoms associated with Phelan-McDermid Syndrome (PMS), which include hypotonia, speech delay and autistic behaviour. Indeed, we recently demonstrated that SHANK3 is essential to mediate mGlu5 receptor signalling by recruiting Homer1b/c, another scaffold protein, to the PSD.
Materials We treated Shank3Δ11 -/- mice with VU0409551 a potent and selective positive allosteric modulator of mGlu5 receptor.
Methods In order to better clarify if positive allosteric modulators (PAMs) of mGlu5 might rescue the synaptic and behavioral alterations of Shank3 KO mice we tested the ability of VU0409551 to rescue behavioural and synaptic dysfunction in Shank3Δ11 -/- mice. To measure protein translation, we used SUnSET methodology.
Results We found that the acute treatment with VU0409551 rescued repetitive and stereotyped behaviours, social impairments and intellectual inflexibility observed in Shank3Δ11 -/- mice. Moreover, we found a specific reduction of activity dependent protein translation, in cortex and striatum of Shank3Δ11 -/- mice that can was rescued by chronic treatment with VU0409551.
Discussion The use of Shank3 KO mice allowed to clarify, in vivo, how and in which brain areas Shank3 absence affect mGlu5 signaling. Our results suggest that mGlu5 signalling is impaired in cortex and striatum of Shank3Δ11 -/- mice. Moreover, treatment with mGlu5 PAMs is able to rescue functional and behavioural defects due to Shank3 absence.
Conclusion The results of our study pave the way for possible pharmacological therapies for PMS based on mGlu5 positive modulation

P65 - Tetraspanin7 (TSPAN7) knock-out mice: spotlight on hippocampal and lateral habenula function

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Object: Mutations in many genes have been linked to increased risk of developing Intellectual disability (ID) and autism spectrum disorders (ASD) so far, including Tm4sf2 that encodes for tetraspanin7 (TSPAN7) protein. Often, ID and ASD share clinical and genetic components resulting in high comorbidity. In line with these observations, patients displaying mutated tm4sf2 gene has been diagnosed for ID and ASD. The hippocampal formation has been suggested as one of the main regions involved in ID and ASD. On the other hand, recently, the lateral habenula (LHb) has emerged as master regulator of several brain areas, such as the limbic system and monoaminergic nuclei, known to regulate behaviors that are impaired in neuropsychiatric disorders, including behavioral flexibility and sociability that are impaired in ID and ASD patients. For these reasons, we decided to

investigate the TSPAN7 function in hippocampus and lateral habenula (LHb) of Tm4sf2$^{-/y}$ mice.
Materials and Methods: For our purpose we used a knock-out animal model for TSPAN7 (Tm4sf2$^{-/y}$). Tm4sf2$^{-/y}$ mice were generated by replacing the first coding exon with a LacZ-pGK-Neor cassette. Tm4sf2$^{+/y}$ and Tm4sf2$^{-/y}$ mice were characterized through behavioral tests, biochemistry, electron microscopy and electrophysiology.
Results: We observed defects in hippocampal function and related behaviors in Tm4sf2 knock-out (Tm4sf2$^{-/y}$) mice that has been rescued by the PICK1-GluA2 interaction inhibitor (pep2-EVKI) and by positive modulation of AMPA receptors through the ampakine CX516 (Murru L. et al. 2017). In preliminary results, Tm4sf2$^{-/y}$ mice showed a minor sociability, increased self-grooming, altered marble burying, decreased sucrose preference and increased depressive-like state. Furthermore, functional experiments showed a strong hypo-excitability, an aberrant neuronal firing pattern and altered potassium and sodium conductances in LHb neurons of Tm4sf2$^{-/y}$ mice.
Discussion and conclusions: With our data, we demonstrated that AMPA receptors positive modulation ameliorates Tm4sf2$^{-/y}$ mice hippocampal phenotype. Moreover, preliminary data highlighted altered LHb activity in Tm4sf2$^{-/y}$ mice, suggesting that also this brain region could play a role in neurodevelopmental disorders.

P66 - Predisposition to alcohol drinking and alcohol consumption alter expression of calcitonin gene-related peptide, neuropeptide Y, and microglia in bed nucleus of stria terminalis in a subnucleus-specific manner

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Object. Excessive alcohol consumption can lead to alcohol abuse and dependence. Understanding the biological basis of excessive alcohol drinking can be useful to unravel signaling mechanisms and molecular signatures that underlie abnormal drinking behaviors. Sardinian alcohol-preferring (sP) and -non preferring (sNP) rats have been selectively bred for opposite alcohol preference and consumption under the standard, homecage, two-bottle “alcohol vs water” choice regimen with unlimited access. sP rats voluntarily consume large amounts of alcohol, resulting in significant blood alcohol levels and producing psychopharmacological effects (including anxiolysis and motor stimulation). Conversely, sNP rats avoid alcohol virtually completely. sP and sNP rats have been characterized for different phenotypes: in comparison with sNP rats, alcohol-naive sP rats displayed (1) more anxiety-related behaviors; (2) higher initial sensitivity to the locomotor stimulating and sedative/hypnotic effects of alcohol; and (3) lower sensitivity to the aversive effects of alcohol.
Materials and Methods. Calcitonin Gene-Related Peptide (CGRP) and neuropeptide Y (NPY) are two pleiotropic neuropeptides involved in modulation of anxiety-related behaviors. We aimed at analyzing whether CGRP (exerting anxiogenic effects in BNST) and NPY (exerting mainly anxiolytic effects) expression were differentially regulated in sP and sNP rats. In addition, we analyzed microglia activation, an emerging crucial process in substance abuse. The analysis focused on extended amygdala (AMY), a system influencing anxiety-related behaviors. Our analysis was carried out on CGRP/NPY-immunoreactive terminals present in and microglia activation occurring in bed nucleus of the stria terminalis (BNST) and AMY. Adult, male alcohol-naive sP and sNP rats were anesthetized and perfused by paraformaldehyde. Their brains were dissected, embedded in sucrose, frozen, cut at cryostat and sections processed for immunofluorescence and analyzed at confocal microscope. The analysis was conducted on anterior BNST, namely anterolateral (AL), anteromedial (AM), and anteroventral (lateral + medial subdivisions: AVl, AVm) subnuclei.
Results. CGRP-immunofluorescent fibers/terminals did not differ between alcohol-naïve sP and sNP rats. Fiber/terminal NPY-immunofluorescence intensity was lower in BNST-AM and BNST-AVm of alcohol-naive sP rats. Activation of microglia (revealed by morphological analysis) was decreased in BNST-AM and increased in BNST-AVm of alcohol-naive sP rats. Prolonged (30 consecutive days), voluntary alcohol intake under the homecage 2-bottle “alcohol vs. water” regimen strongly increased CGRP intensity in BNST of sP rats in a subnucleus-specific manner: in BNST-AL, BNST-AVm, and BNST-AM. CGRP area sum, however, decreased in BNST-AM, without changes in other subnuclei. Alcohol consumption increased NPY expression, in a subnucleus-specific manner, in BNST-AL, BNST-AVl, and BNST-AVm. Alcohol consumption increased many size/shapes parameters in microglial cells, indicative of microglia de-activation. Finally,

microglia density was increased in ventral anterior BNST (BNST-AVl, BNST-AVm) by alcohol consumption. Discussion-Conclusion. Genetic predisposition to high alcohol intake of sP rats (which display an "anxious" phenotype) could be in part mediated by anterior BNST subnuclei showing lower NPY expression and differential microglia activation. Alcohol intake in sP rats (producing an anxiolytic effect) induced complex subnucleus-specific c hanges in BNST, affecting CGRP and NPY expression as well as microglia de-activation and increased density: it can thus be hypothesized that these changes might contribute to the anxiolytic effects of voluntarily consumed alcohol repeatedly observed in sP rats.

P67 - Development of a protocol for whole brain fluorescence imaging in mouse and zebrafish to quantitatively detect cell activation

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Object. The study of central nervous system (CNS) can be greatly boosted by taking advantage from an approach that allows to analyze CNS as a whole, by leaving intact its structure. By using light, which can be easily separated in different wavelength to achieve labelling of multiple specific and easily differentiable markers, it is possible to collect an enormous amount of data from whole CNS circuitry at once, providing crucial information on, e.g., activation of multiple specific markers in several areas of the complex CNS circuitry at a given time. However, light penetration depth in biological tissues is limited by scattering and adsorption. Possible solutions can be obtained either by using physical methods (such as refractive index matching solutions) or by chemical methods (such as removal of scatterers), based on tissue transformation protocols. The advent of light sheet microscopy (LSM) has opened the possibility of performing fast volumetric imaging with optical sectioning capability, largely expanding the potential to image whole brains (and whole body) of small animals. Indeed, this technique drastically reduces acquisition time and photobleaching, achieving good resolution at high penetration depths. However, it requires that the sample be transparent. For this reason, LSM is the major technique exploiting ex-vivo clearing for bulk sample analysis. Furthermore, the achievement of a quantitative approach requires not only strict standardization of the whole procedure, but also elimination of all artifacts (e.g., autofluorescence due to aldehyde fixation) that corrupt specific signal and increase measure variability.

Materials and methods. Two animal species were used, mouse and zebrafish, to develop a protocol for ex vivo CNS quantitative analysis via brain clearing. We used iDISCO+ as reference technique (Renier et al., 2016) and imaged whole brains by LSM. Immunofluorescence against GFAP was performed as previously described (Rossetti et al., 2018).

Results. Ex-vivo brain labeling is most often obtained by aldehyde fixation which, however, increase autofluorescence. This can become a dramatic problem in whole mount preparation. In our hands, zebrafish produced a higher level of autofluorescence than mouse. Thus, we used zebrafish to develop a protocol for reduction of autofluorescence using, as agent, glycine, hydrogen peroxide, sodium borohydride, ammonia and Sudan Black. In immunofluorescent labeled sections, autofluorescence was reduced by all agents, with decreasing potency: Sudan Black > ammonia > sodium borohydride > hydrogen peroxide > glycine. Sudan Black, however, achieved its effect by covering the sections by a precipitate (which decreased specific immunolabeling also), what makes it not useful for whole mount fluorescence imaging. Each agent is thought to affect a specific molecular counterpart: however, ammonia and glycine are both thought to modify amine residues, and thus, we discarded glycine as ammonia was found more effective. In subsequent experiments we combined all others in different sequences and obtained a reduction of about 80% of control autofluorescence with a protocol that used hydrogen peroxide (as first or last) + ammonia followed by sodium borohydride.

Experiments were also performed to analyze penetration of antibodies in deep structures of CNS. To perform this experiment, we used the larger mouse brain. Following first antibody incubation (anti GFAP), the brain was frozen, cut and brain sections incubated with a) only secondary, fluorescently-labeled antibody or b) again anti GFAP antibody followed by secondary, fluorescently-labeled antibody. The results showed that 1) first antibody penetrated even into deep brain structures, 2) no difference was detected if primary antibody was added to sections, 3) no penetration gradient was detectable between surface (cortex) and deep structures (hippocampus).

Finally, we performed a brain clearing experiment (without immunolabeling) by using organic solvents: the results showed an excellent transparency of

whole mouse brain. The same complete transparency was also obtained in zebrafish brain.
Discussion-Conclusion. Once the methodology will be fully developed, the present technique has the potential to allow a quantitative analysis of immunolabeled markers in a whole brain structures, thus preserving whole CNS complex circuitry.

P68 - Pro- and anti-inflammatory markers are both decreased by calcitonin gene-related peptide (CGRP) in experimental autoimmune encephalomyelitis (EAE)

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Object. Multiple sclerosis (MS) is a complex neuroinflammatory disease whose pathogenic mechanisms involve an autoimmune activation against central nervous system (CNS) antigens. We decided to investigate molecular mechanisms that occur during the induction phase of MS, in order to evaluate molecules that, released inside CNS, act on both brain-resident and infiltrated immune cells. We focused our attention on calcitonin gene-related peptide (CGRP, a neuropeptide mainly synthesized by neuronal cells) that can influence the activity of microglia and astrocytes, but also immune cells.
Materials and Methods. We released CGRP intrathecally by osmotic minipumps during the induction phase of experimental autoimmune encephalomyelitis (EAE) in mice (Rossetti et al., 2018): two days after EAE induction with MOG_{35-55} in C57BL/6 mice, a catheter (attached to an osmotic minipump) was implanted in a subdural location of the spinal canal. CGRP was delivered at 50 pmol/h: administration lasted 14 days. Control mice received artificial CSF (aCSF). The animals were then perfused, spinal cord cut at a cryostat and sections processed for immunofluorescence. The morphological and biochemical correlates of the CGRP action was analyzed by using antibodies against the following markers: Iba1 or CD11b (for microglia), GFAP (for astrocyte), iNOS (that in macrophage/microglia has been associated to a so-called inflammatory activation), Ym1 (that in macrophage/microglia has been associated to a so-called protective activation), CD206 or CD68 (macrophage/microglia activation markers), phosphorylated ERK1/2 and p38.
Results. We had already shown that the neuropeptide dampens the disease course and changes the 2D shape of microglial cells (Sardi et al., 2014). A 3D analysis of microglia ramifications showed that CGRP treatment significantly increased the Max End Radius of microglia, indicative of inhibition of activation. Focusing on the ventral and dorsal median aspects of spinal cord (the most consistently infiltrated areas), we found that CGRP leads to a reduction in pial cell infiltrates, but also in the percentage of CD68+ and Ym1+ cells in pial membrane, but not parenchyma.
Following extraction of fresh CNS tissue regions, a Real-Time PCR analysis showed that CGRP caused a reduction of IL-1beta expression in the encephalon (without cerebellum) and cerebellum (whereas it let unchanged levels in the spinal cord), and of IL-6 in the encephalon (without cerebellum) (unchanged levels in the cerebellum and spinal cord). No differences were detected in TNF-alpha and Ym1 expression in all three regions. It is worth mentioning that in the spinal cord homogenate for RT-PCR the pial membrane is likely to have been scratched out during forced expulsion of the spinal cord from the canal. These results suggest that the neuropeptide inhibits the expression of molecules associated with inflammatory, but also, unexpectedly, of those associated to protective roles.
Using a mixed astrocyte-microglia culture we compared Ym1 expression in microglia following 24h CGRP stimulation: the results showed that CGRP caused a reduction in Ym1 expression. It is worth mentioning that microglial cells in culture are in an activation state: thus, this result seem to be consistent with the reduction in Ym1 percentage observed in the pial membrane. Finally, since CGRP was shown to mediate tolerance to morphine-induced analgesia by activation of ERK (in astrocytes) and p38 (in microglia) in the spinal cord, we analyzed the same intracellular cascade: during EAE development the protective effect of CGRP was associated with ERK activation in astrocytes, but not p38 activation in microglia.
Discussion-Conclusion. The present results show that CGRP protective effect during EAE induction is associated to inhibition of microglial cells, reduced cell infiltration in pial membrane, and, most importantly, down regulation of both types of markers, inflammatory and protective, although in a highly context-dependent manner. These findings show, for the first time, that amelioration during EAE can be accompanied by a reduction of molecules associated to macrophage/microglia activation with opposite activities (both of the so-called

inflammatory and protective roles) rather than to a switch from inflammatory to protective function.

P69 - Abnormal behaviors and neuronal functions in mice lacking Shank3 in parvalbumin-expressing interneurons

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Object Shank1, Shank2 and Shank3 are scaffold proteins located in the postsynaptic density of excitatory glutamatergic synapses, essential for synaptic function and development. Mutations in SHANK genes are strongly associated with Autism Spectrum Disorders (ASDs); in particular, SHANK3 haploinsufficiency is considered the major cause of the neurological symptoms of Phelan McDermid Syndrome (PMS), a neurodevelopmental disorder characterized by expressive language delay, intellectual disability, hypotonia, autistic-like behaviour, and epilepsy. Since an imbalance between excitatory and inhibitory systems may lead to autism symptoms, we hypothesized that some neurological pathological features described in PMS patients might be due to alterations of the inhibitory system. Indeed, the function of Shank3 in excitatory synapses of inhibitory neurons has been not fully explored.

Materials We generated PV-cre$^{+/-}$ Shank3$^{fl/wt}$ mice, in which Shank3 is deleted selectively in Parvalbumin positive (PV+) interneurons

Methods We performed behavioural tests in adult mice.

Results We found an impairment in grooming, learning, and memory tasks. Furthermore, we demonstrated that treatment with Ganaxolone, a positive modulator of $GABA_A$ receptors, improves the altered phenotypes of PV-cre$^{+/-}$ Shank3$^{fl/wt}$ mice.

Discussion The use of the PV-Cre$^{+/-}$-Shank3$^{floxed/-floxed}$ conditional mice, a cell restricted conditional mice allowed us to clarify, in vivo, how Shank3 absence affect PV+ neurons development and function. Moreover, our results showed that treatment with $GABA_A$ positive modulators was able to ameliorate the behavioral defects due to Shank3 deletion.

Conclusion Our study reveals a new role for Shank3 in modulating PV+ interneurons and suggest that GABAergic system might represent a pharmacological target to develop new treatment for patient with PMS.

P70 - Rescuing epilepsy associated with SYN1 and SCN1A gene mutations by inhibiting eEF2K/eEF2 pathway

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Object Eukaryotic Elongation Factor 2 Kinase (eEF2K) is a ubiquitous Ca2+/Calmoduline-dependent kinase that regulates protein translation by catalyzing the phosphorylation of eEF2 at Thr56. In neurons, eEF2K is activated by Ca2+ influx via glutamate receptors stimulation and modulates the expression of certain proteins involved in synapse formation and plasticity. We recently demonstrated that eEF2K activity regulates the excitation/inhibition ratio in the brain. In particular, eEF2K-/- mice display enhanced GABAergic transmission and tonic inhibition and are less susceptible to epileptic seizures. Accordingly, we propose eEF2K/eEF2 pathway as a possible target for antiepileptic therapies.

Materials and Methods Using biochemical, electrophysiological and behavior experiments we studied the possibility to rescue epileptic and behavioral defects in the Scn1a+/- mice, the animal model of Dravet syndrome, by genetically and pharmacologically inhibiting eEF2K/eEF2 pathway.

Results and discussion We found that both genetic deletion and pharmacological inhibition of eEF2K can reduce epilepsy in two models of human genetic epilepsy, the Synapsin I knock-out mice (SynI -/y) (Heise et al, 2017) and the voltage-gated sodium channel (Scn1a) knock-ou mice (Scn1a+/-), the animal model of Dravet syndrome. We also found that the activity of eEF2K/eEF2 pathway (measured by the levels of phospho-eEF2) is enhanced in these two genetic mouse models of epilepsy and also in a pharmacologically induced model of epilepsy.

Therefore, the alteration of this pathway might represent a common molecular epileptogenic

mechanism responsible for the hyperexcitability of the neuronal network described in epilepsy.
Using the Scn1a+/- mice, we also found that motor coordination defect, memory impairments, and stereotyped behavior are reverted by eEF2K deletion. The analysis of spontaneous inhibitory postsynaptic currents (sIPSCs) suggested that the rescue of the pathological phenotype was driven by the potentiation of the GABAergic synapses.
Conclusions Thus our data indicate that the pharmacological inhibition of eEF2K could represent a novel therapeutic strategy for treating epilepsy and related comorbidities.

P71 - Oxytocin receptor changes in neurodevelopment: sex differences and behavioural consequences

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Object Oxytocin (OT) is a small neuropeptide secreted by the hypothalamus. In the CNS, it regulates many social and nonsocial functions. We previously reported that an altered expression level of oxytocin receptor (OTR) is present in two different mouse models of neurodevelopmental disorders, Oprm1-/- and Dys+/- mice (Gigliucci et al, 2014; Ferretti et al, 2019). To understand if these OTR alteration is a common feature of neurodevelopmental disorders in general, we are now studying the Magel2 +m/-p mouse model of Prader-Willi Syndrome, characterized by an abnormal feeding behaviour, failure to thrive, and altered social skills; in this model, a postnatal OT treatment prevents social deficits in adult mice (Meziane et al, 2015). Our aim is to understand how OTR levels change in the absence of Magel2, and to clarify if the early OT treatment can also restore OTR levels alterations.
Materials and methods We tested 4-months-old WT mice, KO mice treated with vehicle and KO mice treated at birth with OT, using receptor autoradiography to evaluate OTR expression in brain areas known to be involved in the regulation of social behaviors. In all the groups, males and females were analyzed separately.
Results Our analysis revealed sex and genotype-dependent differences in several brain areas. Interestingly, we also highlighted that in specific brain regions the OT treatment restored normal OTR levels in KO males, but had no effect on KO females.
Discussion These findings are indicative of a complex relationship between OTR alterations and behavioural deficits. The strong differences between males and females hint a sexually dimorphic regulation mechanism of the receptor. We're now investigating the effects of an OT treatment at birth immediately after its administration, by including in the analysis a group of treated or non-treated PN8 mice.
Conclusion OTR levels in the brain are often altered in neurodevelopmental disorders accomunated by social deficits. In this context, we've underlined the direct correlation between the receptor levels and the behavioural phenotype of adult male mice, as the beneficial OT treatment at birth could also revert OTR levels abnormalities. Lastly, we've revealed a strong difference in receptor expression in males and females, a difference that could have a crucial influence on the response to OT therapies.

P72 - Glial microvesicles in motion at the neuron surface

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Question Extracellular Vesicles (EVs) released from astrocytes and microglia are key players in glia-neuron communication in the healthy and diseased brain. However, how EVs move across the extracellular space to reach target neurons and whether EVs interact with neurons at preferential sites remain elusive.
Method To elucidate whether EVs produced by astrocytes interact with neurons at preferential sites, we placed single EVs on different compartments of hippocampal neurons (cell body, dendrite and axon) by using optical manipulation and followed EV-neuron interaction by live microscopy. EVs were added to neuronal medium, trapped by the infrared laser tweezers and positioned on the chosen compartment. The trapping laser was switched off and time-lapse images were collected at 2-20 Hz for 20 min to monitor EV-neuron dynamics.
Results We found that glial EVs efficiently bind to both the cell body and neurites of primary hippocampal neurons. Surprisingly, after the neuron contact, a

large fraction of EVs move along the surface of axons and dendrites in both retrograde and anterograde directions. By the use of vesicles released from prion protein (PrP) knock out astrocytes we showed that extracellular vesicle motion is driven by binding of neuronal receptor, i.e. the cellular prion protein, that drifts on the plasma membrane following cytochalasin-sensitive, nocodazole-insensitive cytoskeleton rearrangements. However, a fraction of extracellular vesicles contain actin filaments and have an independent capacity to actively move at the neuron surface in an actin-dependent manner.
Conclusion Our results provide initial evidence for the existence of a dual mechanism (active and passive) underlying extracellular vesicle motion of glial EVs at the neuron surface. These data suggest that also in vivo EVs may move not randomly but following an anatomically defined pattern of connections and extracellular motion for medium/large-EVs may represent a specific route to spread misfolded proteins from the brain region where they first originate to progressively larger brain areas over time in neurodegenerative conditions.

Neuroimaging and Methodological Research

P73 - Neurofunctional correlates of body-ownership and sense of agency: a meta-analytical account of self-consciousness

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Object Self-consciousness consists of several dissociable experiences, including the sense of ownership of one's body and the sense of agency over one's action consequences (Gallagher, 2000). The relationship between body-ownership and the sense of agency has been described by different neurocognitive models, each providing specific neurofunctional predictions. According to an "additive" model, the sense of agency entails body-ownership, while an alternative "independence" hypothesis suggests that they represent two qualitatively different processes, underpinned by distinct brain systems (Tsakiris et al., 2010). We propose a third "interactive" model, arguing the interdependence between body-ownership and the sense of agency: these constructs might represent different experiences with specific and exclusive brain correlates, but they also could partly overlap at the neurofunctional level.
Materials Here we sought to test these three neurocognitive models by reviewing the available neurofunctional literature of body-ownership and the sense of agency, with a quantitative meta-analytical approach that allowed us to compare their neural correlates statistically.
Methods We interrogated the Pubmed database in March 2019, by using both general-domain ("fMRI" or "PET") and specific-domain ("body-ownership" or "sense of agency") keywords. We performed a detailed inspection of the resulting manuscripts, and we included in the current meta-analysis 17 studies investigating neural correlates of body-ownership, for a total of 205 peaks of activation, and 14 experiments assessing neural correlates of sense of agency, for a total of 106 peaks of activation.
Results We identified (i) a body-ownership-specific network including the left inferior parietal lobule and the left extra-striate body area, (ii) a sense-of-agency-specific network including the left SMA, the left posterior insula, the right postcentral gyrus, and the right superior temporal lobe and (iii) a shared network in the left middle insula.
Discussion These results provide support for the interactive neurocognitive model of body-ownership and the sense of agency. Body-ownership involves a sensory network in which multisensory inputs are integrated, to be self-attributed. On the other hand, the sense of agency is specifically associated with premotor and sensory-motor areas, typically involved in generating motor predictions and in action monitoring. Finally, body-ownership and the sense of agency interact at the level of the left middle insula, a high-level multisensory hub engaged in body and action awareness in general.
Conclusions Our findings show that, even if body-ownership and the sense of agency are partly grounded on the same basic multisensory integration processes, they can be regarded as different experiences underpinned by partially distinct brain networks.

P74 - Effect of metformin as add-on therapy in a high-grade glioma mouse model

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Object: Glioblastoma (GBM) is the most aggressive malignant brain tumor refractory to conventional treatments, resulting in poor life expectancy. Genetic mutations and cancer-related inflammation influence therapy response. Our previous data with Gli36ΔEGFR cell line indicated that metformin (MET) addition to Temozolomide (TMZ) increases the survival of orthotopic GBM bearing-mice inoculated with human EGFR-mutated glioma cell lines, and Positron Emission Tomography (PET) imaging allowed to monitor cell proliferation and inflammation. Here, we evaluated the effect of metformin as add-on therapy in orthotopic GBM models inoculated with patient-derived EGFR+ GBM cancer stem cells (CSCs), L0627.

Materials: L0627 CSCs were exploited both for in vitro and in vivo experiments. In vitro, TMZ and MET were tested alone and in combination, at different concentrations. In vivo, athymic nude mice were intracranially injected. Mice were divided into different treatment groups, and tumor response was monitored before therapy and after 7 and 28 days using imaging. At sacrifice, brains were processed for immunohistochemistry.

Methods: Cell proliferation/survival was measured by MTT incorporation assay. In vivo, tumor growth was monitored with T2-weighed MRI and PET imaging with [^{18}F]FLT (cell proliferation) and [^{18}F]VC701 (inflammation).

Results: After 72 h, the treatment regimen with TMZ alone didn't show any effect on cell proliferation at any different concentration, whereas MET 5 mM significantly reduced cell proliferation compared to vehicle-treated cells. Combination of drugs increased the efficacy of single administration. Different to what observed in vitro, MET alone didn't improve mice survival compared to vehicle-treated mice, whereas mice treated only with TMZ increased their survival. In any case, all mice displayed increased tumor growth visible at MRI. Only in TMZ+MET treated group, this increase was not observed after 150 days. No changes in [^{18}F]FLT uptake were detected at the end of treatment, while TMZ-treated mice displayed significant increase in [^{18}F]VC701 uptake.

Discussion: As previously observed in Gli36ΔEGFR cell line, the add-on of metformin improves the effect of TMZ. Tumor obtained by Gli36ΔEGFR cell line displayed high [^{18}F]FLT uptake, correlating to tumor response to TMZ treatment. In this case, tumor masses were more diffuse in the parenchyma and no effect on [^{18}F]FLT uptake was observed. In both models, we detected a different modulation of inflammation after TMZ+MET treatment compared to TMZ alone.

Conclusion: Our data indicate that add-on metformin increases TMZ efficacy in GBM EGFR+ models possibly acting on the inflammatory microenvironment, but further studies are needed.

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P75 - PET and MRI evaluation of experimental stroke: a new SPM analysis

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Object: Advanced imaging techniques as PET and MRI are applied in stroke to monitor disease and guide therapy. In this work, Middle Cerebral Artery Occlusion (MCAO) is performed in mouse as ischemia model and followed over time using dedicated MRI and PET/CT with [^{18}F]FDG and [^{18}F]VC701 as radioligands, for glucose metabolism and microglia/macrophages activation, respectively. Image analysis was implemented to obtain an automatic operator-independent method.

Materials: A group of ten C57BL/6 male mice underwent surgery for MCAO using a 45 minutes occlusion protocol. Seven animals completed PET/CT and MR imaging study, following this scheme: [^{18}F]VC701-PET at day 1; T1-w and T2-w MRI and [^{18}F]FDG-PET at day 2; MRI and PET with [^{18}F]VC701

and [^{18}F]FDG at 1 and 2 weeks post-ischemia. A second group of ten control mice performs a PET/MRI session using the same sequences.
Methods: PET/CT images were reconstructed and analyzed using two protocols: a region of interest (ROI) and a statistical parameter mapping (SPM) based approach. For ROI analysis, a spherical region of 1 mm diameter was manually drawn based on co-registered MR images. The automatic SPM analysis is built on a statistical method: all images are realigned to a standardized anatomical space obtained from controls, scaled to the global mean of the whole brain and a voxel-wise student t-test is performed to identify regions with abnormal uptake at single subject level.
Results: ROI quantification found a maximum decrease in glucose metabolism in the ipsilateral versus contralateral hemisphere at d2 (-62% of [^{18}F]FDG uptake), partially restored thereafter to -32% at d9 and d16. Maximum inflammation was observed at d9, with a 50% increase of [^{18}F]VC701 uptake, remaining stable the week after, (+34% at d16). SPM analysis showed a large volume of reduced [^{18}F]FDG uptake, with mean peak t-values of 17, 7.8 and 13 at d2, d9 and d16, respectively. Applied to [^{18}F]VC701 tracer, SPM found no significant uptake at d1, as for the ROI analysis, and significant uptake in the ipsilateral hemisphere at d8 and d15 (mean t-value of 8.5 and 9.5).
Discussion: The results of the two PET images analysis are consistent. The introduction of an SPM based pipeline allowed a more streamlined and standardized analysis method, automatically detecting the regions affected by stroke. This avoids operator-dependent errors and calculate automatically lesion volume.
Conclusions: SPM single subject analysis provides an optimal tool to automatically estimate damage in ischemia models, with application in therapy evaluation.
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P76 - Hungry brains: A meta-analytical review of brain activation imaging studies on food perception and appetite in obese individuals

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OBJECT The dysregulation of food intake in chronic obesity has been explained by different theories. The Reward Surfeit Theory (RST) suggests that a predisposing factor to excessive food intake is the fact that food ingestion has a particular rewarding value. A refinement of this theory can be found in the Incentive Sensitization Theory (IST) which posits that obese would show an up-regulation of the reward system in the long run, which is driven by visual cues. The Reward Deficit Theory (RDT) suggests that obese people keep overeating because their reward system is less sensitive to dopaminergic signals. Differently, the Inhibitory Control Deficit Theory (ICDT) calls into play higher-level control functions: these would be not as good in obese patients who would over-react to food-related cues. To assess their explanatory power, we meta-analyzed 22 brain-activation imaging studies.
MATERIALS AND METHODS We combined a novel unique-solution hierarchical clustering (HC) algorithm implemented in a Matlab toolbox called CluB with the GingerALE algorithm to perform a meta-analysis on visual and gustatory perception of food stimuli in obese (OB) and healthy weight (HW) individuals varying in satiety state. The dataset was composed of 650 activation foci. The clusters returned by the HC algorithm were retained only if they were overlapping with the resulting ALE map: those clusters were submitted to a cluster composition analysis, which allows to test the specificity for a particular level of a factor or an interaction between them.
RESULTS 38 clusters survived the intersection analysis with the ALE map. Three were specific for OB (superior frontal gyrus, SFG; ventral striatum, VS; anterior insula, AI), 2 for HW (midbrain; thalamus). Only one cluster was associated with the visual modality (AI), whereas 5 with the gustatory (globus pallidus; mid-posterior insula; VS; postcentral gyrus; thalamus). The right SFG, orbitofrontal cortex and superior medial frontal cortex clusters were specific for the fed condition, while the mid-posterior insula was specific for the fasting state. Finally, the left Nucleus Accumbens/Caudate Head displayed a significant sensory modality-by-satiety interaction, whereas the left VS displayed a significant group-by-sensory modality interaction. Three-way interactions were also explored.
DISCUSSION AND CONCLUSION Consistent with a RST, obese individuals exhibit a ventral striatum hyper-

responsivity in response to pure tastes, particularly when fasting. Further, obese subjects display more frequent ventral striatal activation for visual food cues when satiated: this continued processing within the reward system, together with the aforementioned evidence, is compatible with the IST. On the other hand, we did not find univocal evidence in favor of a RDT nor of an ICDT. We conclude that the available brain activation data on the dysregulated food intake and food-related behavior in chronic obesity can be best framed within an IST.

P77 - All-Resolutions Inference for Brain Imaging

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Object Researchers often highlight the patterns of brain activation suggested by the data, but false discoveries are likely to intrude into this selection. It is well-known that humans are very good at finding seemingly convincing patterns even in pure noise. How confident can the researcher be about a pattern that has been found, if that pattern has been selected from so many potential patterns? We propose a novel approach - termed 'All Resolutions Inference' (ARI) - that delivers strong FWER control in any selected set of voxels, e.g. those found by a cluster-forming threshold.

Materials For a real-data illustration, we consider a sample of 80 subjects from the Human Connectome Project database (HCP, S1200 Release). Participants are presented with blocks of trials that either ask them to decide which of two shapes presented at the bottom of the screen match the shape at the top of the screen, or which of two faces presented on the bottom of the screen match the face at the top of the screen, where the faces have either an angry or fearful expression.

Methods ARI estimates the proportion of truly active voxels in any selected set of voxels. The selection process does not have to be declared beforehand; it does not have to be well-described; it may be circular; it does not even have to be repeatable. The selection may be data-driven or knowledge-driven, or any mix of the two.

Results The exploratory search for patterns of activations was performed by a data-driven approach (clusterwise analysis) as well as by using domain knowledge (Harvard-Oxford Atlas cortical structural areas). The task involving matching shapes and emotionally negative seems to engage a consistent activation in Occipital Pole and Temporal Occipital Fusiform, a moderate activation in Lateral Occipital Cortex inferior division and Occipital Fusiform Gyrus.

Discussion We illustrated how ARI can be used to find interesting patterns of effects by learning from what the data shows and from what is expected by domain knowledge.

Conclusions ARI allows a truly interactive approach to selective inference, that does not set any limits on the way the researcher chooses to perform the selection.

P78 - Functional abnormalities in individuals with a desire for healthy limb amputation

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Object Body Integrity Dysphoria (BID) is characterized by a long-lasting and persistent desire for the amputation of a physically healthy limb. In recent years, BID has been interpreted as a body schema/image disorder due to anomalies in the brain networks involved in body ownership and body representation. To date, only a few studies investigated the neural correlates of this disorder, suggesting abnormalities in brain networks involved in body awareness. Nevertheless, these studies included small and heterogeneous samples and mainly ROI-based analyses of structural or functional data.

Materials We studied ten subjects with desire for the amputation of the left leg and 14 healthy subjects. All subject with BID reported a lifelong and persistent desire to have an amputation of a functionally healthy body part.

Methods Participants underwent fMRI measurements of brain activity during tactile stimulation and movements (ME) of hands and feet. In the tactile stimulation task, the experimenter touched the left or right hand or foot of the subjects. These conditions were alternated with rest according to a block design. During the ME task, the participants were asked to perform movements of the right and left hand (thumb-to-finger sequential opposition) or foot (flexion and abduction of the foot's toes) alternated with periods of rest.

Results The comparison within BID and healthy subjects showed reduced activations in the bilateral

somatosensory cortex (SI and SII) and bilateral supramarginal gyrus in individuals with BID, only during the tactile stimulation task. These regions showed a decreased activity in participants with BID during the somatosensory stimulation of the body parts, regardless of which body part was stimulated. No significant differences were found for the motor execution tasks.
Discussion Our results show functional alterations in the brain of individuals suffering from BID during the tactile stimulation of body parts. These alterations concern brain regions involved in different stages of the construction of the body representation such as the somatosensory cortex and the supramarginal gyrus. This reduction of activity concerns the body representation of the whole body and is not confined to the body part affected by the desire of amputation.
Conclusions These results indicate a global alteration of the brain networks involved in multisensory integrations and body representation, but it remains unclear how the observed brain anomalies in the processing of somatosensory stimuli are linked with the desire of amputation of a specific limb and side of the body.

P79- Non-linear microscopy as a tool for medical histopathology: a pilot study.

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Object: Our aim is to develop experimental and numerical methods to help the pathologists in the diagnosis of diseases with minimum invasiveness. Our first aim is to work on biopsies, with a long term goal to implement the same methodology in vivo, possibly in the surgical field.
Materials: we test methods for digital pathology based on the application of high resolution multimodal non-linear microscopy on biopsies of xenograft cancers in mice. In this pilot study, we will investigate the presence and progression of the tumor by monitoring the microscopic state of Collagen, whose organization changes during several pathological processes. Since Collagen can be non-invasively visualized by Second Harmonic Generation (SHG) microscopy, we can exploit its micro-structure as an early diagnostic marker in experimental and clinical medicine. Non-linear SHG microscopy allows to quantitatively asses the tissue microstructure, well beyond the morphological information. However, also non-linear autofluorescence microscopy can be advantageously applied to study a variety of tissues, from skin to retina.
Methods. We have recently developed a 2-dimensional phasor-based approach (µMAPPS, Microscopic Multiparametric Analysis by Phasor projection of Polarization-dependent SHG signal) of the polarization dependent second harmonic generation images that is able to quantitative map the features of the collagen architecture (fibril orientation and the local molecular disorder) in tissues at the micrometer scale on two complex planes.
Results. We present here the results of the application of µMAPPS to the microscopic characterization of the complex tumor extra cellular matrix architecture ex-vivo and in-vivo. Three different tissue preparations (fixed sections, biopsies, in-vivo) were selected for a first validation of the method. A more in depth study was performed in tumor biopsies at two stages of tumor progression by exploiting also geometrical features of the phasor plots and an unsupervised machine learning clustering algorithm, which automatically groups pixels with similar microstructural characteristics.
Discussion: Our µMAPPS analysis can be performed on wide (2mmx2mm) sections, providing the microstructural features of entire tumor sections. By introducing a 'tumor entropy' parameter, that describes the tissue order at the level of the whole section, and by combining it with the µMAPPS clustering approach, we highlight regions of the whole tumor sections characterized by similar microscopic behavior, opening the possibility to describe the organization/de-structuring trend of the tissues during the development of pathologies and to characterize the stage of the tumor growth.
Conclusions. Non-linear excitation microscopy offers wide possibilities to investigate the microscopic structure of tissues in vivo, avoiding the use of extrinsic labeling. Our preliminary study shows that these optical imaging techniques coupled to machine learning algorithms, is particularly promising for the evaluation of pathological states of various tissues.

P80 - Quantitative analysis of OCT-a retina scans from healthy and AMD vascular plexa according to signal amount and dispersion of caliber-classified vessels

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Object To apply a novel image-analysis approach to retinal tissues developing a semi-automatic quantitative protocol for descriptions of microvascular angioarchitectures on the basis of a small set of interdependent parameters.

Materials Angio-OCT scans from healthy individuals and AMD patients, classified as borderline or evident by qualified experts.

Methods The method foresees that vascular 3D angioarchitectures from samples could be summarized as a near-linear relationship on the basis of the amount, dispersion and caliber of the observed vascular trees. The slope of the resulting lines correlates with the angiogenic potential of the samples. This approach has been applied to xenotransplanted experimental tumors [1] and cerebral vessels of the twitcher mouse (a model of Krabbe disease) [2].

OCT-A images were obtained with a TOPCON scanner and elaborated using the freely available ImageJ application.

Results After a preliminar registration step, much effort was devoted to develop a rationale for an automatic application able to isolate the binary representation of retinal vascular plexa. The resulting signals were then classified according to the minimal vascular cross-section observed on the XY Cartesian planes and OCT-A artefacts removed assigning to vessels a depth not greater of their planar caliber. The final analysis was carried out on a set of partially reconstituted vascular trees, obtained combining vessels with different calibers but belonging to the same plexus.

In some of the AMD patients, we quantified a vascular reduction in the deep plexus of pathological eyes together with an increase in the microvascularization of corresponding choroidal tissues.

Discussion In addition to contributing to the identification of vascular alterations, this approach candidates itself as an useful tool to monitor disease progression and the effectiveness of anti-AMD therapies.

Conclusions Results supported the feasibility of the analysis, and pave the way to the use of this approach for the study of disease progression in AMD and in neurodegenerative diseases affecting retinal vasculature.

[1] Righi et al., Sci. Rep. (2018), 8:17520 | doi:10.1038/s41598-018-35788-4
[2] Righi et al., Int. J. Mol. Sci. (2019), 20:2384 | doi:10.3390/ijms20102384