



ISTITUTO DI
NEUROSCIENZE

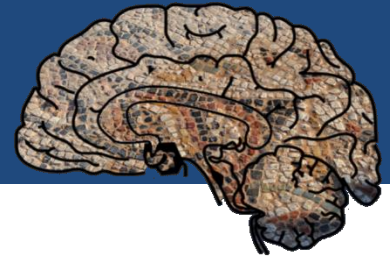


Consiglio Nazionale
delle Ricerche

IN CNR Annual Retreat 2017

28–30 September 2017

*Hotel Flamingo Resort
S. Margherita di Pula (CA)*



Abstracts

- I. Plenary Lectures (PL)**
- II. Oral Communications (OC)**
- III. Training Course (TC)**
- IV. Posters (P)**

INDEX

INNOVATIVE TECHNOLOGIES FOR NEUROELECTRONIC INTERFACES	5
WHAT THE GENES TELL US ABOUT OUR ORIGINS, EVOLUTIONARY HISTORY AND OUR CURRENT HEALTH	6
BEYOND MOTONEURONS: TRANSYNAPTIC ACTION OF BONT/A	7
THE INNATE IMMUNE MOLECULE PTX3 ENHANCES THE SYNAPTIC CONTENT OF AMPA RECEPTORS VIA EXTRACELLULAR MATRIX REMODELING	8
MATERNAL IMMUNE ACTIVATION DELAYS EXCITATORY-TO-INHIBITORY GABA SWITCH	9
MICROGLIA-DERIVED EXTRACELLULAR VESICLES REGULATE THE RECRUITMENT, PROLIFERATION AND DIFFERENTIATION OF OLIGODENDROCYTE PRECURSOR CELLS	10
UNVEILING THE MECHANISMS UNDERLYING OLIGODENDROCYTE DIFFERENTIATION: IMPLICATIONS FOR DOWN SYNDROME	11
LONG-TERM POTENTIATION (LTP) REQUIRES SYNAPTIC GLIA FOR PROBDNF PROCESSING AND RECYCLING OF THE ISOLATED PRO-PEPTIDE (BDNFPRO)	12
THE ROLE OF THE FEMALE EPILEPSY PROTEIN PCDH19 BETWEEN SYNAPSE AND NUCLEUS	13
USING FRET-BASED SENSORS TO STUDY THE CAMP/PKA AXIS AT THE OUTER MITOCHONDRIAL MEMBRANE	14
MCERULEAN3-BASED CAMELEON SENSOR TO EXPLORE MITOCHONDRIAL Ca^{2+} DYNAMICS <i>IN VIVO</i>	15
WHEN TIMING MATTERS: THE MIRROR MECHANISM OBSERVED THROUGH THE LENS OF INTRACEREBRAL RECORDINGS	16
IN VIVO OPTICAL IMAGING OF REHABILITATION-INDUCED CORTICAL PLASTICITY AFTER STROKE	17
PRE- AND POST-SYNAPTIC ALTERATIONS INDUCED BY BDNF OVER-EXPRESSION IN THE 6-OHDA-LESIONED RAT MODEL OF PARKINSON'S DISEASE	18
LOSS-OF-FUNCTION MUTATIONS IN THE <i>SIGMAR1</i> GENE CAUSE DISTAL HEREDITARY MOTOR NEUROPATHY BY IMPAIRING ER-MITOCHONDRIA TETHERING AND Ca^{2+} SIGNALING	19
LONG LASTING NEUROTOXIC EFFECTS OF MDMA ADMINISTRATION DURING ADOLESCENCE	20
TAAR1 MEDIATES THE PROTECTIVE EFFECT OF 3-IODOTHYRONAMINE AGAINST B-AMYLOID-DEPENDENT	21
BIDIRECTIONAL NEURON-GLIOMA INTERACTIONS: EFFECTS OF GLIOMA CELLS ON SYNAPTIC ACTIVITY AND ITS IMPACT ON TUMOR GROWTH	22
PLASTICITY IN RETINITIS PIGMENTOSA AND RETINAL PROSTHESES	23
A MULTIDISCIPLINARY APPROACH TO CHARACTERIZE THE PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS OF THE KETAMINE ANALOG METHOXETAMINE (MXE) IN RATS	24
INVOLVEMENT OF DOPAMINE IN THE DIFFERENCES IN SEXUAL BEHAVIOUR BETWEEN ROMAN HIGH AND LOW AVOIDANCE RATS: BEHAVIORAL, PHARMACOLOGICAL AND NEUROCHEMICAL FINDINGS	25
INFLUENCE OF JWH-018 REPEATED ADMINISTRATION ON THE RESPONSIVENESS OF MESOLIMBIC AND MESOCORTICAL DOPAMINE TRANSMISSION	26
RAT 50-KHZ ULTRASONIC VOCALIZATIONS AND GLUCOCORTICOID SIGNALING: EFFECTS OF CORTICOSTERONE, MIFEPRISTONE AND METYRAPONE ON CALLING INITIATION AND ON CALLING STIMULATED BY SOCIAL CONTACTS OR AMPHETAMINE	27
ROLE OF THE ODORANT RECEPTOR AT THE AXON TERMINAL	28
MECHANISMS OF THE IMPAIRED INCRETIN ACTION IN TYPE 2 DIABETES: A MODELLING STUDY	29
AUTOPHAGY CONTROLS NEONATAL MYOGENESIS BY REGULATING THE GH/IGF1 SYSTEM	30
A WINDOW WITH A VIEW: ORDER AND CHAOS IN THE BRAIN VISUALIZED BY TWO PHOTON MICROSCOPY	31
OPTOGENETIC DISSECTION OF INTERNEURON-TYPE-SPECIFIC SIGNALING TO ASTROCYTES	32
DIVING IN FOR DEEPER INSIGHTS	33
CONCURRENT MULTI-SITE CALCIUM RECORDINGS AND BRAIN IMAGING: STUDY OF DYNAMIC CONNECTIVITY RELATED TO SYSTEM AND FLUCTUATION STATES	34
NEUROIMAGING INSIGHTS INTO NETWORK-BASED NEURODEGENERATION	35

INVOLVEMENT OF TG2 IN CALCIUM HOMEOSTASIS IN RAT PRIMARY HIPPOCAMPAL NEURONS	36
THE INNATE IMMUNE MOLECULE PTX3 ENHANCES THE SYNAPTIC CONTENT OF AMPA RECEPTORS VIA EXTRACELLULAR MATRIX REMODELING	37
CHRONIC TREATMENT WITH BIFIDOBACTERIUM (<i>LONGUM</i> , <i>BREVE</i> , <i>INFANTIS</i>) CHANGES GABA _A SUBUNITS EXPRESSION AND EXCITABILITY IN THE HIPPOCAMPUS OF ADULT MALE RATS	38
ASTROCYTE SIGNALING IN BRAIN REWARD SYSTEM	39
BRAIN CHANGES IN DOPAMINE AND SEROTONIN LEVELS IN RATS EXPOSED TO THE ACTIVITY-BASED MODEL OF ANOREXIA	40
INTRA-VTA MICROINJECTION OF BACLOFEN AND CGP7930 SUPPRESSES ALCOHOL SELF-ADMINISTRATION IN RATS	41
ALTERATIONS IN 5-ALPHA REDUCTASE AND NEUROSTEROIDS IN THE PREFRONTAL CORTEX MEDIATE THE PSYCHOTIC- AND MANIA-LIKE PHENOTYPES INDUCED BY SLEEP DEPRIVATION	42
EARLY LIFE STRESS INCREASES ALCOHOL INTAKE AND IMPAIRS COGNITIVE BEHAVIOR IN C57B/6J MICE: POSSIBLE INVOLVEMENT OF NUCLEUS ACCUMBENS AND HIPPOCAMPAL FORMATION	43
INTRAVENOUS SELF-ADMINISTRATION OF THE SYNTHETIC CANNABINOID JWH-018 IN ADOLESCENT MICE	44
NOVEL TARGETS AND THERAPEUTICS FOR SOCIAL IMPAIRMENTS	45
CHRONIC STRESS EXPERIENCED BY PARENTS DURING ADOLESCENCE CAN INCREASE RISK FOR AUTISM SPECTRUM DISORDER IN OFFSPRING: A PROPOSED ANIMAL MODEL	46
CHRONIC TREATMENT WITH ETHINYL ESTRADIOL AND LEVONORGESTEL BLUNTS THE RESPONSE TO STRESS IN FEMALE RATS	47
MOUSE MODEL OF POLYMICROGYRIA: PHARMACOLOGICAL AND CELLULAR THERAPEUTIC APPROACHES	48
EXPLORING THE ROLE OF ASTROCYTIC CA ²⁺ SIGNALING IN ALZHEIMER'S DISEASE	49
PISA SYNDROME IN A DRUG-NAIVE PARKINSON'S DISEASE PATIENT	50
TRANSCRIPTIONAL EFFECT OF THE ACETYLCHOLINESTERASE DONEPEZIL ON THE HUMAN-RESTRICTED CHRFAM7A GENE IN IMMUNE AND NEURONAL CELL MODELS: IMPLICATIONS IN THE REGULATION OF THE A7 NACHR ACTIVITY	51
INSULIN CLEARANCE IS INDEPENDENTLY MODULATED BY INSULIN SENSITIVITY, BMI AND GLUCOSE TOLERANCE	52
BENEFICIAL AND DETRIMENTAL CONSEQUENCES OF A PRIMARY INCREASE IN MITOCHONDRIAL ROS FORMATION ON MITOCHONDRIAL FUNCTION, CA ²⁺ HOMEOSTASIS AND CARDIOMYOCYTE VIABILITY	53
MAO-DEPENDENT ER-MITOCHONDRIA DYSFUNCTION AND MAST CELL DEGRANULATION LEAD TO ADVERSE CARDIAC REMODELING IN DIABETES	54
THE ROLE OF MONOAMINE OXIDASES IN DIFFERENTIATION AND MATURATION OF IPSC-DERIVED CARDIOMYOCYTES	55
MITOCHONDRIAL CAMP SIGNALLING IS INVOLVED IN METABOLIC FLEXIBILITY	56
ENDOPLASMIC RETICULUM CA ²⁺ HANDLING PROTEINS IN ZEBRAFISH BRAIN: A FOCUS ON CALSEQUESTRIN	57
<i>IN VITRO</i> CHARACTERIZATION OF MIRNA-MEDIATED CONTROL OF SATB2 EXPRESSION DURING CORTICOGENESIS	58
UNVEILING THE MECHANISMS UNDERLYING OLIGODENDROCYTE DIFFERENTIATION: IMPLICATIONS FOR DOWN SYNDROME	59
ALTERED MIGRATION OF INHIBITORY INTERNEURONS IN A MOUSE MODEL OF INTELLECTUAL DISABILITY	60
SYNAPTIC ACTIVITY MIGHT REPRESENT A PHYSIOLOGICAL TOOL TO MODULATE GLIOMA GROWTH	61
INHIBITION OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE SUPPRESSES NICOTINE-INDUCED ACTIVATION OF MESOLIMBIC DOPAMINE TRANSMISSION	62
CHRONIC SYNTHETIC CANNABINOID WIN 55,212-2 ADMINISTRATION INDUCES NEUROMOLECULAR CHANGES AND CROSS-SENSITIZATION BEHAVIORAL EFFECTS TO COCAINE IN ADOLESCENT RATS	63
EFFECT OF EXPOSURE TO THE ACTIVITY-BASED MODEL OF ANOREXIA NERVOSA ON LIVER OF FEMALE RATS	64
MATERNAL Δ ⁹ -TETRAHYDROCANNABINOL BIASES DOPAMINE SYSTEM TOWARDS BEHAVIORAL METAPLASTICITY	65

OXYTOCIN INDUCES PENILE ERECTION AND YAWNING WHEN INJECTED INTO THE BED NUCLEUS OF THE STRIA TERMINALIS: INVOLVEMENT OF GLUTAMIC ACID, DOPAMINE AND NITRIC OXIDE	66
ADOLESCENT ALCOHOL EXPOSURE INCREASES NICOTINE AND COCAINE REWARD ONLY IN RATS GENETICALLY VULNERABLE TO DRUGS OF ABUSE	67
IMPAIRED FEAR RESPONSE IN PAIN INSENSITIVITY CAUSED BY NGF MUTATION	68
THE 5A-REDUCTASE INHIBITOR FINASTERIDE REDUCES DYSKINESIA IN A RAT MODEL OF PARKINSON'S DISEASE	69
COMPARISON OF THE EFFICACY OF 5A-REDUCTASE INHIBITORS ON L-DOPA INDUCED DYSKINESIA IN A MODEL OF PD	70
METABOLIC ALTERATIONS, CELLULAR STRESS AND NEURODEGENERATIVE PROCESSES	71
IMPACT OF ACUTE-PHASE COMPLICATIONS AND INTERVENTIONS ON 6-MONTH SURVIVAL AFTER STROKE. A PROSPECTIVE STUDY	72
GABAERGIC NETWORK ACTIVITY AND POTASSIUM ELEVATIONS AT THE ONSET OF SEIZURE-LIKE EVENTS IN ENTORHINAL CORTEX SLICES	73
CALCITONIN GENE-RELATED PEPTIDE (CGRP) ACTS AS AN HOMEOSTATIC FACTOR ON MICROGLIAL CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS	74
THE ITALIAN STROKE-APP: ICTUS 3R	75
MCU KNOCK-DOWN IMPAIRS SKELETAL MUSCLE AND MOTOR NEURON DEVELOPMENT IN ZEBRAFISH EMBRYOS	76
INDUCTION OF AUTOPHAGY BY THE PLANT PHENOL PTEROSTILBENE: MECHANISMS AND BIOMEDICAL POTENTIAL	77
PGE1 DELIVERED BY LIPOSOMES ANTAGONIZED DENDRITIC SPINE LOSS AND REDUCTION OF VEGF AND VEGF-R2 IN FRONTAL CORTEX AND HIPPOCAMPUS OF DIABETIC RATS	78
PCDH19 C-TERMINUS IS CLEAVED IN RESPONSE TO NMDAR ACTIVATION AND HOMEOSTATICALLY REGULATES IEGS	79
NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: NEW THERAPEUTIC TARGETS OF GLIOMA AND GLIOBLASTOMA CELLS?	80
GLIOBLASTOMA EXOSOMES SHAPE TUMOR ENVIRONMENT ENHANCING CANCER CELL INVASIVENESS	81
LIPOSOMES TREATMENT ANTAGONIZED DENDRITIC SPINE LOSS AND REDUCTION OF NEUROGENESIS IN HIPPOCAMPUS OF CHRONICALLY STRESSED RATS	82
GENDER DIFFERENCES IN A NEW POTENT 5HT2A AGONIST EFFECTS:	83
GAMMA-HYDROXYBUTYRIC ACID INDUCES CHANGES IN RELATIVE CEREBRAL BLOOD VOLUME	84
BEHAVIORAL EFFECTS OF THE NOVEL PSYCHOACTIVE SUBSTANCE (NPS) METHOXETAMINE IN RATS	85
ANXIOLYTIC EFFECT OF A STANDARDIZED EXTRACT OF <i>SALVIA MILTIORRHIZA</i> IN RATS	86
BEHAVIOURAL AND NEUROCHEMICAL CHANGES INDUCED BY Δ^9 -	87
MECHANISMS UNDERLYING THE SUPPRESSING EFFECT OF COR659 ON ALCOHOL AND CHOCOLATE SELF-ADMINISTRATION	88
DIFFERENTIAL RESPONSIVENESS OF NUCLEUS ACCUMBENS DOPAMINE TO STIMULI INSTRUMENTALLY CONDITIONED TO CONVENTIONAL OR DRUG REINFORCERS	89
INVOLVEMENT OF CORE CLOCK GENES IN LITHIUM RESPONSE	90
INTEGRATED ANALYSIS OF CONVERGING GENOME-WIDE GENOTYPING AND TRANSCRIPTOMIC DATA TO IDENTIFY GENES ASSOCIATED WITH LITHIUM RESPONSE IN BIPOLAR DISORDER	91
HEROIN ADDICTION AND LEUKOCYTE TELOMERE LENGTH	92
A NOVEL THERAPEUTIC STRATEGY FOR THE PREVENTION OF THE ONSET OF DYSKINESIA IN THE THERAPY OF PARKINSON'S	93
LYS49 PHOSPHOLIPASE A2 FROM <i>BOTHROPS ASPER</i> (MT-II) FORMS CELL SURFACE AMYLOID-LIKE STRUCTURES THAT COLOCALIZE WITH NUCLEOLIN: A NEW FUNCTIONAL AMYLOID?	94
DEVELOPMENT OF A MULTIPARAMETRIC PROGNOSTIC INDEX FOR DETERMINING REHABILITATION OUTCOME IN STROKE PATIENTS	95
A NOVEL CAUSATIVE GENE FOR AUTOSOMAL DOMINANT LATERAL TEMPORAL EPILEPSY (ADLTE)	96

INNOVATIVE TECHNOLOGIES FOR NEUROELECTRONIC INTERFACES

Annalisa Bonfiglio

Department of Electrical and Electronic Engineering, University of Cagliari, Italy

The introduction in the early 70s of the Ion Sensitive Field Effect Transistor (ISFET) represented a real turning point in several fields since it allowed the realization of compact, label-free, and versatile sensors and biosensors based on the concept of transistor meant as a charge sensor. Despite their great potentiality, the high costs, the restricted range of employable materials associated to the silicon technology, and the need for a reference electrode, have reduced the applicability of such devices in the bio-sensing field, in particular in *in vivo* applications. We have developed a reference-less sensor based on an organic semiconductor device, called Organic Charge-Modulated Field-Effect Transistor (OCMFET), that thanks to its peculiar transduction principle and structure, offer the possibility to modulate its sensitivity by acting on geometry-related parameters of the device itself. The proposed approach has been applied to several sensing tasks as, for instance, pH, DNA detection, thus giving rise to a new family of highly sensitive, reference-less, and low-cost devices for a wide range of bio-sensing applications.

Electrophysiological monitoring of neuronal assemblies both *in vitro* and *in vivo* is of great importance in disciplines like computational neuroscience, brain-machine-interfaces, and pharmacology. To date, the two main types of electrophysiological tools (i.e. Micro Electrode Arrays and the mentioned Ion Sensitive FET), though widely employed, present issues such as the high cost of production, the rigidity of the materials, and the need of a reference electrode in the liquid medium where the sensing takes place. Here we demonstrate that the Organic Charge Modulated FET device, developed in our lab, besides being flexible, low cost, transparent, and reference-less, is able to sense different parameters of biomedical interest depending on how a particular part of its structure (called sensing area) is functionalized.

We therefore propose here an OCMFET specifically designed to sense both electrical activity of electroactive cells (such as cardiomyocytes and neurons) and the metabolic cellular activity *in vitro*. We will demonstrate that the charge perturbation induced by the cellular electrical activity on the OCMFET sensing area leads to a marked variation of the output current of the device, giving the possibility to clearly record cardiac and neuronal field potentials. Moreover, we will also demonstrate that the same structure can be employed for monitoring pH fluctuations of the culture medium induced by cells metabolic activity, thus giving important clues on cells conditions and viability. In fact, in the particular application of *in vitro* electrophysiology, an alteration of the acidity of the culture medium can induce changes in the electrical behavior of the cell culture itself, thus representing a parameter of interest in pharmacological testing. The sensing mechanism relies on the presence of a plasma activated, sub micrometric (750 nm) layer of Parylene C deposited on the sensing area. Besides turning the device pH-sensitive, the presence of such a sensing layer makes the sensing area an ideal surface for cell growth and development. The proposed device has been fully characterized as a pH sensor, and, in order to prove the possibility of using it for *in vitro* applications, the device was preliminary tested with 3T3 cells (an immortalized fibroblast cell line), in an experiment aimed at inducing and measuring a variation of their metabolic activity, which have been monitored by measuring the (low-buffered) medium acidification. The presented results represent the first example of direct metabolic monitoring using an organic field effect transistor and it is an interesting example on how organic electronics can pave the way to innovative low-cost, multi-sensing approaches to *in vitro* electrophysiology and pharmacology.

What the genes tell us about our origins, evolutionary history and our current health

Francesco Cucca

Institute for Genetics and Biomedical Research (IRGB), CNR, Cagliari, Italy

Recent advances in the characterization of human genetic variation, culminating in the complete sequence of the genome of thousands of individuals, have dramatically improved our understanding of the way genes and their products function in health and disease. They have also provided a powerful tool to reconstruct the past history of humanity on an evolutionary timescale, providing information about population movements and adaptation to different environments. Isolated human populations have long been of particular interest because of their potential to yield unique insight into the ancestral history of a region, and because finding genes and gene variants that predispose to disease is more feasible in some of these populations. During my presentation, I will use the example of the isolated, ancient island population of Sardinia to show the impact of genomics to investigate, in a systematic manner, the bases of diseases and the origin of human populations.

BEYOND MOTONEURONS: TRANSYNAPTIC ACTION OF BoNT/A AT CENTRAL CHOLINERGIC BOUTONS

Matteo Caleo, PhD¹ Matteo Spinelli¹, Francesca Colosimo¹, Ivica Matak, PhD², Ornella Rossetto, PhD³, Zdravko Lackovic, MD², Laura Restani, PhD¹

¹CNR Neuroscience Institute, Pisa, Italy; ²Laboratory of Molecular Neuropharmacology, Department of Pharmacology, University of Zagreb School of Medicine, Zagreb, Croatia; ³Department of Biomedical Sciences, University of Padova, Italy

Intramuscular injections of BoNT/A are widely employed in clinical neurology and represent an excellent treatment for several neuromuscular pathologies (dystonia, spasticity, muscle spasms) characterized by hyperexcitability of peripheral nerve terminals. Substantial experimental and clinical evidence indicates that not all BoNT/A effects can be explained solely by the silencing of the neuromuscular junction. In particular, there are cases in which the clinical benefit does not parallel the extent of muscle weakness, e.g. exceeding the duration of peripheral chemo-denervation.

There is no consensus, however, on how these central actions arise.

Here we demonstrate that catalytically active BoNT/A is retrogradely trafficked from the nasolabial musculature (whisker pad) to the facial nucleus of the brainstem in rats and mice. Our data further indicate that BoNT/A is not retained within motoneurons but transcytosed preferentially into cholinergic terminals impinging onto motoneuron cell bodies. The intoxicated cholinergic terminals become larger, suggesting impaired neuroexocytosis. Since cholinergic boutons (also known as C-boutons) provide excitatory input to the motoneurons, the present findings reveal a novel pathway by which BoNT/A reduces motoneuron drive, i.e. via the silencing of a specific set of afferent connections.

These data provide evidence for synapse-specific central effects of BoNT/A. This knowledge is important for a complete understanding of the mechanisms of action of this neurotoxin. Indeed, these results not only provide a mechanistic explanation for the clinical cases in which the therapeutic benefit is apparent despite little neuromuscular blockade, but also they are mandatory to devise a mechanism-based use of the neurotoxin which translates into more effective therapies for patients.

THE INNATE IMMUNE MOLECULE PTX3 ENHANCES THE SYNAPTIC CONTENT OF AMPA RECEPTORS VIA EXTRACELLULAR MATRIX REMODELING

Giuliana Fossati¹, Davide Pozzi^{1,2}, Alice Canzi², Sonia Valentino¹, Elsa Ghirardini^{1,3}, Filippo Mirabella², Milena Moretti³, Giulia Taraboletti⁴, Barbara Bottazzi^{1,2}, Cecilia Gotti⁵, Cecilia Garlanda^{1,2}, Alberto Mantovani^{1,2}, Michela Matteoli^{1,5}, Elisabetta Menna^{1,5}

¹IRCCS Humanitas, Rozzano (MI), Italy; ²Hunimed University, Rozzano (MI), Italy; ³University of Milano, via Vanvitelli 32, 20129 Milano, Italy; ⁴Tumor Angiogenesis Unit, Department of Oncology, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; ⁵IN CNR, Milano, Italy

In the developing central nervous system, the control of synapse number and function is critical to the formation of neural circuits. Astrocytes play a key role in this process by releasing factors which promote the formation of excitatory synapses. Astrocyte-secreted thrombospondins (TSPs) induce the formation of structural synapses, which are however postsynaptically silent. The possibility that additional astrocyte-derived factors may promote the functional switch of the thrombospondin-induced synapses has never been explored. We identified the humoral innate immune molecule PTX3, which is expressed in the rodent brain in a developmental window corresponding to the late embryonic–early postnatal period, as a key molecule which plays a crucial role in the formation of post-synaptically active CNS synapses. This is attained by PTX3 ability to increase the surface levels and clustering of the AMPA glutamate receptors through the remodeling of the perineuronal network via a $\beta 1$ integrin-dependent process. Of note, PTX3 was found to bind TSP-1 and -2, which are expressed in the brain in the same temporal window, but not TSP-4, which is expressed in the spinal cord and dorsal root ganglia. These data unveil a fundamental crosstalk between the immune and nervous systems to establish the first wave of synaptogenesis and the organization of the early functional circuits in the developing brain

MATERNAL IMMUNE ACTIVATION DELAYS EXCITATORY-TO-INHIBITORY GABA SWITCH

Irene Corradini^{1,2#}, Elisa Focchi^{1,3#}, Marco Rasile^{2,4}, Raffaella Morini², Genni Desiato^{2,5}, Romana Tomasoni², Michela Lizier^{2,6}, Elsa Ghirardini^{2,3}, Diego Morone², Isabella Barajon⁴, Flavia Antonucci³, Davide Pozzi^{2,4}, Michela Matteoli^{1,2*}
#equal contribution

¹IN CNR, Milano, Italy; ²IRCCS Humanitas, Rozzano (MI), Italy; ³University of Milano, via Vanvitelli 32, 20129 Milano, Italy; ⁴Hunimed University, Rozzano (MI), Italy; ⁵Università degli studi di Bicocca, Milano, Italy; ⁶IRGB CNR, Milano, Italy

The association between maternal infection and neurodevelopmental defects in the progeny is well-established, although the biological mechanisms and the pathogenic trajectories involved have not been defined yet. We provide the evidence that maternal immune activation hits a key neurodevelopmental process, the excitatory to inhibitory GABA switch, whose defects are univocally linked to diseases such as autism or epilepsy. In particular, prenatal exposure at GD9 to poly-inosinic:poly-cytidylic acid (PolyI:C) causes, in the offspring, the unbalanced expression of the Na⁺-K⁺-2Cl⁻ cotransporter 1 (NKCC1) and the K⁺-Cl⁻ cotransporter 2 (KCC2), which results in higher resting chloride intracellular concentrations, delayed GABA switch and higher susceptibility to seizures, which endures up to the adulthood. Chromatin immunoprecipitation experiments reveal increased binding of the repressor factor REST/NRSF to position 509 of the KCC2 promoter in the prenatally exposed offspring, leading to the down regulation of KCC2 transcription. The KCC2/NKCC1 unbalance is prevented when IL-1RI KO mice, which display braked immune response and lack of brain cytokine elevation upon maternal immune activation, are implanted in a wild type dam and prenatally exposed. We finally provide the evidence that pretreatment of pregnant dams with magnesium sulfate is sufficient to prevent the early inflammatory state and the GABA switch delay associated to maternal immune activation. These data open the avenue for a safe pharmacological treatment which, when applied in specific pregnancy time windows, may prevent the neurodevelopmental defects consequent to the prenatal immune activation.

MICROGLIA-DERIVED EXTRACELLULAR VESICLES REGULATE THE RECRUITMENT, PROLIFERATION AND DIFFERENTIATION OF OLIGODENDROCYTE PRECURSOR CELLS

M. Lombardi¹, M. Fumagalli², R. Parolisi, E. Bonfanti², F. Scaroni², F. Filippello¹, A. Buffo³, C. Verderio^{1,4}

¹IRCCS Humanitas, Rozzano (MI), Italy; ²Dept of Pharmacology and Biomolecular Sciences, University of Milano, Italy; ³Università degli Studi di Torino, Dept. of Neuroscience and Neuroscience Institute Cavalieri Ottolenghi (NICO), Torino, Italy; ⁴CNR Institute of Neuroscience, Milano, Italy

Microglia are very plastic cells that acquire multiple activated phenotypes in response to brain insults, participating not only in mechanisms of injury, but also in tissue remodeling. In multiple sclerosis, a chronic demyelinating disease, microglia contribute to the onset and the acute phase of the disease by secreting cytokines, reactive oxygen species and complement proteins, but also exert pro-regenerative functions, by providing neurotrophins and promoting oligodendrogenesis in the restorative phase of the disease. However, the mode of action of these cells in fostering or inhibiting remyelination repair is still largely unclear. Here, we investigated the action of extracellular vesicles (EVs) shed from microglia with diverse activation states on Oligodendrocyte Precursor Cells (OPCs), the glial cell type able to generate mature, myelinating oligodendrocytes. Fluorescence analysis of OPCs co-exposed to EVs and the proliferative marker EdU showed that EVs produced by pro-inflammatory cells (M1-EVs) limit OPC proliferation, while EVs released from M2 pro-regenerative microglia (M2-EVs) tend to increase it. Immunocytochemistry and western-blot of mature oligodendrocyte markers revealed that M1-EVs or M2-EVs, but not EVs derived from unstimulated microglia (M0-EVs), promote OPC maturation, with M2-EVs displaying higher differentiation activity. However, only M2-EVs significantly foster myelin deposition in an *in vitro* system of OPCs co-cultured with DRG neurons. Despite not influencing OPC proliferation and differentiation, M0-EVs are able to act as chemoattractants for OPCs, similarly to M2-EVs. Globally, these results show that through EVs, M2 microglia and, to a lesser extent, M1 and M0 cells may exert a beneficial action on OPCs, promoting myelin repair. A beneficial role for microglia-derived EVs is also indicated by preliminary data in an *in vivo* model of lysolecithin-induced corpus callosum demyelination. Immunofluorescence analysis indicated that M2-EVs increase the density of OPCs at the lesion site, especially under prolong EV delivery, and promote OPC proliferation, but not differentiation one week after administration. Analysis at longer time points (15-20 days after EV administration) are required to better assess the action of EVs on OPC remyelination. Collectively, these results unveil EVs as key players in microglia-OPCs cross-talk and suggest that microglia derived-EVs contain signals able to influence OPC proliferation and trigger their terminal maturation.

Preliminary analysis of the action of EVs derived from peripheral macrophages showed that EVs derived from inflammatory cells inhibit OPC migration and differentiation. This suggests that infiltrating macrophages, rather than resident microglia, block the pro-regenerative activity of OPCs in demyelinating disease.

UNVEILING THE MECHANISMS UNDERLYING OLIGODENDROCYTE DIFFERENTIATION: IMPLICATIONS FOR DOWN SYNDROME

Alessandro Ulivi¹, Veronica Meraviglia¹, Nicol Colombo¹, Maria Pia Abbracchio², Renata Bartesaghi³, Stefania Ceruti², Ilaria Prada¹, Meritxel Pons Espinal⁴, Davide De Pietri Tonelli⁴, Roberta Benfante¹, Patrizia Rosa¹

¹CNR Neuroscience Institute, Milano, Italy; ²University of Milan, Italy; ³University of Bologna, Italy
⁴IIT, Genova, Italy

Oligodendrocytes have attracted growing interest because of evidence showing that many brain disorders are associated with defective myelination, from “classic” demyelinating diseases such as multiple sclerosis, to stroke, schizophrenia, depression, Down syndrome (DS) and autism. Oligodendrocyte progenitor cells post-natally differentiate through various pre-myelinating stages before becoming mature oligodendrocytes, a process that involves the complex interplay of a number of different intrinsic and extrinsic factors. Among these, it is known that G protein-coupled receptor 17 (GPR17) acts as an intrinsic timer of oligodendrocyte differentiation by keeping pre-myelinating oligodendrocytes in an immature state until its down-regulation allows full oligodendrocyte maturation. The mechanisms controlling GPR17 expression/stability are only partially known, and one aim of our work is to clarify the mechanisms underlying the tight regulation of GPR17 during oligodendrocyte maturation. We have recently concentrated on receptor endocytic trafficking and demonstrated that GPR17 can be sorted to lysosomes or recycled to the plasma membrane via a Rab4-dependent pathway in differentiating cells. As it can be expected that the cell surface levels of GPR17 are modulated by the balance between degradation and recycling, and that this will affect receptor signalling and, consequently oligodendrocyte differentiation, we further characterised the mechanisms of GPR17 sorting in the endosomal pathway by focusing on the role of its C-terminal PDZ binding motif. Our findings demonstrated: i) that the PDZ binding motif is required for GPR17 recycling to the cell surface by means of interactions with the retromer complex-associated protein SNX27; and ii) that SNX27 knockdown accelerates receptor sorting into lysosomes. This rapid GPR17 degradation led to the early expression of myelin proteins and accelerated the kinetics of oligodendrocyte differentiation *in vitro*. Given that recent data have shown that SNX27 is indirectly down-regulated by the over-expression of miR-155 in DS brains, we analysed the effects of miR-155 over-expression in oligodendroglia cells and found that increased levels of this trisomic microRNA inhibited GPR17 expression as well as cell differentiation. These results correlate with data showing altered myelination and a decreased number of GPR17⁺ cells and in the brains of Ts65Dn mice (a model of DS) and suggest that a defective oligodendrocyte differentiation occurs in trisomic brain. We are currently seeking to identify the molecular mechanisms and the possible miR-155 target(s) that may play a role in myelination defects of DS brain.

LONG-TERM POTENTIATION (LTP) REQUIRES SYNAPTIC GLIA FOR proBDNF PROCESSING AND RECYCLING OF THE ISOLATED PRO-PEPTIDE (BDNFpro)

Beatrice Vignoli¹, Robert Blum², Roberto Rimondini³, Spartaco Santi⁴, Marco Canossa^{1,5}

¹*Centre for Integrative Biology (CIBIO), University of Trento, Povo (Trento), Italy;* ²*Institute for Clinical Neurobiology, University Hospital, Julius-Maximilians-University Würzburg, Würzburg, Germany;*

³*Department of Medical and Surgical Sciences (DIMEC), University of Bologna, Bologna, Italy;* ⁴*CNR, Institute of Molecular Genetics (IGM), IOR, Bologna, Italy ;* ⁵*EBRI European Brain Research Institute- Rita Levi-Montalcini, Rome, Italy*

Long-term potentiation (LTP) requires synaptic glia for (pro)-Brain-derived Neurotrophic Factor (proBDNF) clearing and recycling. While glial recycling of the neurotrophin involves proBDNF proteolysis to increase the availability of mature BDNF at synaptic sites, the fate of isolated BDNF pro-peptide (BDNFpro) remains elusive. Here we present evidences that BDNFpro is secreted from peri-synaptic glia following long-term potentiation (LTP)-inducing electrical activity. Once released, BDNFpro binds the sortilin family member SorCS2 on adjacent post-synaptic neurons to induce TrkB sorting and to increase TrkB phosphorylation, a mechanism that is required for LTP maintenance. These data offer fundamental information about the mechanisms by which LTP requires synaptic glia for proBDNF enzymatic processing and recycling of the isolated products, and reveal BDNFpro as a critical mediator of TrkB synaptic tagging.

THE ROLE OF THE FEMALE EPILEPSY PROTEIN PCDH19 BETWEEN SYNAPSE AND NUCLEUS

Silvia Bassani¹, Laura Gerosa¹, Giulia Maia Serratto¹, Andrzej Cwetsch², Alessandra Folci¹, Erika Pizzi³, Fernando Hall-Balcazar¹, Michele Mazzanti³, Laura Cancedda², Maria Passafaro¹

¹CNR Neuroscience Institute, Milan, Italy; ²Italian Institute of Technology, Genoa, Italy; ³University of Milan, Bioscience Dept., Milan, Italy

Mutations in the *PCDH19* gene on chromosome X (Xp22.1) cause a female-limited epilepsy (PCDH19 Female Epilepsy, PCDH19-FE) that is frequently associated with intellectual disability and autism.

Epilepsy affects heterozygous females and spares hemizygous males, with the exception of few mosaic males. In adulthood, the non-epileptic symptoms become the most disabling issues.

Since the discovery of its involvement in PCDH19-FE in 2008, *PCDH19* has rapidly become the second most clinically relevant gene in epilepsy after the Dravet Syndrome causative gene *SCN1A*. To date, more than 100 inherited or *de novo* mutations have been reported in *PCDH19*, including point mutations and partial or whole gene deletions. Despite this, a comprehensive understanding of *PCDH19* biological function as well as its role in the PCDH19-FE pathogenesis is lagging behind.

PCDH19 encodes for protocadherin-19 (PCDH19) that is a calcium-dependent cell-adhesion molecule belonging to the non-clustered Δ 2-protocadherin subclass of the cadherin superfamily. PCDH19 has six conserved extracellular cadherin repeats, a transmembrane region and an intracellular C-terminus (CT).

We found that *PCDH19* is expressed at both excitatory and inhibitory synapses of hippocampal neurons and regulates neuronal excitability via two distinct mechanisms: by modulating GABA_AR transmission at synapses and gene expression in the nucleus.

Our data indicate that PCDH19 CT interacts with the GABA_AR alpha subunits; upon PCDH19 shRNA-mediated downregulation, GABA_AR surface expression is reduced and fast GABAergic transmission impaired. Since *PCDH19* expression increases throughout embryonic development and peaks in the first postnatal days when GABA signaling orchestrates neuronal migration and arborization, we downregulated PCDH19 in rat hippocampus via shRNA *in utero* electroporation. Consistently with an impairment of GABAergic transmission, PCDH19 downregulation during brain development affects the migration and morphological maturation of pyramidal neurons and increases rat's seizure susceptibility.

In addition, upon sustained NMDAR activation, PCDH19 CT is cleaved by gamma secretase and enters the nucleus. In the nucleus, PCDH19 CT associates with the CoREST complex and represses the transcription of immediate early genes (IEGs), which are key regulators of neuronal plasticity and excitability. Conversely, PCDH19 shRNA-mediated downregulation increases IEGs transcripts. Notably, PCDH19 cleavage occurs *in vivo* upon epileptogenic stimuli, as demonstrated by CT generation in hippocampal homogenates from mice that experienced pilocarpine induced-seizures. We hypothesize that PCDH19 cleavage might represent a homeostatic mechanism in response to strong neuronal activation that prevents IEGs overactivation.

In conclusion, PCDH19 emerges as a new GABA_AR binding partner that controls GABAergic transmission and simultaneously exerts a homeostatic control of excitability via IEGs expression regulation, thus suggesting new pathogenic mechanisms for PCDH19-FE.

USING FRET-BASED SENSORS TO STUDY THE cAMP/PKA AXIS AT THE OUTER MITOCHONDRIAL MEMBRANE

Alex Burdyga², Nicoletta C. Surdo², Stefania Monterisi², Giulietta Di Benedetto^{1,3}, Elisa Penna³, Luca Pellegrini³, Mario Bortolozzi³, Pawel Swietach², Tullio Pozzan^{1,3}, Konstantinos Lefkimmiatis^{1, 2, 3}

¹CNR Neuroscience Institute, Padova, Italy; ²Department of Physiology Anatomy and Genetics, Oxford University, UK; ³Foundation for Advanced Biomedical Research, Venetian Institute of Molecular Medicine, Padova, Italy

The second messenger cyclic AMP (cAMP) achieves its functional pleiotropy thanks to the compartmentalization of its signaling cascade. This is accomplished *via* the coordinated actions of two protein families, phosphodiesterases (PDEs) and A-kinase anchoring proteins (AKAPs). According to this paradigm, PDEs selectively hydrolyze cAMP allowing it to reach cellular domains of enriched PKA (protein kinase A) generated by AKAPs. However, another group of proteins can regulate the cAMP/PKA axis, the phosphatases. These enzymes dephosphorylate the proteins modified by PKA to effectively terminate the cAMP cascade. While the role of phosphatases in regulating PKA is intuitively important, their contribution into shaping the cAMP/PKA pathway is underappreciated. Here we developed and validated a number of FRET-based sensors to monitor cAMP, PKA and phosphatase activity in the cytosol and outer mitochondrial membrane (OMM). Using as model co-cultures of neonatal cardiac myocytes we performed a comprehensive live single cell imaging study and demonstrated that differences between the cAMP/PKA axis present at the cytosol and OMM did not depend on PDEs but rather on differential phosphatase activity between the two compartments. Our imaging data, which were also supported by classic biochemistry, demonstrate that phosphatases are required for sculpting functionally distinct cAMP/PKA domains and that this occurs because of the kinetics of the bimacromolecular interaction between phosphatases and PKA-phosphorylated proteins.

mCerulean3-BASED CAMELEON SENSOR TO EXPLORE MITOCHONDRIAL Ca^{2+} DYNAMICS *IN VIVO*

Elisa Greotti^{1,2}, Diana Pendin^{1,2}, Ilaria Fortunati⁴, Camilla Ferrante⁴, Lorena Zentilin⁵, Nina Kaludercic¹, Letizia Mariotti^{1,2}, Annamaria Lia², Marta Gómez Gonzalo^{1,2}, Michele Sessolo^{1,2}, Giorgio Carmignoto^{1,2}, Renato Bozio⁴, Tullio Pozzan^{1,2,3}

¹Neuroscience Institute, Padova Section, National Research Council, Italy; ²Dept. Biomedical Sciences, University of Padova, Padova, Italy; ³Venetian Institute of Molecular Medicine (VIMM), Padova, Italy; ⁴Chemical Science Dept., University of Padova and INSTM, Padova, Italy; ⁵International Centre for Genetic Engineering and Biotechnology (ICGEB), Trieste, Italy

Genetically encoded calcium indicators (GECIs) are widely employed for the quantitative measurements of Ca^{2+} levels both in the cytosol and in organelles. Organelle-specific targeting signals in GECI's sequence allow selective targeting of the probes to a specific cell subcompartment and thus permit a detailed investigation of the spatial aspects of Ca^{2+} signalling. Different types of GECIs have been created and among them one of the most used is a class of FRET (Förster resonance energy transfer)-based Ca^{2+} sensors, called Cameleons. Cameleons targeted to the mitochondrial matrix are still quite popular, despite the available probes are plagued by different problems, from low fluorescence intensity to substantial mistargeting that represent major obstacles to their extensive *in vivo* usage. Indeed, although both cameleons and other mitochondrial targeted GECIs have been extensively used to investigate mitochondrial Ca^{2+} dynamics in cultured living cells, only a few *in vivo* studies are available, mainly in small organisms or easily accessible tissues. Last, but not least, in the most commonly used mitochondrial GECI, the donor protein ECFP is characterized by a double exponential lifetime that makes Fluorescence Lifetime Imaging (FLIM) analysis difficult to quantitate. Thanks to recent technical advancements FLIM is becoming a popular and robust alternative method to study cellular signaling. FLIM measurements, compared to intensity-based images, have the advantage of being totally independent of fluorophore concentration, excitation intensity fluctuation, sample thickness, or photobleaching. We have modified the classical mitochondria-targeted Cameleon GECIs by: substituting the donor ECFP with mCerulean3, a brighter and more stable fluorescent protein with a single exponential lifetime; extensively modifying the N-terminal of the constructs to improve the mitochondrial targeting efficiency; and extending the linker between two Ca^{2+} responsive elements to ameliorate the fluorescence changes caused by Ca^{2+} binding. The new probes have been thoroughly characterized *in situ* employing both intensity- and lifetime-based approaches. Furthermore, their usage *in vivo* in neurons and cardiac cells has been achieved inserting the cDNAs into adeno associated viral vectors and cell specific promoters. Data on the use of these new Cameleons in adult cardiac myocytes or *in vivo* in the mouse brain are presented.

WHEN TIMING MATTERS: THE MIRROR MECHANISM OBSERVED THROUGH THE LENS OF INTRACEREBRAL RECORDINGS

Pietro Avanzini

¹*CNR Neuroscience Institute, Parma, Italy*

A fine-grained description of the spatiotemporal dynamics of human brain activity is a major goal of neuroscientific research. Limitations in spatial and temporal resolution of available non-invasive recording and imaging techniques have hindered so far the acquisition of precise, comprehensive four-dimensional maps of human neural activity. Here I will first illustrate the procedure our group employed to solve the sparse sampling problem, achieving quantitative and four-dimensional neuroimaging-like results on a large set of patients undergoing pre-surgical stereo-EEG.

I will move forward showing how this 4D approach allows to deconstruct the cortical responses to action observation in time, thus not only differentiating the involvement of different cortical networks, but also defining an internal hierarchy based on each region time course.

IN VIVO OPTICAL IMAGING OF REHABILITATION-INDUCED CORTICAL PLASTICITY AFTER STROKE

Anna Letizia Allegra Mascaro^{1,2}, Emilia Conti², Stefano Lai³, Antonino Paolo Di Giovanna², Cristina Spalletti¹, Claudia Alia⁴, Eros Quarta², Alessandro Panarese³, Leonardo Sacconi^{2,5}, Silvestro Micera^{3,6}, Matteo Caleo¹, Francesco Saverio Pavone^{2,5,7}

¹CNR Neuroscience Institute, Pisa, Italy; ²European Laboratory for Non-linear Spectroscopy University of Florence, Italy; ³Scuola Superiore Sant'Anna, Pisa, Italy; ⁴Scuola Normale Superiore, Pisa, Italy; ⁵CNR National Institute of Optics, Italy; ⁶Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland; ⁷Department of Physics and Astronomy, University of Florence, Italy

Neuro-rehabilitation is one of the most effective treatment for recovering motor deficits in stroke patients. Nevertheless, the neural basis of recovery associated with rehabilitative intervention is debated. We are interested in studying the multiple facets of cortical remodeling induced by combined physical and pharmacological rehabilitative treatment. By longitudinal wide-field fluorescence imaging of cortical activity while training, we address the recovery of motor representation in the peri-infarct area. Coupling of the spared cortex to the injured hemisphere is investigated by an all-optical approach that combines calcium imaging and optogenetic neuronal activation. In addition, by using two-photon microscopy both in vivo and ex vivo on cleared cortices, we analyze how vascular remodeling accompanies synaptic plasticity. In this seminar I will show how, by combining optical tools of visualization and manipulation of neuronal activity, the impact of rehabilitation on cortical plasticity can be dissected at multiple scales.

PRE- AND POST-SYNAPTIC ALTERATIONS INDUCED BY BDNF OVER-EXPRESSION IN THE 6-OHDA-LESIONED RAT MODEL OF PARKINSON'S DISEASE

Elisabetta Tronci¹, Francesco Napolitano^{2,3}, Ana Muñoz⁴, Camino Fidalgo^{1,5}, Francesca Rossi¹, Anders Björklund⁶, Alessandro Usiello^{2,7}, Manolo Carta¹

¹Department of Biomedical Sciences, University of Cagliari, Italy; ²University of Naples "Federico II", Italy; ³Ceinge Biotechnologie Avanzate, Naples, Italy; ⁴University of Santiago de Compostela, Spain; ⁵University of Zaragoza, Teruel, Spain; ⁶Lund University, Sweden; ⁷University of Campania "Luigi Vanvitelli", Caserta, Italy

In addition to its role in neuronal survival, the neurotrophin BDNF has been shown to influence serotonin transmission and synaptic plasticity, events strongly implicated in the appearance of L-DOPA-induced dyskinesia (LID), a motor complication occurring in parkinsonian patients after long-term treatment with the drug.

In order to evaluate a possible influence of BDNF in the appearance of LID, 6-OHDA-lesioned rats received a striatal injection of different concentrations of an adeno-associated viral (AAV) vector over-expressing either BDNF or GFP, as control vector. Starting eight weeks later, animals received repeated daily treatments with L-DOPA (4-6 mg/kg plus benserazide 4-6 mg/kg s.c.) or saline, and dyskinesias as well as L-DOPA-induced rotations were evaluated. Moreover, molecular changes in striatal D1 receptor-dependent cAMP/PKA and ERK/mTORC signaling pathways, as well as, sprouting of the striatal serotonin axons were assessed. Results showed that the AAV-BDNF vector injection induced, in 6-OHDA-lesioned rats, striatal over-expression of BDNF, as well as striatal and pallidal serotonin axon hyperinnervation. Moreover, rats that over-expressed BDNF were more prone to develop LID and L-DOPA-induced rotations, compared to the GFP-treated control group. Finally, the higher susceptibility of rats to develop dyskinesia and rotations was associated with alterations in striatal D1R-dependent signalling cascade. This study suggests that BDNF over-expression, by inducing changes in pre-synaptic serotonin axons trophism, is able to exacerbate maladaptive responses to L-DOPA administration.

LOSS-OF-FUNCTION MUTATIONS IN THE *SIGMAR1* GENE CAUSE DISTAL HEREDITARY MOTOR NEUROPATHY BY IMPAIRING ER-MITOCHONDRIA TETHERING AND Ca²⁺ SIGNALING

Giorgia Pallafacchina^{1,2}, Sofia Zanin^{1,2}, Elisa Gregianin³, Antonio Petrucci⁴,
Rosario Rizzuto², Giovanni Vazza³

¹CNR Neuroscience Institute; ²Department of Biomedical Sciences, University of Padova, Padova, Italy;

³Department of Biology, University of Padova, Padova, Italy; ⁴Neuromuscular and Rare Neurological Diseases Centre, San Camillo-Forlanini Hospital of Rome, Rome, Italy

Distal Hereditary Motor Neuropathies (dHMN) are clinically and genetically heterogeneous neurological conditions characterized by degeneration of the lower motor neurons. To date, 19 dHMN genes have been identified but 80% of dHMN cases remain without genetic description. By a combination of autozygosity mapping, identity-by-descent segment detection and whole-exome sequencing approaches we identified two novel homozygous mutations in the *SIGMAR1* gene (p.E138Q and p.E150K) in two distinct Italian families affected by an autosomal recessive form of HMN.

Sigma non-opioid intracellular receptor 1 (σ 1R) is a 28 kDa chaperone protein of the endoplasmic reticulum (ER) that localizes at the mitochondria-associated ER membrane (MAM). It is involved in several aspects of cellular homeostasis in the nervous system, including regulation of ion channels, neurite growth and Ca²⁺ signalling. Functional analyses in several neuronal cell lines strongly support the pathogenicity of the σ 1R mutations and provide insights into the underlying pathological mechanisms involving the regulation of ER-mitochondria tethering, Ca²⁺ homeostasis and autophagy. We demonstrated that σ 1R substitutions behave as “loss-of-function” mutations affecting cell viability and altering Ca²⁺ homeostasis due to a derangement of ER-mitochondrial contact sites. Preliminary data obtained in primary skin fibroblasts from patients with homozygous E150K mutation also showed MAM disorganization, an upregulation of basal autophagy, and an altered global Ca²⁺ handling compared to controls. Interestingly, we observed an increased mitochondrial Ca²⁺ uptake and cytosolic Ca²⁺ elevation upon agonist stimulation, which is in apparent contradiction with previous results obtained in the overexpressing neuroblastoma cells. Importantly, patient fibroblasts showed a reduced amount of the mutated σ 1R protein compared to controls, similarly to a knock-down phenotype. This could explain the increased Ca²⁺ response recorded in E150K mutant cells and at least in part explain the differences in Ca²⁺ dynamics observed with the overexpression model. Our data definitively demonstrate the involvement of *SIGMAR1* in motor neuron maintenance and survival by correlating, for the first time in the Caucasian population, mutations in this gene to distal motor dysfunction and highlight the role of σ 1R in MAM maintenance and in Ca²⁺ signals modulation as a critical aspect of motor neuron degeneration in dHMN pathology.

LONG LASTING NEUROTOXIC EFFECTS OF MDMA ADMINISTRATION DURING ADOLESCENCE

Giulia Costa¹, Lucia Frau¹, Pier Francesca Porceddu¹, Nicola Simola¹, Micaela Morelli^{1,2}

¹*Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy;* ²*CNR Neuroscience Institute, Cagliari, Italy*

Several results have shown that the adolescent brain may be vulnerable to long-term neurotoxic effects induced by drugs acting at the central nervous system level.

In line with these studies, previous results from our research group demonstrated that chronic exposure to 3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) of adolescent mice exacerbates dopamine neurotoxicity and neuroinflammatory effects elicited by 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP) in motor areas such as *substantia nigra pars compacta* and caudate–putamen at adulthood and that combined administration of MDMA plus caffeine during adolescence may worsen the neurotoxicity and neuroinflammation elicited by MDMA in the same brain areas.

In order to study the neurotoxic effects of MDMA administered chronically at different post-natal days, the present study evaluated NET, DAT and TH -positive fibers and GAD-67 and TH-positive neurons in different motor and limbic areas. Mice received MDMA (10 mg/kg, i.p.), twice a day/twice a week from post-natal day (PND) 60 to PNDs 82, 107 or 124, and were then sacrificed at different time-points after discontinuation (PNDs 85, 110, 138, or 214). A reduction of DAT-positive fibers in the caudate–putamen and medial prefrontal cortex associated to a reduction of TH-positive nigral neurons, and a reduction of GAD-67-positive neurons in the striatum, medial prefrontal cortex and hippocampus were detected in mice treated from PND 60 to 107 (28 administrations). In contrast an increase in NET-positive hippocampal fibers was found in the same group. In addition to eliciting these effects, MDMA reduced TH-positive striatal fibers and nigral neurons in mice treated from PND 60 to 124 (36 administrations). Finally, the effects of MDMA on nigrostriatal DA system and GABAergic transmission persisted up to 3 months after discontinuation. Results suggested that MDMA produces long-term changes in neurotransmitter systems regulating both motor and cognitive performances.

TAAR1 MEDIATES THE PROTECTIVE EFFECT OF 3- IODOTHYRONAMINE AGAINST β -AMYLOID-DEPENDENT

Grazia Rutigliano^{1,2}, Alice Accorroni^{1,2}, Martina Sabatini³, Riccardo Donzelli³, Alessandro Saba³, Riccardo Zucchi³, Nicola Origlia¹

¹CNR Neuroscience Institute, Pisa, Italy; ²Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy; ³Department of Pathology, University of Pisa, Pisa, Italy

Beta-Amyloid (A β) oligomers, which accumulate in vulnerable brain areas, such as the entorhinal cortex (EC), early in the course of Alzheimer's disease (AD), have been proven to be involved in the impairment of synaptic plasticity. Also, it has been suggested that brain thyroid hormone (TH) metabolism may be altered in AD with a reduction of the active form of the hormone, while acute administration of the TH derivative 3-iodothyronamine (T1AM) stimulates memory acquisition in mice. Therefore, we investigated the effect of T1AM exogenous administration on EC synaptic plasticity and EC-dependent associative memory in wild type mice exposed to A β and in AD mice expressing human mutations of the APP gene (hAPP-J20 line), and if trace-amine associated receptor 1 (TAAR-1, T1AM main receptor) was involved in T1AM effects.

To this aim, we performed extracellular in vitro recordings of Long-term Potentiation (LTP) either in EC slices from wild type (WT) mice and treated with A β oligomers or taken from 3-4 month old hAPP-J20 mice. A β oligomers were exogenously applied on slices at a concentration of 200 nM, previously demonstrated to inhibit EC-LTP, while T1AM was administered at a concentration (5 μ M) shown to affect neither basal synaptic transmission nor LTP induction and maintenance. Our results indicate that T1AM perfusion completely prevented LTP impairment in A β -treated WT slices. Moreover, LTP was completely rescued in hAPP-J20 slices perfused with T1AM, with respect to transgenic untreated slices and was comparable to LTP recorded in control non-transgenic slices. Similarly, the administration of the TAAR1 agonist RO5166017 (250nM) protected from A β -dependent LTP impairment. However, T1AM protective effects were completely abolished in presence of the TAAR1 antagonist EPPTB (5nM). These findings suggest that TAAR1 mediates the T1AM rescue of LTP in an A β enriched environment. To confirm the neuroprotection in vivo, 10 μ l of T1AM solution was injected (i.c.v.; 1.32 μ g/ Kg b.w.) in WT and hAPPJ20 mice. T1AM treatment improved associative memory functions in hAPPJ20 mice, as assessed by the novel object place/context recognition test.

Our results suggest that T1AM/TAAR1 pathway plays a neuroprotective effect rescuing A β -induced neuronal dysfunction. A more thorough understanding of the neuroprotective properties of T1AM and underlying mechanisms might lead to the identification of new pharmacological targets to delay disease progression.

BIDIRECTIONAL NEURON-GLIOMA INTERACTIONS: EFFECTS OF GLIOMA CELLS ON SYNAPTIC ACTIVITY AND ITS IMPACT ON TUMOR GROWTH

Elena Tantillo^{1,3}, Chiara Cerri², Chiara Maria Mazzanti³, Mario Costa², Matteo Caleo²

¹*Scuola Normale Superiore, Pisa, Italy*; ²*CNR Neuroscience Institute, Pisa, Italy*; ³*Pisa Science Foundation-ONLUS, Pisa, Italy*

Introduction

Gliomas are the most common primary brain tumors of the central nervous system. The development of novel therapies requires a better understanding of the biology of glioma cells and their interactions with resident brain cells. A successful brain tumor treatment should indeed aim at halting tumor growth and at the same time protecting neuronal cells to prevent functional deficits and cognitive deterioration. Currently, there is little information available in literature on functional changes during glioma growth and investigations on the role of neural activity in glioma progression have yielded contradictory results. Here we provide a detailed understanding of how tumor growth reverberates on the function of neuronal networks and how it is influenced by neural activity.

Methods

We transplanted GL261 cells into the primary visual cortex of syngeneic C57BL/6 mice and we performed chronic recordings of visual evoked potentials (VEPs) and local field potentials (LFPs) in awake, head-fixed mice. A subset of mice with sham surgery were used as controls. Field potential responses were evoked by photic stimulation. A detailed analysis of VEPs and LFPs was performed in order to follow changes induced by tumor growth. To investigate the role of neural activity in controlling glioma progression, we manipulated neural activity in glioma-bearing mice and we analyzed glioma cell proliferation by either Ki67- or BrdU-immunostaining. We used botulinum neurotoxin A (BoNT/A) to block synaptic activity and visual deprivation (dark rearing) to reduce physiological activity. A group of mice was treated with the neurotoxin BoNT/A (or vehicle) delivered in the visual cortex 7 days after GL261 cell injection; a second group was reared in total darkness (or in normal environment) starting from day 11 after glioma induction. Tumor analyses were performed at day 14.

Results

We observed a progressive increase in the VEP amplitude over the recording sessions, indicative of stimulus-dependent response potentiation (SRP). In glioma-bearing mice, an initial phase of SRP was followed by rapid decay and deterioration of visual response, that completely disappeared by day 25 after cell transplant. LFPs recordings revealed the occurrence of seizures in a subset of the glioma-bearing animals. Alterations of the LFP power spectra were detected, with an increase in slow activity (delta band) during glioma development. Long-lasting blockade of synaptic activity by BoNT/A leads to a higher density of proliferating cells. The reduction of physiological activity by dark rearing also increases significantly glioma proliferation with respect to controls. These data indicate that neural activity restrains glioma proliferation.

Altogether, these findings demonstrate that glioma impacts on the surrounding neurons dampening visually-evoked activity. This reduction, in turn, promotes glioma proliferation and might trigger a vicious feedback loop that exacerbate glioma progression.

PLASTICITY IN RETINITIS PIGMENTOSA AND RETINAL PROSTHESES

Guido Marco Cicchini¹, Elisa Castaldi², Claudia Lunghi², Paola Binda², Maria Concetta Morrone²

¹CNR Neuroscience Institute Pisa, Italy; ²University of Pisa, Italy

Nearly 300 million people worldwide have severe loss of visual acuity with about 40 million being totally blind. Recent advances in retinal prosthesis technologies generates hope for partially restoring vision in this people, however, little is known on the residual capacity of the visual system to elaborate signals after many years of deprivation. To this aim we studied two aspects of visual plasticity in two cohorts of patients with severe visual deficits.

In the first study we focused on relatively short term cortical plasticity and measured the response to a brief period of monocular deprivation in a group of RP patients with residual foveal vision. These measures revealed that despite the massive loss of visual input, ocular dominance shift was spared and was in line with age matched controls. Interestingly we found a correlation between residual visual acuity and deprivation effect, indicating that the subjects with poorer vision were also those who responded most to deprivation.

In a second study we tested psychophysical and physiological responses in a population of late RP patients who underwent implantation with Argus II Retinal Prosthesis and the follow up training program. Behavioural results indicated that all the subjects were able to detect high contrast stimuli using the prosthetic implant. Interestingly their performance correlated with the amount of training after the surgery with a learning curve that spans over years. Interestingly also physiological responses displayed important changes before and after surgery. Before the implant, the BOLD activity in V1 and LGN was very weak or absent but surprisingly, after prolonged use of Argus II the BOLD response to visual inputs was enhanced.

Overall our results show that cortical plasticity is a crucial factor in cases of retinal degeneration, dampen the effects of the pathology during the course of the illness and enabling sight recovery after implantation of retinal prostheses.

A MULTIDISCIPLINARY APPROACH TO CHARACTERIZE THE PHARMACOLOGICAL AND TOXICOLOGICAL EFFECTS OF THE KETAMINE ANALOG METHOXETAMINE (MXE) IN RATS

Liana Fattore

CNR Institute of Neuroscience-Cagliari

The use of new psychoactive substances (NPS) is increasing worldwide. One commonly used NPS is methoxetamine (MXE), a ketamine analogue with a high affinity for the N-methyl-D-aspartate (NMDA) receptor, widely available through on-line sources and not detectable by routine urine drug screens (Zanda et al., 2016). Although several adverse effects and fatal intoxications have been reported following MXE exposure, very little is known about the psychopharmacological effects of this compound or its toxicity.

After an initial screening of non-toxic doses, we first characterized the effects of an acute intraperitoneal (i.p.) administration of MXE (0.5-5 mg/kg) in a battery of behavioral test in rats and found that it (i) significantly alters motor activity in a dose-dependent manner, (ii) induces perseverative behavior and/or obsessive-compulsive, (iii) differentially affects anxiety-like states and (iv) may induce a rapid antidepressant effects at high doses (Zanda et al., 2017). In collaboration with Prof. Chiamulera and collaborators (University of Verona) we showed that MXE also substitutes for ketamine in a drug self-administration substitution paradigm (Mutti et al., 2016) and fully generalizes to ketamine interoceptive stimulus in a two-lever operant drug discrimination paradigm in rats (Chiamulera et al., 2016), thus showing to induce rewarding and discriminative effects similar to ketamine. In support to its abuse liability, following intravenous (i.v.) administration of MXE we observed an enhanced level of dopamine in the rat nucleus accumbens shell and a dose-dependent stimulation of the firing rate and burst firing of ventral tegmental area dopamine neurons projecting to the NacS (Mutti et al., 2016), findings that highlight an electrophysiological and neurochemical profile predictive of its addictive properties. Moreover, immunohistochemical analysis showed that behaviourally active doses of MXE (1 and 5 mg/kg) increased the phosphorylation of ribosomal protein S6 (rpS6) in the medial prefrontal cortex and hippocampus, revealing rapid neuroadaptive molecular changes similar to those reported recently for ketamine (Tedesco et al. 2013). More recently, we have started a collaboration with Micaela Morelli and collaborators (University of Cagliari) to investigate the effect of a chronic intermittent exposure to MXE (0.5 mg/kg i.p.) at both behavioral and molecular levels. We found that repeated MXE administration affects animals' ultrasonic vocalizations and their performance in the novel object recognition test, the elevated plus maze and marble burying test, but not in the Y-maze spontaneous alternation task. In these animals, immunohistochemical analysis revealed modified levels of (i) the serotonin transporter (SERT) in the nucleus accumbens (NAc) core but not in the shell, medial prefrontal cortex (mPFC) and caudate putamen (CPu), (ii) the dopamine transporter (DAT) in the PFC and NAc shell but not in the NAc core, and (iii) tyrosine hydroxylase in the CPu, substantia nigra pars compacta (SNc) and ventral tegmental area (VTA) of rats.

Overall, our combined studies revealed important behavioral effects of this new psychoactive substance and provided a 'molecular snapshot' of neuroadaptive molecular effects of MXE at behaviorally active doses.

References

- Chiamulera C, Armani F, Mutti A, Fattore L (2016) *Behav Pharmacol* 27(2-3 Spec Issue):204-10.
- Mutti A, Aroni S, Fadda P, Padovani L, Mancini L, Collu R, Muntoni AL, Fattore L, Chiamulera C (2016) *Psychopharmacology* 233:2241-51.
- Tedesco V, Ravagnani C, Bertoglio D, Chiamulera C (2013). *Neuroreport* 24: 388-393.
- Zanda MT, Fadda P, Chiamulera C, Fratta W, Fattore L (2016) *Behav Pharmacol* 27:489-96.
- Zanda MT, Fadda P, S Antinori S, di Chio M, Fratta W, Chiamulera C, L Fattore (2017) *Br J Pharmacol*, in press.

INVOLVEMENT OF DOPAMINE IN THE DIFFERENCES IN SEXUAL BEHAVIOUR BETWEEN ROMAN HIGH AND LOW AVOIDANCE RATS: BEHAVIORAL, PHARMACOLOGICAL AND NEUROCHEMICAL FINDINGS

Fabrizio Sanna¹, Jessica Bratzu¹, Maria Antonietta Piludu², Maria Giuseppa Corda², ¹Maria Rosaria Melis¹,
Osvaldo Giorgi², Antonio Argiolas^{1,3}

¹Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari; ²Department of Life and Environmental Sciences, Section of Pharmaceutical, Pharmacological and Nutraceutical Sciences, University of Cagliari and ³CNR Institute of Neuroscience, Cagliari Section, Italy

The Roman lines of rats (High – RHA, and Low Avoidance - RLA) display opposite behavioral traits: RHA rats are active copers, impulsive and prone to abuse drugs while RLA rats are reactive copers, hyperemotional and prone to develop depressive-like symptoms. These differences are linked to differences in brain monoamine function and neuroendocrine responses to stress. RHA and RLA rats differ also in sexual behavior, with RHA rats displaying higher motivation and better copulatory performances than RLA rats [1].

Recently, we found that the differences observed in sexual behavior between the two Roman lines occur concomitantly to differences in the activation of the mesolimbic dopaminergic system, which plays a key role in motivated behaviors (i.e. goal-directed behaviors), as assessed by the differences in dopamine concentrations in the dialysates obtained from the nucleus accumbens shell by means of intracerebral microdialysis before and during sexual activity [2]. Together with the well-known role of dopamine in sexual behavior [3], these findings suggest that the sexual differences between RHA and RLA rats may be due to differences in dopamine neurotransmission.

Another brain area involved in motivated behaviors is the medial prefrontal cortex (mPFC), which receives, as the nucleus accumbens, dopaminergic projections from the ventral tegmental area (VTA). The mPFC is involved in several functions, from decision making to coping to stress, from behavioral inhibition to incentive evaluation. This raises the possibility that the mPFC might be another brain area in which the Roman lines display differences in dopaminergic (monoaminergic) activity related to the observed differences in sexual behavior.

In order to test this hypothesis, naïve (never exposed to a receptive female) and sexually experienced (which underwent five copulation tests) RHA and RLA rats implanted with a microdialysis probe aimed at the mPFC, were used in a classical appetitive/consummatory test of sexual behavior, during which copulatory parameters were recorded and dialysate aliquots collected from the mPFC for the determination of dopamine and noradrenaline by HPLC-ECD.

The results show that the higher sexual motivation and better performance of RHA vs. RLA rats occurred concomitantly with a higher dopamine and noradrenaline release in the mPFC, as shown by the higher concentrations found in the mPFC dialysates of RHA vs. RLA rats. These differences between the two lines were greater in naïve animals and persisted, although attenuated, in experienced animals. Furthermore, sexually experienced rats of both lines displayed higher dopamine, and to a lesser extent noradrenaline, release in the mPFC compared to their sexually naïve counterparts.

These findings confirm that a different mesocorticolimbic dopaminergic tone exists in RHA and RLA rats, which may be responsible, together with differences in noradrenaline release, at least in part, for their different copulatory patterns, and could provide further insights into the differences among individuals in the neural basis of motivated behaviors and their relationship with vulnerability to abuse natural and/or drug rewards or to develop depressive disorders.

References

- [1] Sanna F et al., *Physiol Behav*, 127: 27-36, 2014
- [2] Sanna F et al., *Behav Brain Res*, 281: 177-186, 2015
- [3] Melis & Argiolas, *Neurosci Biobehav Rev*, 19: 19-38, 1995

Work partially supported by grants to FS from MIUR (SIR 2014, RBSI14IUX7), to OG from RAS (CRP-59842) and to AA and MRM from the Department of Biomedical Sciences (PRID 2015, FIR 2016).

INFLUENCE OF JWH-018 REPEATED ADMINISTRATION ON THE RESPONSIVENESS OF MESOLIMBIC AND MESOCORTICAL DOPAMINE TRANSMISSION

Nicholas Pintori¹, Cristina Miliano¹, Marta De Felice¹, Claudia Sagheddu¹, Giulia Margiani¹, M. Paola Castelli¹, M. Grazia Ennas¹, Marco Pistis^{1,2}, Gaetano Di Chiara^{1,2}, Maria Antonietta De Luca¹

¹ *Department of Biomedical Sciences, University of Cagliari, Italy;* ² *CNR Neuroscience Institute, Cagliari, Italy*

Since 2004, herbal mixtures containing synthetic cannabinoids (SC), broadly known as Spice/K2, have been marketed as a legal marijuana surrogate. The SC 1-pentyl-3-(1-naphthoyl)-indole (JWH-018) has been detected in several samples of Spice/K2 drugs. Previous studies of our group showed that JWH-018 has CB1-receptor dependent reinforcing properties and increases dopamine (DA) transmission in the shell of the nucleus accumbens (NAc). Other studies showed that taste stimuli increase extracellular DA in the NAc and in the medial prefrontal cortex (mPFC) of rats. This effect shows single-trial habituation in NAc shell but not in core or in mPFC. However, mPFC 6-OHDA lesions abolish habituation of DA responsiveness to taste stimuli in NAc shell. Such findings support the hypothesis of an inhibitory influence of mPFC DA on NAc DA, and its putative role in the loss of control of the motivational value of stimuli and in impulsivity. In order to test if the repeated administration of JWH-018 is able to modulate the activity of DA terminal areas and is associated to changes in the responsiveness to taste stimuli, rats were administered once a day for 14 consecutive days with JWH-018 (0.25 mg/kg i.p.) or with vehicle. After a week of wash out, the DA extracellular levels were measured by *in vivo* brain microdialysis in the NAc shell, core, and mPFC of rats either naive or pre-exposed to chocolate (1ml/5min i.o.); behavioral taste reactions were also recorded. JWH-018 administration inhibited the increase of DA in the NAc shell of animals naive to chocolate, abolished habituation of DA responsiveness to repeated chocolate exposure in the same area while induced it in the mPFC. In the NAc core, the treatment with JWH-018 potentiated, delayed and prolonged the stimulatory DA response to taste stimuli of animals pre-exposed to chocolate. Differences in aversive, but not hedonic, taste reactions were observed. Parallel studies of *in vivo* electrophysiology showed that JWH-018 treatment reduces the number of spontaneously active DA neurons of the ventral tegmental area (VTA) and increases their bursting activity. Further studies on neurodegeneration and neuroinflammation (TH in the VTA, GFAP and IBA-1 in the mPFC/NAc) produced by repeated JWH-018 administration showed that JWH-018 induces astrogliosis in mPFC and NAc shell, and induces changes of microglia in NAc core. These data show that JWH-018 is able to change the activity of DA neurons and to induce differential adaptive changes of the responsiveness of DA transmission to taste stimuli in DA terminal areas, similarly to previous results obtained in mPFC 6-OHDA lesioned rats. Moreover, repeated administration of JWH-018 induces changes of astrocytes and microglia, which are an index of neuroinflammation. This study may be useful to understand if such dysfunctions of cortical-limbic-striatal DAergic circuit can lead to specific detrimental effects of recurring use of Spice/K2 drugs.

RAT 50-KHZ ULTRASONIC VOCALIZATIONS AND GLUCOCORTICOID SIGNALING: EFFECTS OF CORTICOSTERONE, MIFEPRISTONE AND METYRAPONE ON CALLING INITIATION AND ON CALLING STIMULATED BY SOCIAL CONTACTS OR AMPHETAMINE

Nicola Simola¹, Elena Paci², Marcello Serra¹, Giulia Costa¹, Micaela Morelli^{1,3}

¹Department of Biomedical Sciences, Section of Neuropsychopharmacology, University of Cagliari, Cagliari, Italy; ²Department of Physiology, Pharmacology and Neuroscience, University of Bristol, Bristol, UK; ³CNR Neuroscience Institute, Cagliari, Italy

Rats emit 50-kHz ultrasonic vocalizations (USVs) to communicate positive emotional states, and these USVs are increasingly being investigated in preclinical studies on reward and motivation. Although it is the activation of dopamine receptors that initiates the emission of 50-kHz USVs, non-dopaminergic mechanisms may modulate calling in the 50-kHz frequency range. To further elucidate these mechanisms, the present study investigated whether the pharmacological manipulation of glucocorticoid signaling influenced calling. Rats were administered corticosterone (1-5 mg/kg, s.c.), the glucocorticoid receptor antagonist mifepristone (40 or 100 mg/kg, s.c.), or the corticosterone synthesis inhibitor metyrapone (50 or 100 mg/kg, i.p.). The effects of these drugs on calling initiation and on calling recorded either during non-aggressive social contacts or after the administration of amphetamine (0.25 or 1 mg/kg, i.p.) were then evaluated. Corticosterone failed to initiate the emission of 50-kHz USVs and did not influence pro-social and amphetamine-stimulated calling. Similarly, mifepristone and metyrapone did not initiate calling. However, metyrapone completely suppressed pro-social calling as well as calling stimulated by a moderate dose (1 mg/kg, i.p.) of amphetamine. Conversely, mifepristone attenuated calling stimulated by a low (0.25 mg/kg, i.p.), but not moderate (1 mg/kg, i.p.), dose of amphetamine, and had no influence on pro-social calling. The present results demonstrate that glucocorticoid signaling modulates calling in the 50-kHz frequency range only in certain situations that may be rewarding for rats, and suggest that metyrapone suppresses calling by mechanisms different from the inhibition of corticosterone synthesis.

ROLE OF THE ODORANT RECEPTOR AT THE AXON TERMINAL

Simona Francia^{1,2} (Lodovichi group)

It is known for more than 20 years that odorant receptors not only detect odors but also determine the convergence of sensory neurons to form glomeruli in specific locations in the olfactory bulb, giving rise to the sensory map. The sensory map has a critical role in encoding odors, that are represented by a spatial pattern of activated glomeruli. Odorant receptors are expressed specifically and exclusively in two locations in sensory neurons: at the cilia, where they detect odors, and at the axon terminal. Why odorant receptors are expressed at the axon terminal? That could be a suitable location for axon guidance molecules. This hypothesis could explain the role of the odorant receptor in the formation of the sensory map. However, the mechanism of activation and function of the odorant receptor at the axon terminal has remained elusive for all this year. The odorant receptor expressed at the cilia, is a G- protein-coupled receptor, that upon binding odors leads to an increase of cAMP and Ca^{2+} .

In a previous work, we found that the odorant receptor at the axon terminus is functional and coupled to local increase of cAMP and Ca^{2+} . The question that remained to be addressed was the mechanism of activation of the odorant receptor at the axon terminus.

We hypothesized that few molecules expressed in the olfactory bulb, could bind and activate the odorant receptor at the axon terminus of olfactory sensory neurons.

Here, by studying the spatio-temporal dynamics of Ca^{2+} in olfactory sensory neurons in response to a pool of molecules extracted from the olfactory bulb, we found that this pool, locally applied, was able to induce Ca^{2+} rise at the axon terminus of olfactory sensory neurons. To ascertain that this Ca^{2+} rise was due to odorant receptor activation, we expressed odorant receptors in HEK cells. We found that the active pool of molecules from the olfactory bulb was able to elicit Ca^{2+} rise in HEK cells transfected with specific odorant receptors, but not in HEK cells transfected only with the vector, used as controls.

By mass spectrometry of the active pool of molecules from the olfactory bulb, we identified a putative ligand of the odorant receptor at the axon terminus. This ligand, locally applied, was able to elicit Ca^{2+} rise in olfactory sensory neuron axon terminus and in HEK cells transfected with specific odorant receptors. Furthermore, it regulates the turning behaviour of sensory axons. Mice carrying a null mutation in the ligand exhibits a deeply perturbed sensory map.

All together our data demonstrated that the odorant receptor at the axon terminus appears to act as an axon guidance molecule, activated by molecules expressed in the olfactory bulb, that contribute in providing the olfactory sensory neurons with instructions to reach the proper target.

The identity of the odorant receptor, therefore, defines the odors a neuron is responsive to and the location in the olfactory bulb where it projects, linking perception to its internal representation. An odor is indeed encoded by a spatial pattern of activated glomeruli.

MECHANISMS OF THE IMPAIRED INCRETIN ACTION IN TYPE 2 DIABETES: A MODELLING STUDY

Eleonora Grespan¹, Toni Giorgino¹, Andrea Natali², Ele Ferrannini³, Andrea Mari¹

¹CNR Institute of Neuroscience, Padova, Italy; ²Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; ³CNR Institute of Clinical Physiology, Pisa, Italy

Background and aims: The mechanisms underlying the reduced incretin effect (IE) in type 2 diabetes (T2D) are still debated. The impairment is often attributed to loss of GIP efficacy, but bolus administration of GIP appears to elicit a stronger response compared to constant infusion. GLP-1 efficacy is better preserved, but to an unclear degree. *In vitro* studies have mostly used GLP-1 and GIP at supraphysiological concentrations in mouse islets, making the extrapolation of the results *in vivo* difficult. A role of GIP and GLP-1 in intracellular calcium has been proposed. To investigate the response to GIP and GLP-1 in IE, we use a recently developed mathematical model of the β cell, capable of explaining *in vitro* and *in vivo* data through consistent and physiologically based mechanisms.

Material and methods: We used *in vivo* data from a study of IE in subjects with normal glucose tolerance (NGT) and T2D, based on an OGTT and an isoglycaemic intravenous glucose infusion (IIGI) test. We also used literature data from a hyperglycaemic clamp with boluses and continuous infusion of GIP at different doses (Test 1) and from a graded glucose infusion test with continuous infusion of GLP-1 at different doses (Test 2). The model features an immediately releasable insulin pool, which is emptied by calcium-mediated exocytosis and refilled by a process controlled by calcium and glucose. This model accurately describes the IIGI test and attributes the β cell dysfunction of T2D to a defect in the refilling process. In the present study, we hypothesized that GIP and GLP-1 increase insulin secretion rate (ISR) by raising calcium levels and potentiating the refilling.

Results: ISR during the OGTT and the IIGI (Fig. 1) was accurately predicted by postulating a transient effect of incretins on intracellular calcium levels, which explains the early IE, and a sustained effect on refilling (Fig. 1, K_{incr}). In T2D, K_{incr} increase was grossly impaired (1.2 in T2D vs 2.0-fold in NGT), while the transient effect on calcium was partly preserved (calcium increment AUC $\approx 63\%$ of NGT). The same mechanism could also reproduce the ISR peaks reported in Test 1, where T2D showed a marked impairment in K_{incr} increase (1.1 in T2D vs 3.0-fold in NGT at the low GIP dose), while the simulated calcium peak was present but reduced to 30% of NGT. In Test 2, the progressive increase in K_{incr} with GLP-1 levels was impaired in T2D (2.4 in T2D vs 3.1-fold in NGT at the lowest GLP-1 dose).

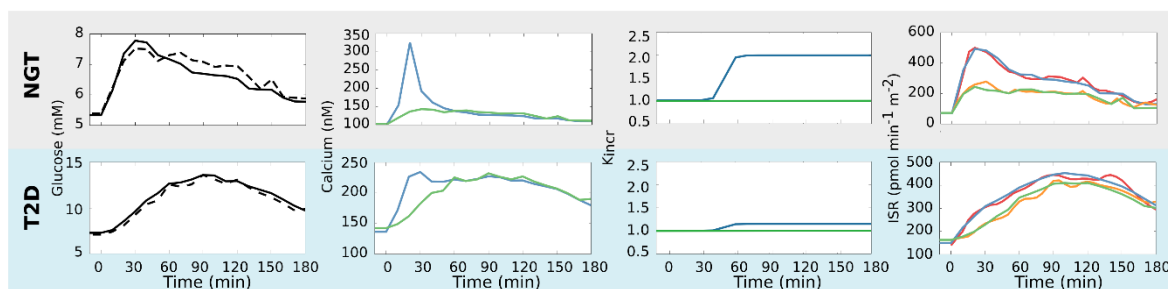


Fig.1: simulation in subjects with normal glucose tolerance (NGT) and type 2 diabetes (T2D). Glucose: OGTT (solid black line) and IIGI (dashed black line); calcium and refilling potentiation (K_{incr}): OGTT (blue line) and IIGI (green line); experimental ISR: OGTT (red line) and IIGI (orange line); model-predicted ISR: OGTT (blue line) and IIGI (green line).

Conclusion: The model suggests that: 1) an initial rise in intracellular calcium underlies the early effects of incretins; 2) sustained effects are mediated by refilling potentiation; 3) in T2D, the defective IE is due to an impairment of both processes and is not explained by the intrinsic beta-cell dysfunction; 4) in T2D, refilling potentiation by GIP is strongly impaired, while the calcium-mediated effect is partly preserved; refilling potentiation by GLP-1 is also reduced, although to a lesser extent.

AUTOPHAGY CONTROLS NEONATAL MYOGENESIS BY REGULATING THE GH/IGF1 SYSTEM

Clara De Palma¹, Silvia Zecchini¹, Matteo Giovarelli², Marco Sandri³, Emilio Clementi^{1,4}

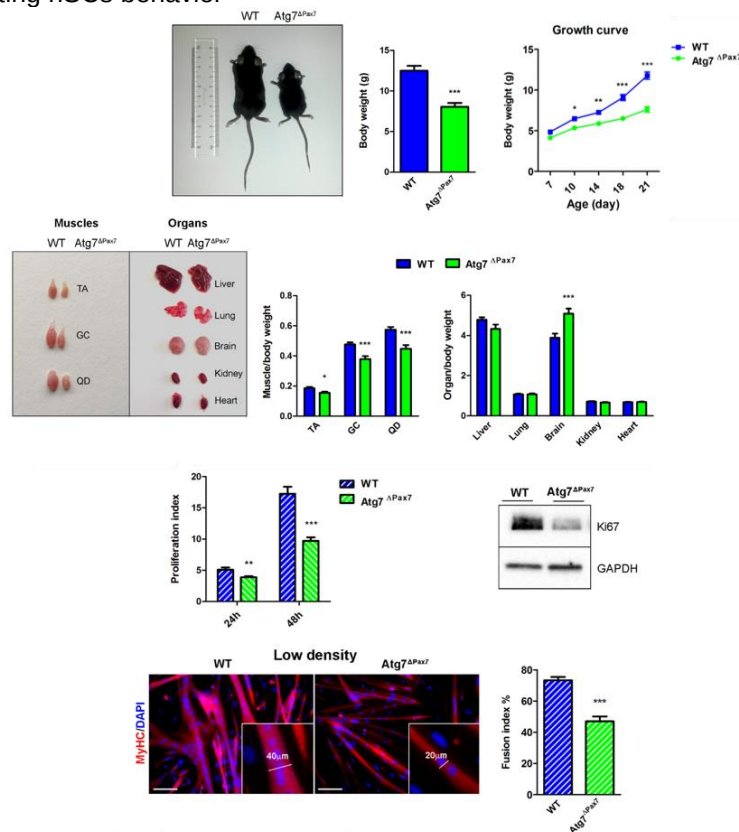
¹Unit of Clinical Pharmacology, University Hospital "Luigi Sacco"- ASST Fatebenefratelli Sacco; CNR-Institute of Neuroscience; ² Department of Biomedical and Clinical Sciences "Luigi Sacco", Università degli Studi di Milano, Milano, Italy; ³ Department of Biomedical Science, University of Padova, Padova, Italy; ⁴IRCCS Eugenio Medea Scientific Institute, Bosisio Parini, Italy

Autophagy is emerging as an important process in adult muscle stem cells functions: it is known to regulate metabolic reprogramming during activation from quiescent state, maintain stemness and prevent senescence. To date little information has been provided on whether autophagy controls muscle development. To study this we deleted Atg7 specifically in satellite cells and found that Atg7 knockdown leads to severe deficiencies in skeletal muscle growth.

Specific deletion of Atg7 in Pax7⁺ precursors led in mice to a dwarf phenotype, with an effect restricted to the neonatal phase of muscle development. Atg7 knockdown suppressed neonatal satellite cells (nSCs) proliferation and differentiation downregulating the GH/IGF-1 functions. When we disrupted autophagy, GH receptor (GHR) expression was specifically reduced in muscle because of a decreased expression of CHOP and lead to impaired local production of IGF-1.

We also examined the correlation between autophagy and GH/IGF-1 pathway immediately after gene deletion. It is interesting to note that the effects of chronic inhibition of autophagy in nSCs are the same of those of acute inhibition.

In conclusion, our results provide in vivo evidence that basal autophagy, by regulating the GH/IGF1 pathway, controls neonatal satellite cells activity, including proliferation and terminal differentiation and impairs neonatal myogenesis. Disrupting autophagy, GHR levels change accordingly with CHOP expression leading to reduced local production of IGF-1. Collectively, we identify autophagy as an important factor for muscle maturation in neonatal phase, regulating nSCs behavior



Atg7^{ΔPax7} mice body weight and growth curve. Specific muscles and organs weights. nSCs proliferation and differentiation analysis

A WINDOW WITH A VIEW: ORDER AND CHAOS IN THE BRAIN VISUALIZED BY TWO PHOTON MICROSCOPY

Gian Michele Ratto^{1,2}

¹Nanoscience Institute, CNR and NEST, Pisa, Italy; ²Scuola Normale Superiore, Pisa, Italy

Two-photon microscopy is a powerful tool to image form and function in the brain in vivo: neurons, glia and dendrites can be visualized by means of an ever expanding range of fluorescent probes sensitive to specific parameters of the intracellular environment such as calcium, chloride, pH and membrane potential. Changes in the intracellular concentration of calcium and chloride ions report on two complementary aspects of neuronal computation: calcium increments are a proxy for neuronal depolarization and firing, while chloride changes report the activation of the main inhibitory synapses. At this time, two photon imaging is the only available tool that allows to peer into neuronal function in the intact brain at the level of cell population, individual cells and at the subcellular level: indeed, in vivo imaging is providing novel insights on brain structure and function with unprecedented temporal and spatial resolution. In addition, the availability of genetic and pharmacological models of brain pathologies, allows to investigate how the disease affects brain computation at the neuronal level, and hopefully this will contribute to enlighten the relationship between cognitive deficits and the biophysics of neuronal computation.

Imaging experiments produces very large data sets and their processing and quantification is not a trivial task. Typically, a crucial step of the analysis is the identification of the image regions (Regions Of Interest, ROIs) that are 'interesting' for the following quantification, imposing an a priori bias on the analysis. An alternative vision is the exploitation of the statistical properties of the images for the extraction of the features of interest in both space and time domain. This alternative point of view requires the convergence of widely different notions originating from the field of image analysis, thermodynamics and information theory. In this talk I will address three questions that are at the core of the problem of linking optical imaging to brain function: 1) What is an 'interesting' event in the fluorescence data and how can we extract events hidden in a noisy environment? 2) What is a ROI and how do we can define it without any a priori hypothesis? 3) What can we learn from the statistics of the fluorescence fluctuations? I will provide an overview of the methodology that we are developing for the interpretation of calcium imaging data, while exploring the characteristics of brain computation in models of epilepsy and disease of the autistic spectra.

OPTOGENETIC DISSECTION OF INTERNEURON-TYPE-SPECIFIC SIGNALING TO ASTROCYTES

Giorgio Carmignoto

CNR Neuroscience Institute, Padova, Italy

The most recent advances in optical technologies have given rise to a new era for biological research. It is now possible to combine different tools, including genetically encoded calcium, optogenetics and patch-clamp recordings in *in vitro* and *in vivo* models with single and two-photon laser-scanning microscopy to see the brain in action. This approach has led to the novel view that the brain should no longer be regarded solely as a neuronal network, but instead as a circuit of interactive neuron and glial cell networks, opening new perspectives for our understanding of brain function and dysfunction. This presentation will be focused on the GABAergic signalling to astrocytes. Notably, the signalling diversity of the different GABAergic interneuron subtypes to post-synaptic neurons is crucial to generate the functional heterogeneity of brain circuits. Whether this diversity applies to other brain cells, such as the glial cell astrocytes, remains totally unexplored. We here reveal that Parvalbumin- and Somatostatin-expressing interneurons, two key interneurons in the brain, differentially signal to astrocytes inducing in these cells weak and robust GABA_B receptor-mediated Ca²⁺ elevations, respectively. Furthermore, astrocytic responses depress upon Parvalbumin interneuron repetitive stimulations and potentiate upon Somatostatin interneuron repetitive stimulations, revealing a distinguished astrocyte plasticity. Remarkably, the potentiated response depends on the neuropeptide Somatostatin, co-released with GABA by Somatostatin interneurons, that activates Somatostatin receptors at astrocytic processes. In the somatosensory cortex of adult mice we have indentified a novel signalling mode of GABAergic interneurons that through the combined release of a neuropeptide and GABA, generates cell-specific interneuronal-astrocytic networks

DIVING IN FOR DEEPER INSIGHTS

Tommaso Cerullo

Leica Microsystems S.r.l., Life Science Research Division, Milano, Italy

Investigation of complex biological processes in living tissues over time by confocal microscopy is a standard imaging approach in neurosciences. Newly engineered transgenic fluorescence markers enable researchers to study function, interaction and development of living cells in their natural tissue environment in a more and more detailed way. Multiphoton microscopy provides further exciting options, like deep tissue and second/third harmonic generations imaging.

The color channels are preferably recorded simultaneously to preserve the temporal and spatial interrelationships among the investigated biological parameters. This requires a number of light sensors detecting in parallel the different color channels and a device able to feed simultaneously each of them with the proper fraction of the light spectrum.

The adoption of spectral separation and detection devices fulfilling easily and flexibly the above reported needs is nowadays common in confocal microscopy whereas the old fashion solution to separate the spectral emission-fractions for simultaneous recording by dichroic beam splitting mirrors and barrier filters is the only one available for non-descanned detection in multiphoton microscopy. Such a design has the disadvantage that, by changing dye combinations, the system must be equipped by additional appropriate filter configurations, making it a costly, slow and inflexible solution.

With the launch of the brand new SP8 DIVE system Leica Microsystems offers the world's first spectrally tunable solution for multi-color, multiphoton deep tissue imaging. DIVE stands for Deep *In Vivo* Explorer and it is based on Leica Microsystems all-new 4Tune technology. It offers four freely and independently tunable spectral channels for non-descanned detection in multiphoton microscopy. The user can define the desired spectral bands easily in an intuitive graphical software interface for the best efficiency and channel separation. It also allows seamlessly tuning the detection for second or third harmonics, whenever changing the illumination wavelength, simultaneous to fluorescence recording.

On top of this unique flexible and effective solution, the SP8 DIVE proposes a new laser beam routing unit to manage up to four multiphoton lasers (OPO included), and the VBE (Vario Beam Expander) device providing the freedom of choice as for the beam size diameter to maximize the penetration depth or the resolution. The VBE offers beam shaping for up to four different IR illumination wavelengths. All four IR beams can be adjusted for resolution and penetration depth and independently for focus correction assuring a perfect coincidence for excitation with different IR colors.

All together these technologies offer full spectral freedom and enables relevant breakthroughs in multicolor deep tissue imaging.

CONCURRENT MULTI-SITE CALCIUM RECORDINGS AND BRAIN IMAGING: STUDY OF DYNAMIC CONNECTIVITY RELATED TO SYSTEM AND FLUCTUATION STATES

Xin Yu

Max Planck Institute for Biological Cybernetics Tübingen, Germany

The resting-state fMRI signal could be characterized at a single-vessel level to map vascular network connectivity in the rat brain. Translational efforts have been made to implement the single-vessel mapping scheme to investigate the vessel-specific fMRI signal fluctuation in the human brain with 3T and 9.4T MR scanners. To characterize neuro-glio-vascular dynamic changes contributing to the fMRI signal fluctuation, the fMRI signal could be simultaneously recorded with the GCaMP6-mediated neuronal and glial calcium signal in the rat cortex. The evoked and spontaneous astrocytic calcium signal represented distinct neurovascular coupling features and correlated to different states of neuronal oscillations. The spontaneous astrocytic calcium spikes may directly contribute to the slow-frequency fMRI signal fluctuation. This work is aimed to identify key coupling signals of the neuron-glial-vessel network as prognostic indicators of brain state changes in the diseased brain.

NEUROIMAGING INSIGHTS INTO NETWORK-BASED NEURODEGENERATION

Federica Agosta

Neuroimaging Research Unit, Institute of Experimental Neurology, Division of Neuroscience, Scientific Institute San Raffaele, Milan, Italy

Current knowledge of neurodegenerative diseases is limited by poor understanding of how they progress through the central nervous system (CNS). It has recently been hypothesized that clinical progression in these conditions involves the systematic spreading of protein misfolding along neuronal pathways. Protein aggregates would trigger misfolding of adjacent homologue proteins in newly-affected regions, and this would propagate in a “prion-like” fashion across anatomical connections. In neurodegenerative diseases, novel neuroimaging techniques can help to elucidate the spatial, time-dependent expansion of the underlying pathology across brain networks. So far, however, a useful framework for conceptualizing and predicting the profiles observed in patients has been lacking. The recent development and application of graph theoretical tools to brain magnetic resonance imaging (MRI) connectivity research offer a unique opportunity to explore principles of network-based neurodegeneration and to address unanswered questions. Just like in natural systems, social interactions, and electrical and telecommunication grids, the brain can be understood as a complex, interconnected network (the human connectome) at both the structural and the functional levels. When graph theory is applied to in this field, a brain network is regarded as a set of connected nodes, with each node representing a specialized neural element, and the connection edges representing measures of structural or functional connectivity between them. The application of graph theory on brain connectivity data put previous MRI findings in a new perspective, suggesting that node properties are likely to play a critical role in the pathophysiology of neurodegenerative diseases. Moreover, a major strength of graph theory is that it can be used to generate and test competing generative models designed to explain observed variations across a range of topological properties in the disease. Network science experiments will pave the way to the development of novel tools for understanding the biological underpinnings of CNS proteinopathies such as Alzheimer’s disease, frontotemporal dementia and Parkinson’s disease, and to identifying individualized, early interventions to modify disease progression.

INVOLVEMENT OF TG2 IN CALCIUM HOMEOSTASIS IN RAT PRIMARY HIPPOCAMPAL NEURONS

Claudia Verderio¹, Elisa Tonoli², Ilaria Prada¹, Elisabetta Verderio²

¹CNR Neuroscience Institute, Milano, Italy; ²School of Science and Technology, Interdisciplinary Biomedical Research Centre, Nottingham Trent University, Nottingham, United Kingdom

Type 2 transglutaminase (TG2) is a calcium-dependent cross-linking enzyme known to be implicated in multiple diseases linked to calcium dysregulation, e.g. oxidative-stress, cell-death and neurodegeneration. Here we investigated TG2 activity in rat hippocampal neurons and its impact on neuronal calcium homeostasis. Neurons treatment with tetrodotoxin (TTX), an inhibitor of synaptic transmission, led to a 2.4-fold increase in TG2 transamidating activity. To verify if this effect could represent a compensatory response aimed at rescuing neuronal firing we analysed how manipulation of TG2 activity impacted synchronous calcium oscillations, a form of intense synaptic activity driven by bursts of neuronal firing. TG2 inhibition decreased calcium oscillations' frequency and the interspike calcium concentrations in fura-2-loaded neurons, suggesting a role for TG2 in sustained excitatory transmission. Conversely, TG2 promoted the onset of calcium transients followed by sustained plateaus and reversible block of calcium oscillations. Overexpression of human TG2 by transient transfection induced a significant rise in basal calcium concentration, evaluated in the presence of TTX. Basal calcium increase mediated by TG2 did not occur in calcium-free medium, revealing that the protein induces influx of extracellular calcium ions. Nimodipine, a blocker of L-type voltage-gated calcium channels (VOCCs), caused a partial recovery of calcium concentration towards resting levels when applied during the TG2-induced plateau phase, suggesting that calcium influx partially occurs through L-type VOCCs. Consistently, VOCCs-mediated calcium influx was enhanced by TG2. However, other channels/transporters may contribute to this process. Few putative TG2 targets identified by a proteomic approach are now under investigation. Collectively these data indicate a possible role of TG2 in the enhancement of basal calcium levels and in the facilitation of calcium transmission through VOCCs in neurons.

THE INNATE IMMUNE MOLECULE PTX3 ENHANCES THE SYNAPTIC CONTENT OF AMPA RECEPTORS VIA EXTRACELLULAR MATRIX REMODELING

Giuliana Fossati¹, Davide Pozzi^{1,2}, Alice Canzi², Sonia Valentino¹, Elsa Ghirardini^{1,3}, Filippo Mirabella², Milena Moretti³, Giulia Taraboletti⁴, Barbara Bottazzi^{1,2}, Cecilia Gotti⁵, Cecilia Garlanda^{1,2}, Alberto Mantovani^{1,2}, Michela Matteoli^{1,5}, Elisabetta Menna^{1,5}

¹IRCCS Humanitas, Rozzano (MI), Italy; ²Hunimed University, Rozzano (MI), Italy; ³University of Milano, via Vanvitelli 32, 20129 Milano, Italy; ⁴Tumor Angiogenesis Unit, Department of Oncology, IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; ⁵CNR Neuroscience Institute, Milano, Italy

In the developing central nervous system, the control of synapse number and function is critical to the formation of neural circuits. Astrocytes play a key role in this process by releasing factors which promote the formation of excitatory synapses. Astrocyte-secreted thrombospondins (TSPs) induce the formation of structural synapses, which are however postsynaptically silent. The possibility that additional astrocyte-derived factors may promote the functional switch of the thrombospondin-induced synapses has never been explored. We identified the humoral innate immune molecule PTX3, which is expressed in the rodent brain in a developmental window corresponding to the late embryonic–early postnatal period, as a key molecule which plays a crucial role in the formation of post-synaptically active CNS synapses. This is attained by PTX3 ability to increase the surface levels and clustering of the AMPA glutamate receptors through the remodeling of the perineuronal network via a $\beta 1$ integrin-dependent process. Of note, PTX3 was found to bind TSP-1 and -2, which are expressed in the brain in the same temporal window, but not TSP-4, which is expressed in the spinal cord and dorsal root ganglia. These data unveil a fundamental crosstalk between the immune and nervous systems to establish the first wave of synaptogenesis and the organization of the early functional circuits in the developing brain.

CHRONIC TREATMENT WITH BIFIDOBACTERIUM (*LONGUM*, *BREVE*, *INFANTIS*) CHANGES GABA_A SUBUNITS EXPRESSION AND EXCITABILITY IN THE HIPPOCAMPUS OF ADULT MALE RATS

¹Rafaela Mostallino, ¹Francesca Biggio, ²Giuseppe Talani, ³Valentina Locci, ¹Laura Boi, ^{1,2}Enrico Sanna, ^{1,2}Giovanni Biggio G, ²Maria Cristina Mostallino

¹Department of Life and Environmental Sciences, University of Cagliari, Monserrato (CA), Italy; ²Institute of Neuroscience, National Research Council (CNR), Monserrato (CA), Italy; ³Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, USA

Increasing evidence suggest the microbiota as a powerful regulator of brain development function and behavior in both humans and rodents. Although, the mechanisms underlying the ability of the gut microbiota to influence environmental responsiveness remain unknown, many lines of evidence point the hippocampus as a possible target for this fine tuning. Recent evidences suggest that structural integrity of the hippocampal formation is contingent on the presence of a healthy gut microbiota. Nevertheless, the mechanism involved in the ameliorating action of microbiota on brain, behavioral and cognitive functions, has not yet been fully understood. It has been recently proposed that changes in microbiota alter the stress responses to the hypothalamic-pituitary-surrenal (HPA) axis, an effect that may involve the inhibitory neurotransmitter GABA, one of the first candidates in the modulation of emotional states. Here, we studied in rats the long-lasting effect of a chronic (1-2 months) treatment with a preparation (TRIBIF) of three different Bifidobacterium (*Longum*, *Breve*, *Infantis*) on GABAergic system and hippocampal excitability as well as HPA axis responsiveness to acute foot-shock stress in adult naïve rats. We first measured stress-related hormones such as allopregnanolone (AP) and corticosterone (CTS) levels in basal condition and after foot-shock stress. TRIBIF treatment induced a decrease of basal plasmatic content of AP while failed to changes foot-shock-induced increase of CTS levels when compared to vehicle group. Immunohistochemistry analysis showed that two months of TRIBIF treatment reduced $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 4$ and δ GABA_AR subunits expression while increasing $\gamma 2$ subunit. Patch-clamp experiments performed in, dentate gyrus granule cells (DGGC) showed no change in synaptic currents whereas significantly decreased the tonic component of GABAergic inhibition. The latter data correlates with an observed increase of neuronal excitability measured in the same neurons of the dentate gyrus as well as with the reduction of δ subunit. The lack of TRIBIF protective action towards acute stress worth to be further consideration given that treatment was carried out in healthy animals suggesting that beneficial effects of TRIBIF could well manifest themselves in organisms with an altered microbiota. Our results, together with recent findings related to apical dendritic atrophy and spine loss in CA3 pyramidal neurons in germ-free mice, further support the crucial role of the microbiota on brain function (Luczynski et al., 2016).

Founded by VALEAS

ASTROCYTE SIGNALING IN BRAIN REWARD SYSTEM

Linda Maria Requeie^{1,2}, Marta Gomez-Gonzalo^{1,2}, Giorgio Carmignoto^{1,2}

¹*University of Padova, Department of Biomedical Sciences, Padova, Italy;*

²*CNR Neuroscience Institute, Padova, Italy*

The mesolimbic dopamine system originating from the Ventral Tegmental Area (VTA) and projecting to different brain regions, such as nucleus accumbens and hippocampus, plays a prominent role in the cognitive processing of aversion, motivation, pleasure and reward, including the development of addiction. Dopaminergic (DA) neurons of VTA switch from a tonic to a phasic, burst firing that is related to a higher release of dopamine in the VTA projecting areas. Glutamatergic innervation to the VTA plays a central role in this transition from tonic to phasic, burst-firing pattern and modulation and plasticity of the glutamatergic transmission onto DA neurons can profoundly alter the output of DA neurons. In the last years astrocytes have emerged as important regulatory elements in the function of different brain areas. They respond to different neurotransmitters with Ca^{2+} elevations through a mechanism that involves the production of IP3 and Ca^{2+} release from IP3-sensitive intracellular stores. In turn, these Ca^{2+} elevations in astrocytes evoke the release of gliotransmitters that regulate neuronal function. Whether a similar mechanism exists in VTA circuitry is unknown.

By combining patch-clamp recording techniques and Ca^{2+} imaging in VTA slices from young mice we aim to answer the following questions. Are VTA astrocytes recruited in response to an imposed long-lasting, high-frequency burst firing of a DA neuron? Can recruited astrocytes modulate the heteroneuronal excitatory transmission? What neurotransmitters and gliotransmitters are eventually involved in this modulation of VTA circuitry by astrocytes?

Our results show that, in female mice, burst firing of a DA neuron induces the release of endocannabinoids and dopamine in VTA that activates astrocytes. In turn, these astrocytes release glutamate that acting on metabotropic glutamate receptors, located at the presynaptic glutamatergic terminals, favors the excitatory transmission in adjacent DA neurons.

The modulatory action of astrocytic signaling on VTA DA neurons function opens a new perspective to a better understanding of the complex cognitive processes in the brain reward system.

BRAIN CHANGES IN DOPAMINE AND SEROTONIN LEVELS IN RATS EXPOSED TO THE ACTIVITY-BASED MODEL OF ANOREXIA

Roberto Collu¹, Maria Scherma¹, Elisa Giunti¹, Matteo Deidda¹, Walter Fratta^{1,2}, Paola Fadda^{1,2}

¹*Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology*
and ²*Centre of Excellence “Neurobiology of Dependence”, University of Cagliari, Italy*

Considerable evidence supports a positive correlation between altered dopamine (DA) and serotonin (5-HT) function and anorexia nervosa (AN) (Foldi *et al.*, 2017). Here we measured brain content of DA and 5-HT in female rats exposed to the activity-based anorexia (ABA), a rodent model of AN, in regions with an important role in the homeostatic and rewarding regulation of feeding behavior (prefrontal cortex, nucleus accumbens, amygdala, hippocampus and hypothalamus). Analysis were conducted both in the acute and recovered states of the ABA condition, in order to evaluate possible fluctuation of DA and 5-HT levels related to the feeding status. Results show that concentration of both DA and 5-HT were markedly increased in the nucleus accumbens of ABA rats and persisted significantly enhanced also after weight gain. Moreover, levels of DA were significantly higher in the prefrontal cortex and of 5-HT in the hippocampus of ABA rats and normalized toward basal levels after recovery. Of note, exclusively recovered ABA rats showed increased 5-HT levels in the hypothalamus. In conclusion, changed DA and 5HT levels that persist beyond the acute and recovered ABA state, could differently contribute to generate the key behavioral traits, typical hallmarks of AN. Further studies are needed to better understand the complex molecular mechanisms underlying the aberrant reward related behavior in AN.

Reference

Foldi CJ, Milton LK, Oldfield BJ (2017) *J Neuroendocrinol*, in press

INTRA-VTA MICROINJECTION OF BACLOFEN AND CGP7930 SUPPRESSES ALCOHOL SELF-ADMINISTRATION IN RATS

Paola Maccioni¹, Irene Lorrai¹, Andrea Contini², Gian Luigi Gessa¹, Giancarlo Colombo¹

¹CNR Neuroscience Institute, Section of Cagliari, Italy;

²Department of Biomedical Sciences, University of Sassari, Italy

Background – Systemic administration of the orthosteric agonist, baclofen, and several positive allosteric modulators (PAMs) of the GABA_B receptor has repeatedly been reported to decrease multiple alcohol-motivated behaviors, including operant oral alcohol-self-administration, in rats and mice. Most of these results have successfully been translated to human alcoholics, making baclofen a promising pharmacotherapy for alcohol use disorder, while GABA_B PAMs are now entering the initial steps of clinical trials.

Aims – The present study was aimed at evaluating the contribution of the mesolimbic dopamine brain “reward” system to the reducing effect of baclofen and GABA_B PAMs on the reinforcing properties of alcohol. To this end, baclofen and the GABA_B PAM, CGP7930, were microinjected into the ventral tegmental area (VTA) of selectively bred, Sardinian alcohol-preferring (sP) rats trained to self-administer alcohol.

Methods – Rats were initially trained to lever-respond for alcohol (15%, v/v) under a fixed ratio (FR) 4 (FR4) schedule of reinforcement. Once lever-responding had stabilized, rats underwent surgery for placement of a unilateral cannula aiming at the left side of posterior VTA. After resumption of pre-surgery levels of lever-responding for alcohol, baclofen (0, 0.03, 0.1, and 0.3 µg) and CGP7930 (0, 5, 10, and 20 µg) were microinjected into the VTA; 10 min later, rats were exposed to self-administration sessions under the FR4 schedule.

Results – Treatment with baclofen resulted in a dose-related suppression of number of lever-responses for alcohol and amount of self-administered alcohol; treatment with baclofen produced also a dose-related, dramatic increase in latency to the first response on the alcohol lever. No dose of baclofen altered rat motor-performance, evaluated by the inverted screen test immediately before the self-administration session. Treatment with CGP7930 halved the number of lever-responses for alcohol and amount of self-administered alcohol, with no effect on rat motor-performance. Site-specificity was investigated testing the effect of microinjection of baclofen and CGP7930 into the left side of deep mesencephalic nucleus: compared to vehicle, neither 0.3 µg baclofen nor 20 µg CGP7930 altered lever-responding for alcohol and amount of self-administered alcohol.

Conclusions – The results of the present study closely reproduced the effect exerted by both drugs after systemic administration. Additionally, they suggest the involvement of GABA_B receptors located in the VTA (a key area of the mesolimbic dopamine brain “reward” system) in the mediation of the reinforcing properties of alcohol in rats.

ALTERATIONS IN 5-ALPHA REDUCTASE AND NEUROSTEROIDS IN THE PREFRONTAL CORTEX MEDIATE THE PSYCHOTIC- AND MANIA-LIKE PHENOTYPES INDUCED BY SLEEP DEPRIVATION

F. Traccis¹, V. Bini¹, A. Soggiu², S. Scheggi³, A. Pardu¹, S. Fanni¹, P. Roncada², M. Puligheddu⁴, F. Marrosu⁴, D. Caruso⁵, P. Devoto¹, R. Frau¹ and M. Bortolato⁶

¹Dept. of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²'L. Spallanzani' Institute, Proteomics Section, University of Milan, Italy; ³Dept. of Molecular and Developmental Medicine, University of Siena, Italy; ⁴Dept. of Public Health, Section of Neurology, University of Cagliari, Italy; ⁵Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Italy; ⁶Dept. of Pharmacology and Toxicology, University of Utah, USA

Alterations of sleep patterns are prominent in a broad array of neuropsychiatric disorders (Benca et al, 1992). Sleep deprivation (SD) leads to several perceptual and cognitive abnormalities (Killgore, 2010; Daviaux et al, 2014); furthermore, psychotic and manic symptoms can be triggered or exacerbated by sleep deprivation (West et al, 1962; Wehr et al, 1987); accordingly, SD-subjected rats exhibit a wide array of manic-like behavioral manifestations (Gessa et al, 1995). Along these lines, it has been shown that sleep-deprived rats and humans develop deficits in the prepulse inhibition (PPI) of the acoustic startle reflex (Frau et al, 2008; Petrovsky et al, 2014), and PPI deficits are observed in schizophrenia and mania (Braff et al, 2001; Perry et al, 2001). Nevertheless, the neurobiological correlation between sleep deprivation and neuropsychological disorders remain largely elusive.

Given the extensive involvement of neuroactive steroids in psychopathology, we hypothesized that 5 α -reductase (5 α R), the rate-limiting enzyme in the conversion of progesterone into the neurosteroid allopregnanolone, may be responsible of the behavioural complications of SD. We first tested whether rats exposed to SD may exhibit brain-regional alterations in 5 α R isoenzymes and neuroactive steroid levels; then, we assessed whether the behavioral and neuroendocrine alterations induced by SD may be differentially modulated by the administration of the 5 α R inhibitor finasteride, as well as progesterone and allopregnanolone. We found that SD selectively enhanced 5 α R expression and activity, as well as AP levels, in the prefrontal cortex; furthermore, finasteride (10-100 mg/kg, IP) dose-dependently ameliorated PPI deficits, hyperactivity, and risk-taking behaviors, in a fashion akin to the antipsychotic haloperidol and the mood stabilizer lithium carbonate. PPI deficits were exacerbated by allopregnanolone (10 mg/kg, IP) and attenuated by progesterone (30 mg/kg, IP) in SD-subjected, but not control rats. Finally, the microinfusions of finasteride (0.5 μ g/side) in the prefrontal cortex were able to ameliorate PPI deficits induced by SD.

Collectively, these results provide the first-ever indication that 5 α R mediates a number of psychosis- and mania-like complications of SD through imbalances in cortical levels of neuroactive steroids.

References

- Benca et al. (1992). *Arch Gen Psychiatry* 49: 651–668
- Killgore (2010). *Prog Brain Res* 185: 105–129
- Daviaux et al. (2014). *Exp Brain Res* 232: 2243–2253
- West et al. (1962). *Ann N Y Acad Sci* 96: 66–70
- Wehr et al. (1987). *Am J Psychiatry* 144: 201–204
- Gessa et al. (1995). *Eur Neuropsychopharmacol* 5 Suppl: 89–93
- Frau et al. (2008). *Int J Neuropsychopharmacol* 11: 947–955
- Petrovsky et al. (2014). *J Neurosci* 34: 9134–9140
- Braff et al. (2001). *Psychopharmacology* 156: 234–258
- Perry et al. (2014). *Biol Psychiatry* 50: 418–424

EARLY LIFE STRESS INCREASES ALCOHOL INTAKE AND IMPAIRS COGNITIVE BEHAVIOR IN C57B/6J MICE: POSSIBLE INVOLVEMENT OF NUCLEUS ACCUMBENS AND HIPPOCAMPAL FORMATION

Giuseppe Talani¹, Gabriele Sarigu², Francescangelo Vedele², Daniele Colombo², Eleonora Loi², Roberta Piras², Enrico Sanna^{1,2}

¹CNR Neuroscience Institute, Section of Cagliari, Italy; ²Department of Life and Environmental Sciences, Section of Neuroscience and Anthropology, University of Cagliari, Italy

Traumatic events occurring in early life may lead, in both humans and rodents, to a high risk for neuropsychiatric disorders as well as increased vulnerability to drug abuse in adulthood. Repeated maternal separation (RMS) in rodents is a powerful model to investigate the consequences of neonatal stress on vulnerability to ethanol (EtOH) intake as well as impairments in cognitive behavior when observed in adulthood. In some cases, females are less affected by this stress paradigm suggesting that sex hormones may play a pivotal role in protection from stress. We here studied the potential mechanisms involved in the long-term effects of RMS on EtOH voluntary consumption as well as learning and memory in C57BL/6J adult mice evaluating the changes in function and plasticity of GABAergic and glutamatergic synapses, in both hippocampus (Hip) and nucleus accumbens (NAcc). Male or female pups were separated daily from their dam for 360 min (from PND 2 to 17). Adult male mice exposed to RMS showed a marked increase on EtOH intake revealed with the two-bottle free choice paradigm, while in females this result is not statistically relevant confirming a RMS-induced gender-dependent effect. Patch-clamp experiments revealed a significant enhancement in the tonic component of GABAergic inhibition recorded in dentate gyrus granule cells from RMS male mice compared with controls, while in females, RMS failed to induce similar changes. RMS is also accompanied with a marked increase in the frequency of GABAergic IPSCs recorded in the same neurons only in male animals compared to controls. Interestingly, all these changes were parallel to an increase of LTD formation in the CA1 subregion of male RMS mice only, an effect paralleled to an impaired performance on the Barnes maze test. In the NAcc, we observed significant RMS-induced changes in synaptic plasticity when studied in GABAergic medium spiny neurons. Interestingly, RMS-induced impairments in synaptic plasticity in both hippocampal formation and NAcc as well as increased EtOH consumption were no more appreciable when RMS male mice that were treated with a single injection of beta-ethinylestradiol at PND3, suggesting that alteration in the hormonal asset strongly influences the action of RMS. Our data strongly support previous findings related to the gender-dependent effect of RMS. Taken together, these data demonstrate that RMS is associated long-lasting effects on synaptic plasticity in both hippocampus and NAcc of C57BL/6J male mice, a brain areas that are crucial in the control of learning and memory as well as EtOH reward effects and goal directed behavior, and may suggest new potential pharmacological strategies to attenuate stress in early life that are predictive for such changes observed in adults male individuals.

Supported by CNR-DISVA-Sardegna Ricerche

INTRAVENOUS SELF-ADMINISTRATION OF THE SYNTHETIC CANNABINOID JWH-018 IN ADOLESCENT MICE

Giulia Margiani, Maria Paola Castelli, Nicholas Pintori, Paola Fadda, Maria Antonietta De Luca

Department of Biomedical Sciences, University of Cagliari, Italy

Cannabis is the most widely consumed illicit drug. However, in the past decade numerous “herbal mixtures”, containing synthetic cannabinoids and broadly known as Spice/K2, have been marketed in an attempt to circumvent legal issues with Cannabis. The synthetic cannabinoid, 1-pentyl-3-(1-naphthoyl)-indole (JWH-018), a potent CB1 and CB2 cannabinoid receptor agonist, is frequently present in Spice/K2.

Adolescents and young people are the major class of population using Spice/K2 and Cannabis. Adolescence is the most critical stage of brain development, which is involved in maturation of cortical and limbic regions through a pruning mechanism. The vulnerability to consume drugs during this period is therefore higher than in adulthood. Whatever stress during adolescence, such as traumatic events and exposure to drugs, could create remarkable damages in adulthood. Moreover, many clinical studies demonstrate that impaired functions in the adolescent brain are related with onset of neuropsychiatric disorders, which appears commonly during adolescence or young adulthood.

In order to investigate on rewarding and abuse properties of JWH-018 during adolescence, we performed intravenous self-administration (IVSA) using CD1 male from postnatal day 30 to 55.

The study aimed at characterized a dose curve for JWH-018 self-administration. The range of doses used was from 2.5 to 15 ug/kg/inf. During the whole adolescence mice consistently acquired IVSA operant behavior. However, significant differences of active lever pressing compared to inactive ones was observed only at the dose of 7.5 ug/kg/inf.

In conclusion, dose response curve of the effect of JWH-018 on operant behavior in adolescent mice had an inverted U-shape trend. This characteristic is congruent with previous studies and is typical for synthetic cannabinoid identified into Spice/K2. Our data also suggest a higher sensitivity of adolescent than adult mice to acquired IVSA behavior by lever pressing. Despite the increasing popularity of Spice/K2 drugs among young people, their long-term effects are unknown. Future studies will elucidate the enduring consequences of adolescent exposure to JWH-018 through the identification of neurotoxicity and neuroinflammation markers. The results obtained will clarify if the exposure to JWH-018 during adolescence induces long-lasting neurodegenerative effects in adulthood.

NOVEL TARGETS AND THERAPEUTICS FOR SOCIAL IMPAIRMENTS

Marta Busnelli^{1,2*}, Francesca Santini², Gunnar Kleinau³, Mariaelvina Sala², Daniela Braida², Markus Muttenthaler⁴, Simona Di Lascio², Peter J McCormick⁵, Maurice Manning⁶, G. Enrico Rovati², Tommaso Bellini², Bice Chini¹

¹CNR Neuroscience Institute, Milan, Italy; ²Università degli Studi di Milano, Italy ³Charité-Universitätsmedizin, Germany; ⁴University of Queensland, Australia, ⁵University of Surrey, UK, ⁶University of Toledo, OH USA

The hypothalamic neuropeptide oxytocin (OXT) regulates a number of socio-emotional behaviors in mice, including parental care, pair-bonding, social memory, and social aggression and it has been recently proposed as treatment for patients affected by neurodevelopmental and psychiatric disorders. OXT activates OXTR receptors (OXTR) that are expressed at the plasma membrane and, in response to OXT, can activate different G-protein subtypes^a. As many other G-protein coupled receptors (GPCRs), OXTR can exist as monomer and dimers in cell systems and in native tissues; i.e. the mammary gland and the brain^{b,c}. To specifically target OXTR dimers we synthesized a series of bivalent ligands, consisting of two identical OXT-like ligands joined by spacers with different lengths^d, and we evaluated their effects on OXTR signaling using a G-protein activation BRET-based assay.

We demonstrated that OXT promoted the direct engagement and activation of Gq and all Gi/o G-protein subtypes, whereas the bivalent ligands behaved as “biased agonists” specifically activating Gq, Gi2 and Gi3. Moreover, we obtained for OXTR/Gi2 and OXTR/Gi3 monophasic concentration-responses curves, whereas for OXTR/Gq we obtained biphasic curves with the two bivalent ligands with spacers C8 and C10. The first part of the curve corresponded to an EC₅₀ that was 1,000 times lower than the EC₅₀ of the second phase of the curve and that was similar to OXT and of the other bivalent ligands EC₅₀s. Using docking-modelling, receptor mutagenesis and synthetic peptides that mimicked transmembrane OXTR helices and interfered with the proper dimer formation, we provided evidence for the presence of a specific OXTR dimeric receptor arrangement having a TM1-TM2 interface in which only the two bivalents, with defined spacer length (~25 Å), fit within the channel-like structure that connects the two protomers of the dimer. We then tested the bivalent ligand with C8 spacer *in vivo* and we observed that it promotes social behavior of mice and zebrafish with a 100- and 40-fold gain in potency as compared to oxytocin and isotocin, the oxytocin-analog in fish, control groups^d.

Our results demonstrated that OXTRs dimers are expressed in a high affinity state and for the first time, we were able to demonstrate that a GPCR dimer possess a different coupling respect to a monomer. Moreover, *in vivo* studies indicated that OXTRs are expressed as dimers in the CNS and contribute to determine the central effects of OXT.

In general, our studies demonstrated that bivalent ligands are very promising as new tool to activate OXTR dimers and their “biased signalling” may have important implications for specific behaviours and may contribute to the discovery of compounds with unique pharmacological properties and with better therapeutic profiles.

References

- ^a Busnelli, M. et al. 2012 Functional selective oxytocin-derived agonists discriminate between individual G protein family subtypes. *J Biol Chem*.
- ^b Albizu, L. et al. 2010 Time-resolved FRET between GPCR ligands reveals oligomers in native tissues. *Nat. Chem Biol*.
- ^c Romero-Fernandez W. 2013 Evidence for the existence of dopamine D2-oxytocin receptor heteromers in the ventral and dorsal striatum with facilitatory receptor-receptor interactions. *Mol Psychiatry*
- ^d Busnelli M et al. 2016 Design and characterization of superpotent bivalent ligands targeting oxytocin receptor dimers via a channel-like structure. *J. Med. Chem*.

*M. Busnelli is the recipient of a Fondazione Umberto Veronesi Post-doctoral Fellowship and is funded by a NARSAD Young Investigator Grant

CHRONIC STRESS EXPERIENCED BY PARENTS DURING ADOLESCENCE CAN INCREASE RISK FOR AUTISM SPECTRUM DISORDER IN OFFSPRING: A PROPOSED ANIMAL MODEL

Maria Giuseppina Pisu¹, Giorgia Boero², Francesca Biggio², Mariangela Serra²

¹*CNR Neuroscience Institute, Cagliari, Italy;* ²*Department of Life and Environment Sciences, Section of Neuroscience, University of Cagliari, Cagliari, Italy*

Autism spectrum disorder (ASD) describes a group of developmental disorders that affects social interaction, communication, interests and behavior. The ASD is the most serious neurological childhood disease, affecting 1 child in every 100, mainly males than females (4:1) (Fombonne, 2003) and occurs usually within the first three years of life. Due to the different symptoms that make up the ASD it is thought that multiple causes can play a crucial role in etiopathology of these disorders, as genetic susceptibility and the interactions between genetic and environmental factors (Bolkan and Gordon, 2016).

Adverse life experiences during critical periods for central nervous system development may cause epigenetic changes that alter hormones pattern and receptors expression that impact brain circuits.

These modifications have been associated with increased risk for autism in offspring; for instance, women exposed to childhood abuse experience were more likely to have sons with autism spectrum disorder (ASD) than women unexposed (Roberts et al., 2013).

In this study we tested if the exposure parents during adolescence to a chronic mild stress (social isolation for 30 days from weaning) could reproduce some ASD symptoms in the subsequent generation. Offspring of socially isolated rats showed a decrease in plasma oxytocin levels, a peptide that plays a crucial role in social behavior that has been shown to be altered in ASD patients. In agreement, in the resident-intruder test these rats spent more time in cage exploration and self-grooming activity rather than interacting with the intruder animal. Moreover, offspring of socially isolated parents showed behavioral inflexibility in a rodent cognitive task. Conversely, learning and spatial memory were not affected; in agreement, hippocampal Brain Derived Neurotrophic Factor (BDNF) levels were increased in offspring of socially isolated rats.

Furthermore, these animals showed an increase of plasma corticosterone and ACTH levels, suggesting an increase of hypothalamic-pituitary-adrenal (HPA) axis basal activity; nevertheless, this increase was not related to an anxiety-like behavior in anxiometric animal tests. Social deficit, behavioral perseveration, HPA hyperactivity, low oxytocin and high BDNF levels are peculiar characteristics of ASD patients and some established genetic animal models. Thus, offspring of socially isolated animals meets the attribute of face validity for ASD animal model. The next step of this research is to provide a comprehensive assessment of epigenetic alterations in selective genes related with these systems in parents and to test if they are heritable in the form of genomic imprinting. The advantage of this animal model could be to allow studies of molecular mechanisms underlying ASD, without genetic modifications that could trigger cell adaptation mechanisms and lead to changes in other brain circuits, not involved in the pathology.

References

- Bolkan S and Gordon JA. Neuroscience: Untangling autism. *Nature* 352: 45-46, 2016
- Fombonne E. Epidemiological surveys of autism and other pervasive developmental disorders: an update. *J Autism Dev. Disord* 33: 365-382, 2003
- Roberts AL et al. Association of maternal exposure to childhood abuse with elevated risk for autism in offspring. *JAMA Psychiatry* 2013

CHRONIC TREATMENT WITH ETHINYL ESTRADIOL AND LEVONORGESTEL BLUNTS THE RESPONSE TO STRESS IN FEMALE RATS

Patrizia Porcu¹, Alessandra Concas^{1,2}

¹CNR Neuroscience Institute, Cagliari, Italy; ²Department of Life and Environment Sciences, Section of Neuroscience and Anthropology, University of Cagliari, Cagliari, Italy

Endogenous gonadal hormones regulate brain development as well as neuronal plasticity and excitability, thus affecting mood, emotional disorders or cognition. By contrast, effects of exogenous synthetic steroids, such as those used in hormonal contraceptives (HC) formulations, on brain function and plasticity are poorly investigated. 10-40% of HC users report mood changes, with increased rates of depression and anxiety, that are among the leading causes for HC discontinuation. HC decrease brain allopregnanolone content in rats and prevent the rise in allopregnanolone levels during the luteal phase of the menstrual cycle in women. HC also increase basal cortisol levels and blunt the hypothalamic-pituitary-adrenal (HPA) axis response to stress, a condition similar to that found in depressed patients. However, the effects of HC on HPA axis function at the central nervous system level are still unknown. We investigated if long-term treatment with HC alters HPA axis function in female rats, a factor that may increase vulnerability to development of mood disorders.

Adult female rats were orally treated with a combination of ethinyl estradiol (EE, 0.020 mg) and levonorgestrel (LNG, 0.060 mg) once a day for 4 weeks. One group of rats was subjected to the elevated plus maze test at the beginning of the 4th week of treatment to assess anxiety-like behavior; treatment resumed for few days and rats were sacrificed 24 hours after the last administration to assess plasma allopregnanolone levels. We confirmed that this EE-LNG treatment regimen induced anxiety-like behavior (time in open arms, -72% $p < 0.005$; open arms entries, -66% $p < 0.001$) and decreased allopregnanolone concentrations (-43%, $p < 0.01$), similar to effects previously observed using higher doses (EE, 0.030 mg; LNG, 0.125 mg). A second group of EE-LNG treated rats was subjected to 30 min acute restraint stress, 24 hours after the last administration, and sacrificed thereafter to assess HPA axis function. EE-LNG treatment increased basal plasma corticosterone levels (+43%, $p < 0.05$), and blunted the corticosterone response to acute restraint stress. In fact, corticosterone levels following 30 min restraint stress increased by 866% in vehicle-treated rats ($p < 0.0001$), but only by 410% in EE-LNG-treated rats ($p < 0.0001$), resulting a 53% decrease in such response, compared to vehicle-treated rats ($p < 0.01$). EE-LNG treatment also increased the abundance of mineralocorticoid receptors in the hippocampal lysate (+54%, $p < 0.05$), without altering that of glucocorticoid receptors. Overall, these results suggest that long-term treatment with EE-LNG alters stress sensitivity and induces adaptations in HPA axis function. Altered stress sensitivity leads to behavioral and neurobiological adaptations that increase susceptibility to mental illness, including depression and anxiety disorders. Thus, these results might be relevant to understand the etiology of mood changes, which are the leading cause for HC discontinuation in women.

MOUSE MODEL OF POLYMICROGYRIA: PHARMACOLOGICAL AND CELLULAR THERAPEUTIC APPROACHES

Silvia Ferrara¹, Giuliana Fossati², Cristina Zalfa³, Flavia Antonucci¹, Luisa Ponzoni¹, Mariaelvina Sala^{1,4}, Angelo L. Vescovi³, Michela Matteoli^{2,4}, Elisabetta Menna^{2,4}

¹Dept. of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy; ²Humanitas Clinical and Research Center; Milan, Italy; ³Biotechnology and Bioscience Department, Bicocca University, Milan, Italy; ⁴CNR Neuroscience Institute, Milan, Italy

Polymicrogyria (PMG) is a condition characterized by abnormal prenatal brain development and excessive number of ectopic small gyri in the cerebral cortex. PMG patients present an excessive number of abnormally small gyri separated by shallow sulci, associated with fusion of the overlying molecular layer of the cerebral cortex. The topographic distribution of PMG may be focal, multifocal or diffuse; unilateral or bilateral; symmetric or asymmetric. Clinical manifestations have a large spectrum, ranging from isolated selective impairment of cognitive function to severe encephalopathy and intractable epilepsy. The severity of neurological manifestations and the age at presentation are in part influenced by the extent and localization of the cortical malformations but may also depend on its specific aetiology. The pathogenesis is still poorly understood, several causative gene mutations have been recently found, but also other causes have been identified (prenatal infections, ipoxia).

Experimentally, the pathophysiological aspects of PMG can be reproduced by the focal freeze-lesion (FFL) model in neonatal rodents, resulting in the formation of microgyri in the mouse cortex. Previous studies in the FFL model showed enhanced excitatory and inhibitory synaptic transmission accompanied by increased connectivity in the paramicrogyral cortex and higher susceptibility to epilepsy.

Biochemical, morphological and behavioural analysis of the PMG model revealed, besides the alteration in the cortical laminar structure, a significant astrogliosis and microglial activation, indicating the occurrence of an inflammatory process. In addition, a diffuse cortical hypomyelination is evident in brain slices stained for myelin basic protein (MBP). Furthermore, PMG mice displayed altered EEG profile and defective motor skills such as reduced brawn.

Transplantation of human CNS neural stem cells, which has been demonstrated to exert positive effects on inherited or acquired myelinating disorders and to dampen brain inflammation, play a beneficial effect in the pathological condition of PMG ameliorating the myelination defect by promoting oligodendrocyte precursors proliferation and remodelling of myelin fibers. Our data also show that hNSC transplantation restores normal EEG brain activity and improves motor performances. Pharmacological inhibition of IL-1R pathway by the IL-1R antagonist anakinra, leads to a significant improvement of EEG and motor skills in adult PMG mice thus suggesting a possible role of inflammation at the root of the pathology and identifying a therapeutic time window for the treatment.

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

Annamaria Lia¹, Micaela Zonta^{1,2}, Angela Chiavegato¹, Gabriele Losi^{1,2}, Cristina Fasolato¹ and Giorgio Carmignoto^{1,2}

¹*Department of Biomedical Sciences, University of Padova, Padova, Italy;*

²*CNR Institute of Neuroscience, Padova, Italy*

Alzheimer's disease (AD) is a chronic incurable neurodegenerative disease, characterized by severe and progressive memory loss and cognitive dysfunctions. While numerous experimental studies in AD mouse models revealed remarkable dysfunctions at the level of both single neuron and neuronal network, very little is known about possible dysfunctions in astrocytes.

These glial cells are largely recognized to play fundamental roles in brain function, from the control of tissue homeostasis to the modulation of neurovascular coupling and synaptic transmission. This plethora of astrocytic roles raises the possibility that neurodegenerative diseases depend on both neuronal and astrocytic dysfunctions rather than on neuronal dysfunctions alone.

Instrumental to the study of astrocyte roles in AD are two mouse lines based on a mutant presenilin-2 (PS2-N141I) linked to familial Alzheimer's disease (FAD), i.e., a single homozygous mouse line expressing the PS2-N141I mutant (PS2.30H), under the prion protein promoter, and a double homozygous mouse line expressing also the amyloid precursor protein Swedish mutant (PS2APP, B6.152H), under the thy-1 promoter.

In our study, we addressed the hypothesis that astrocytic Ca^{2+} alterations occur at early disease stages by performing experiments before and after the development of plaque deposition in the PS2APP model, at the age of 3 and 6 months, respectively.

To investigate Ca^{2+} signal dynamics in astrocytes in transgenic mice, we performed *in situ* two-photon imaging experiments in Somatosensory Cortex (SSCx) astrocytes expressing the genetically encoded Ca^{2+} indicator GCaMP6f. This approach allowed us to analyze with unprecedented definition the spatio-temporal properties of Ca^{2+} signals at the different astrocytic compartments including the fine processes.

We found that at 3 months of age, at fine astrocytic processes, activity in Ca^{2+} microdomains was significantly increased in both AD models. In contrast, this astrocyte activity was drastically reduced in 6-month-old PS2APP mice with respect to WT (C57BL/6J).

In conclusion, our preliminary findings reveal that Ca^{2+} microdomains in astrocytes from the SSCx of PS2-based AD mouse models change their basal activity along with the progression of the disease. The aims of our future experiments are to understand the molecular mechanisms at the basis of these Ca^{2+} signal alterations in astrocytes and to validate our observations in the living intact brain.

PISA SYNDROME IN A DRUG-NAIVE PARKINSON'S DISEASE PATIENT

G. Orofino¹, P. Solla¹, A. Cannas², L. Polizzi², M. Meloni¹, F. Marrosu¹

¹PhD Program in Neuroscience, Dept. of Public Health, Section of Neurology, University of Cagliari, Italy;

²AOU Cagliari, Neurological Clinic, Monserrato (CA), Italy

Background:

The term Pisa Syndrome (PS) is used to indicate a tonic lateral flexion of the trunk often associated with slight spinal rotation along the sagittal plane, described in patients on neuroleptic drugs and in idiopathic Parkinson's disease (PD) patients on dopaminergic drugs, but never been described in drug-naïve PD patients as a presenting symptom. We report on a patient who presented a reversible PS without any dopaminergic treatment at onset and before PD diagnosis.

Aims:

Demonstrate that Pisa Syndrome may be present at the onset of Parkinson Disease and not only in the complicated and overtreated disease forms.

Methods:

A 81-year-old woman presented with a 1-year clinical history of PD and no history of trunk lateral flexion. At the neurological examination, she presented a moderate lateral flexion to the right side (Figure 1a) alleviated completely by passive mobilization or on lying supine, compatible with a diagnosis of PS. Brain MRI was normal, while [¹²³I]FP-CIT-SPECT showed a marked reduction of tracer uptake of the right side of putamen (Fig. 2).

She was then treated with levodopa/benserazide with beneficial effects on motor rigidity and tremor, and satisfactory improvement of trunk flexion (Fig. 1b).



Results:

The diagnosis of PS requires a pronounced (at least 10°) lateral flexion, which can be alleviated by passive mobilization or on lying supine.

The onset of PS in PD patients has been described following a change in dopaminergic treatment, on the contrary, the introduction of central dopamine receptor blockers. In most of these cases, PS often represents a motor complication of advanced forms of disease.

Conclusions:

This report shows that PS may be present at the onset of PD and in addition to cardinal motor symptoms. In this case, the response to dopaminergic treatment and the asymmetry showed in the brain [¹²³I]FP-CIT-SPECT, omolateral to the direction of truncal deviation, strongly suggests a mechanism of dopaminergic unbalance as a probable key factor in the development of this postural disturbance.

TRANSCRIPTIONAL EFFECT OF THE ACETYLCHOLINESTERASE DONEPEZIL ON THE HUMAN-RESTRICTED CHRFAM7A GENE IN IMMUNE AND NEURONAL CELL MODELS: IMPLICATIONS IN THE REGULATION OF THE A7 nAChR ACTIVITY

Roberta Benfante^{1,2}, Simona Di Lascio², Annalisa Maroli², Silvia Cardani², Massimo Locati², Lorenzo Drufuca², Diego Fornasari^{1,2}

¹CNR Neuroscience Institute, Milan, Italy; ²Dept. of Medical Biotechnology and Translational Medicine (BIOMETRA), University of Milano, Milano, Italy

In the last years, increasing evidence has linked several neurodegenerative and psychiatric disorders to inflammation. Controlling neuro-inflammation has indeed become a promising approach to treat neurodegenerative diseases. The Central Nervous System exerts a control on innate immunity through the “Cholinergic Anti-Inflammatory Pathway”, in which the splenic terminals of the Vagus nerve induce an anti-inflammatory response by stimulating the release of acetylcholine (ACh). The ACh interacts with the $\alpha 7$ Nicotine Receptor (CHRNA7) expressed by the macrophages, thus inducing down-regulation of pro-inflammatory cytokines.

Recent studies indicated that AChE inhibitors, widely used for the symptomatic treatment of Alzheimer's disease and other dementias, can cause a significant modulation of innate immunity as a side effect.

In human, besides of CHRNA7, an isoform of the $\alpha 7$ nicotinic receptor can be transcribed by the CHRFAM7A gene. This gene is a hybrid product of a partial duplication and fusion of exons 5-10 of CHRNA7 gene with the novel FAM7A gene and maps 1.6 Mb apart from CHRNA7 in inverted orientation. The CHRFAM7A gene transcript undergoes alternative splicing, giving rise to two proteins of 46 and 35 KDa; the former differs from the $\alpha 7$ conventional subunit for the N-terminus domain, whereas the latter is a truncated form of the $\alpha 7$ subunit since it shares with CHRNA7 the four transmembrane domains, the cytoplasmic loop and the C-ter domain. These proteins seem to have a dominant negative effect on the conventional $\alpha 7$ function. Moreover, we have previously shown that CHRFAM7A is down-regulated upon LPS treatment in THP-1 cell model and primary monocytes and macrophages by a transcriptional mechanism reliant on NF- κ B (Benfante et al., 2011).

In this work we explored the link between the “Cholinergic Anti-Inflammatory Pathway” and the AChEI Donepezil, by focusing on the regulation of CHRFAM7A and CHRNA7 expression in neuronal and immune cell models, to better understand their role in peripheral and central inflammation, and define a human restricted mechanism modulating the inflammatory response in neurodegenerative diseases.

Reference

Benfante et al. (2011) *J Neuroimm.* 230 (1-2), 74-84

INSULIN CLEARANCE IS INDEPENDENTLY MODULATED BY INSULIN SENSITIVITY, BMI AND GLUCOSE TOLERANCE

Roberto Bizzotto¹, Andrea Natali², Amalia Gastaldelli³, Ralph A. De Fronzo⁴, Silva Arslanian⁵, Ele Ferrannini³, Andrea Mari¹

¹CNR Neuroscience Institute, Padova, Italy; ²Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy; ³CNR Institute of Clinical Physiology, Pisa, Italy; ⁴Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, USA; ⁵Division of Weight Management, Children's Hospital of Pittsburgh, University of Pittsburgh Medical Center, and Division of Pediatric Endocrinology, Diabetes and Metabolism, Children's Hospital of Pittsburgh, Pittsburgh, PA, USA

The study of the determinants of whole-body endogenous insulin clearance (EIC) is complicated by saturation mechanisms, as elevated insulin secretion rates (ISR) may indirectly modulate EIC through the attendant hyperinsulinemia. We developed a physiologically-based mathematical model of insulin kinetics in humans that describes EIC over a wide range of insulin concentrations produced by glucose-stimulated ISR and exogenous insulin administration. We used in vivo data from: A) tests stimulating ISR in different patterns and levels of glucose infusion (6 different studies, N = 346); B) one- or two-step hyperinsulinaemic euglycaemic clamps with different insulin infusion rates (3 studies, N = 1473). Subjects were non-diabetic (ND, N = 1519) or had type 2 diabetes (T2D, N = 300). Insulin kinetics were described by a circulatory model in which fractional insulin extraction is saturable in the liver and constant in extra-hepatic organs. ISR was estimated from C-peptide, model parameters were estimated using a population (a.k.a. mixed-effect) approach: inter-individual distributions were assessed for all parameters and then used to identify the individual values, even in tests with sparse data. The model calculated EIC at standardised, constant ISRs. In a subset of subjects receiving clamp studies (N = 1425), insulin sensitivity was measured as the M/I index (at 240 pmol·min⁻¹·m⁻² insulin infusion rate). In all studies, the model predicted insulin concentration adequately, with homogeneous parameters. The mean EIC computed at ISR of 100 and 400 pmol·min⁻¹·m⁻² (representing basal and glucose-stimulated ISR, respectively) was 0.98 and 0.73 L·min⁻¹·m⁻². In the subjects receiving clamp studies, EIC standardised at an ISR of 100 pmol·min⁻¹·m⁻² (EIC100) was positively correlated with M/I and negatively with BMI in univariate regression. In a multiple regression model adjusting for glucose tolerance and type of test, M/I and BMI were both independent *positive* predictors (adjusted $r^2 = 0.24$, $p < 0.0001$), while T2D had higher EIC compared to ND ($p < 0.0001$). Values of EIC at an ISR of 400 pmol·min⁻¹·m⁻² showed a similar pattern of relationships with M/I and BMI. Increasing ISR from 60 to 100 pmol·min⁻¹·m⁻² (representing basal ISR in insulin-sensitive and insulin-resistant subjects, respectively) predicted an EIC reduction of 4% due to saturation and a reduction of 28% due to insulin resistance. Thus, the role of EIC saturation is minimal under basal ISR conditions and is quantitatively more relevant under conditions of glucose-stimulated hyperinsulinaemia. The direct relationship of EIC with insulin sensitivity is primary and not an indirect effect of saturation of insulin degradation. Insulin resistance is the most relevant determinant of EIC.

BENEFICIAL AND DETRIMENTAL CONSEQUENCES OF A PRIMARY INCREASE IN MITOCHONDRIAL ROS FORMATION ON MITOCHONDRIAL FUNCTION, Ca^{2+} HOMEOSTASIS AND CARDIOMYOCYTE VIABILITY

Salvatore Antonucci¹, Roberta Menabò², Moises Di Sante^{1,2}, Soni Deshwal¹, Richard C. Hartley³, Thomas Krieg⁴, Michael P. Murphy⁵, Fabio Di Lisa^{1,2}

¹Department of Biomedical Sciences, University of Padova, Italy; ²CNR Neuroscience Institute, Padova, Italy;

³Centre for the Chemical Research of Ageing, WestCHEM School of Chemistry, University of Glasgow, Glasgow, UK; ⁴Department of Medicine, University of Cambridge, Cambridge, UK ; ⁵Mitochondrial Biology Unit, MRC, Cambridge, UK

Background and aim. Since changes in intracellular $[\text{Ca}^{2+}]$ and reactive oxygen species are generally intertwined, it is difficult to establish causal relationships. Here, we aimed at elucidating the consequences of a primary increase in mitochondrial ROS formation induced by a paraquat analogue targeted to mitochondria (MitoPQ). This compound, that elicits mitochondrial ROS formation by means of redox cycling, was investigated in neonatal ventricular cardiac myocytes (NRVMs).

Results.

- (i) High doses (≥ 500 nM) induce an increase in ROS levels ($p < 0.001$) and a decrease of mitochondrial membrane potential (mmp) ($p < 0.001$) followed by cell death ($p < 0.001$);
- (ii) Moderate doses (between 50 nM and 100 nM) induce an increase in H_2O_2 levels ($p < 0.01$), still resulting in a decrease of mmp ($p < 0.01$). Both frequency and amplitude of Ca^{2+} transients are decreased. Notably, cell viability is not affected and cells become unexcitable after caffeine eaddition Ca^{2+} amplitude is increased at low doses of this range (50 nM) and widely decreased at higher doses (100 nM).;
- (iii) Low doses (10 nM) induce a mild accumulation of H_2O_2 ($p < 0.01$) without affecting mmp. Ca^{2+} amplitude is increased ($p < 0.01$) while frequency is not modified. Importantly, not only cell viability is not affected, but also 2 hour pretreatment with this low dose decreases the susceptibility to anoxia/reoxygenation injury ($p < 0.001$).

Conclusions. Cardioprotection, derangements in Ca^{2+} homeostasis and loss of cell viability are caused by low, moderate and high doses of MitoPQ, respectively.

MAO-DEPENDENT ER-MITOCHONDRIA DYSFUNCTION AND MAST CELL DEGRANULATION LEAD TO ADVERSE CARDIAC REMODELING IN DIABETES

Soni Deshwal¹, Marleen Forkink², Chou-Hui Hu², Guido Buonincontri², Salvatore Antonucci¹, Moises Di Sante³, Roberta Menabò³, Michael P Murphy⁴, Nazareno Paolocci^{5,6}, Daria Mochly-Rosen⁷, Thomas Krieg², Nina Kaludercic³, Fabio Di Lisa^{1,3}

¹Department of Biomedical Sciences, University of Padova, Padova, Italy; ²Department of Medicine, University of Cambridge, Cambridge, UK; ³CNR Neuroscience Institute, Padova, Italy; ⁴Mitochondrial Biology Unit, MRC, Cambridge, UK; ⁵Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, USA; ⁶Department of Experimental Medicine, University of Perugia, Perugia, Italy; ⁷Department of Chemical and Systems Biology, Stanford University, School of Medicine, Stanford, USA

Cardiovascular complications are the major cause of mortality among diabetic patients. Reactive oxygen species (ROS), inflammation, mitochondrial dysfunction and endoplasmic reticulum (ER) stress contribute to the structural and functional alterations in the diabetic heart. Although numerous reports hint towards a cross-talk occurring between these factors, the precise interplay remains unknown. Here we investigated whether monoamine oxidases (MAO), mitochondrial flavoenzymes capable of generating H₂O₂, might represent the missing link. Using both genetic and pharmacological approaches we show that exposure of primary cardiomyocytes to high glucose and pro-inflammatory stimuli leads to MAO-dependent increase in ROS formation (≥ 1.5 -fold vs control, $p \leq 0.05$). This is accompanied by reduced mitochondrial membrane potential and perturbed ER homeostasis, the latter evidenced by the upregulation of ER stress markers ATF4, GRP78 and GADD34 (≥ 2 -fold vs control, $p \leq 0.05$). Remarkably, MAO inhibition prevented mitochondrial dysfunction and ER stress, highlighting the novel role of these flavoenzymes in the cross-talk between mitochondria and ER in diabetes. *In vivo*, administration of the MAO inhibitor pargyline to streptozotocin (STZ)-induced type 1 diabetic mice abolished oxidative stress (4-hydroxynonenal), reduced the upregulation of ER stress markers ATF4 and GADD34, and prevented LV diastolic dysfunction (2-fold decrease in diastolic stiffness vs STZ mice, $p \leq 0.05$). Furthermore, we report that MAO inhibition reduced cardiac mast cell degranulation in STZ-mice, likely accounting for the decreased fibrosis observed in STZ-mice following MAO inhibition. Taken together, these results highlight the critical role of MAO in diabetic cardiomyopathy and provide novel insights into the mechanisms underlying MAO-induced changes in the diabetic heart.

THE ROLE OF MONOAMINE OXIDASES IN DIFFERENTIATION AND MATURATION OF iPSC-DERIVED CARDIOMYOCYTES

Moises Di Sante¹, Soni Deshwal², Salvatore Antonucci², Carmen Troiano², Elisa Greotti^{1,2}, Diana Pendin^{1,2}, Fabio Di Lisa^{1,2}, Nina Kaludercic¹

Monoamine oxidases (MAOs) are mitochondrial flavoenzymes that exist in two isoforms, MAO-A and -B, and are responsible for neurotransmitter and biogenic amines catabolism. In pathological conditions MAO activity contributes to cardiac damage, mainly due to excessive formation of H₂O₂ and aldehydes. Yet, the role and contribution of MAOs during early development and cardiomyogenesis has not been investigated and remains elusive. Therefore, we established a human model based on cardiomyocytes derived from human induced pluripotent stem cells (iPSCs) in which MAO expression was genetically manipulated. During cardiomyocyte differentiation, mRNA and protein levels of both isoforms increased progressively. MAO-A appeared to be the most abundant isoform, suggesting that the presence of this protein is essential in the early stages. In order to assess whether MAOs might affect cardiomyocyte differentiation and function, iPSCs were treated with siRNA against either MAO-A or MAO-B. Genetic inhibition of either MAO isoform expression resulted in lower frequency and amplitude of calcium transients in iPSC-derived cardiomyocytes. Moreover, only cells treated with siRNA against MAO-A displayed dyssynchronous beating and loss of the regular striated staining pattern of α -actinin. Taken together, these results highlight the physiological role of MAOs in human cardiomyogenesis. Further studies are necessary to clarify whether derangements in calcium homeostasis, rhythm and ultrastructure resulting from MAO-A ablation in iPSC-derived cardiomyocytes are due to the accumulation of MAO substrates or whether products of MAO activity, i.e. H₂O₂ and aldehydes, may play a role in the signalling cascade involved in cardiomyogenesis.

MITOCHONDRIAL cAMP SIGNALLING IS INVOLVED IN METABOLIC FLEXIBILITY

Giulietta Di Benedetto^{1,2} and Tullio Pozzan^{1,2,3}

¹CNR Neuroscience Institute, Padova, Italy; ²VIMM (Venetian Institute of Molecular Medicine), Padova, Italy;

³Department of Biomedical Sciences, University of Padova, Italy

Cytosolic cAMP cannot reach the mitochondrial matrix but is synthesized *in situ* by a mitochondrial isoform of the soluble adenylylate cyclase (sAC). The presence of an autonomous cAMP signalling cascade is accepted in the matrix, although the molecular players of this pathway and their precise sub-mitochondrial localization are not yet unequivocally defined. To solve this issue we are currently setting up a new split-green fluorescent protein (GFP)-based tool, which will help to uncover the localization, and its possible regulation, of key molecules participating in the intra-mitochondrial cAMP (mt-cAMP) signalling pathway. Moreover, the function and local targets of mt-cAMP remain largely unknown. We developed a FRET-based biosensor to measure mt-cAMP dynamics within mitochondria of living cells, showing that mt-cAMP synthesis through the sAC activity is stimulated by raises in matrix $[Ca^{2+}]$. In addition we found that increases in mt-cAMP result in increased efficiency of mitochondrial ATP synthesis. We concluded that OXPHOS regulation, which modulates ATP generation in response to nutrient availability and cellular demand, is at least partially achieved by mitochondrial cAMP. These findings opened a number of questions regarding the identity of the cAMP effectors, targets and regulator proteins within the mitochondrial matrix, as well as the existence of additional cellular functions regulated by mt-cAMP.

A working hypothesis is the involvement of mt-cAMP in the ability of living cells to adapt fuel preference to changes in nutrient availability termed as *metabolic flexibility* (MF). MF is a hallmark of healthy metabolism, and mitochondria are emerging as determinant in its regulation; indeed, MF involves the control of mitochondrial biogenesis and activity, which results in the fine-tuning of oxidative metabolism. The transcriptional regulatory networks governing mitochondrial biogenesis, as well as proteins involved in MF and the anabolism/catabolism shift, are highly conserved. Interestingly, cAMP is involved in both bacteria and eukaryotic cells in the regulation of diverse processes, including the response to fasting (e.g.: bacterial catabolite repression and biofilm formation; mammalian glycogen degradation; mitochondrial network re-organization). We are currently testing the hypothesis of the existence of a feedback loop where nutrient availability could regulate mt-cAMP level, which, in turn, would participate in the processes underpinning metabolic flexibility through its action on mitochondrial metabolism. Our preliminary data indicate that sugars, α -ketoacids, aminoacids and lipids modulate cAMP in the mitochondrial matrix.

ENDOPLASMIC RETICULUM Ca²⁺ HANDLING PROTEINS IN ZEBRAFISH BRAIN: A FOCUS ON CALSEQUESTRIN

Sandra Furlan¹, Simone Mosole², Marina Campione¹, Marta Murgia², Pompeo Volpe², Alessandra Nori²

¹CNR Neuroscience Institute, Padova, Italy; ²Dipartimento di Scienze Biomediche, University of Padova, Padova, Italy

Teleost fish display undetermined brain growth throughout life. This makes teleost fish an exciting model to study neuronal heterogeneity and to identify specific stem and progenitor cell markers. In particular, cerebellum has a well-known architecture, contains relatively few cell types with distinct morphological, molecular and physiological characteristics. Calsequestrins (Casq) are Ca²⁺-binding proteins involved in numerous cellular processes. Despite an exhaustive documentation of their roles in the muscle physiopathology, less is known about expression in other tissues. Here, we use the well-established model organism zebrafish (*Danio rerio*) to unravel the expression of Casq, with special focus on the adult brain, by qPCR, ISH, proteomic analysis and immunofluorescence. By qPCR we found three RNA transcripts coding for Casq1a, Casq1b and Casq2. In brain total homogenate, two out of three isoforms were detected at protein level, by western blotting and mass spectrometry. Whole brain fractionation experiments revealed that Casq2 isoform was enriched in a particulate fraction containing microsomes and synaptic vesicles, together with post-synaptic density proteins ITPR1 (Ip3-Receptor 1), SERCA2, Homer1b, cortactin, mGluR1, PSD95; Casq1a isoform was present in the same fraction but was also partially recovered in a cytosolic fraction, as the luminal ER proteins ERP44 and calreticulin. By immunofluorescence we found that Casq1a and Casq2 were both localized in the cerebellum area, with enrichment in Purkinje neurons for Casq2, and in granule cells for Casq1a. For comparison, we labelled the sagittal sections of cerebellum area with anti-IP3 receptor, SERCA2, STIM1, calreticulin and parvalbumin. We found a partial co-localization of Casq2 with ITPR1 and STIM1, whereas Casq1a co-localized with SERCA2 and calreticulin in granule cells layer. Our results suggest that different Casq isoforms contribute to the molecular heterogeneity of cerebellum Ca²⁺ stores of adult zebrafish.

IN VITRO CHARACTERIZATION OF miRNA-MEDIATED CONTROL OF Satb2 EXPRESSION DURING CORTICOGENESIS

Manuella Martins^{1,2}, Marco Terrigno^{1,2}, Federico Cremisi^{1,2}

¹*Scuola Normale Superiore di Pisa*; ²*Istituto di Biofisica del CNR, Pisa*

Although the key genes responsible for the generation of the different pyramidal neurons composing the six cortical layers are known, the mechanisms accounting for their expression in specific progenitor cells and at different times of cortical neurogenesis is not completely understood. *Satb2* is a crucial transcription factor of cortical layering, as its activation specifies upper neuron identity, inhibits the transcription of the deep neuron identity *Ctip2* gene and establishes the end of corticogenesis. The onset of *Satb2* protein detection (E15.5) is two days later than the onset of its mRNA detection (E13.5), suggesting that post-transcriptional mechanisms could account for the time of *Satb2* activation.

We assayed the involvement of miRNA-mediated RNA silencing of *Satb2* in an in vitro system of corticogenesis, using neuralized mouse ESCs. Si-RNA-mediated DICER knock-down at early stages of in vitro ESC neuralization (at day in vitro 5-6, DIV5-6), but not at later stages (DIV7-7), induced significant increase of *Satb2* translation in post-mitotic cells at DIV 14. Moreover, *Satb2* 3'UTR inhibited GFP translation from a reporter vector when it was transfected at early (DIV12) but not at late (DIV 17) stages of in vitro corticogenesis. We looked for candidate miRNAs that could be involved in targeting *Satb2*. Among the 100 most expressed miRNAs in cortical progenitor cells sorted at DIV 10, 12, 16, 20 or 26, 13 and 12 showed decreasing and increasing expression over the in vitro process of corticogenesis, respectively. While 9 out of the 13 decreasing are predicted to bind to *Satb2* 3'UTR, none of the 12 increasing show any predicted binding site. Functional experiments of validation of the 9 miRNAs expected to inhibit *Satb2* translation are in progress. Our observations suggest that the activation of *Satb2*, and thus the control of the completion of the corticogenetic process, might considerably be miRNA-dependent.

UNVEILING THE MECHANISMS UNDERLYING OLIGODENDROCYTE DIFFERENTIATION: IMPLICATIONS FOR DOWN SYNDROME

Alessandro Ulivi¹, Veronica Meraviglia¹, Nicol Colombo¹, Maria Pia Abbracchio², Renata Bartesaghi³, Stefania Ceruti², Ilaria Prada¹, Meritxel Pons Espinal⁴, Davide De Pietri Tonelli⁴, Roberta Benfante¹, Patrizia Rosa¹

¹CNR Neuroscience Institute, Milano, Italy; ²University of Milano, Italy; ³University of Bologna, Italy; ⁴IIT, Genova, Italy

Oligodendrocytes have attracted growing interest because of evidence showing that many brain disorders are associated with defective myelination, from “classic” demyelinating diseases such as multiple sclerosis, to stroke, schizophrenia, depression, Down syndrome (DS) and autism. Oligodendrocyte progenitor cells post-natally differentiate through various pre-myelinating stages before becoming mature oligodendrocytes, a process that involves the complex interplay of a number of different intrinsic and extrinsic factors.

Among these, it is known that G protein-coupled receptor 17 (GPR17) acts as an intrinsic timer of oligodendrocyte differentiation by keeping pre-myelinating oligodendrocytes in an immature state until its down-regulation allows full oligodendrocyte maturation. The mechanisms controlling GPR17 expression/stability are only partially known, and one aim of our work is to clarify the mechanisms underlying the tight regulation of GPR17 during oligodendrocyte maturation. We have recently concentrated on receptor endocytic trafficking and demonstrated that GPR17 can be sorted to lysosomes or recycled to the plasma membrane via a Rab4-dependent pathway in differentiating cells. As it can be expected that the cell surface levels of GPR17 are modulated by the balance between degradation and recycling, and that this will affect receptor signalling and, consequently oligodendrocyte differentiation, we further characterised the mechanisms of GPR17 sorting in the endosomal pathway by focusing on the role of its C-terminal PDZ binding motif. Our findings demonstrated: i) that the PDZ binding motif is required for GPR17 recycling to the cell surface by means of interactions with the retromer complex-associated protein SNX27; and ii) that SNX27 knockdown accelerates receptor sorting into lysosomes. This rapid GPR17 degradation led to the early expression of myelin proteins and accelerated the kinetics of oligodendrocyte differentiation *in vitro*. Given that recent data have shown that SNX27 is indirectly down-regulated by the over-expression of miR-155 in DS brains, we analysed the effects of miR-155 over-expression in oligodendroglia cells and found that increased levels of this trisomic microRNA inhibited GPR17 expression as well as cell differentiation. These results correlate with data showing altered myelination and a decreased number of GPR17⁺ cells and in the brains of Ts65Dn mice (a model of DS) and suggest that a defective oligodendrocyte differentiation occurs in trisomic brain. We are currently seeking to identify the molecular mechanisms and the possible miR-155 target(s) that may play a role in myelination defects of DS brain.

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

Andrea Maset¹, Luisa Galla^{1,2}, Claudia Lodovichi^{1,2,3}

¹Venetian Institute of Molecular Medicine (VIMM), Padova, Italy; ²CNR Institute of Neuroscience, Padova, Italy, ³Armenise-Harvard CDA

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

Neuronal migration is one of the fundamental process that underlies proper assembly and function of neuronal circuits. Migration occurs mostly during embryonic life although it persists in the sub-ventricular zone (SVZ), in adulthood. Neuronal precursors generated in the SVZ, migrate along the rostral migratory stream (RMS) to reach the olfactory bulb (OB), where they become mature interneurons. To understand the impact of OPHN1 on cell migration, we employed a line of mice expressing a null mutation of OPHN1. By combining birthdating experiments and lentiviral vectors to labels progenitors in the SVZ, we found that the progression, the morphology and the directionality of migrating cells is deeply perturbed in OPHN1 ko mice. To investigate the mechanism underlying altered cell migration, we performed time-lapse of migrating neuroblasts, *in vivo*, testing molecular cues that have been shown to modulate cell migration, such as GABA. GABA is abundantly present in the rostral migratory stream. It is produced by migrating neuroblasts and is known to modulate rate of migration acting on neuronal precursor in a paracrine/autocrine manner. We found that the response to GABA of migrating neuroblasts was deeply altered in OPHN1 KO mice.

SYNAPTIC ACTIVITY MIGHT REPRESENT A PHYSIOLOGICAL TOOL TO MODULATE GLIOMA GROWTH

Antonella Colistra^{1,2}, Eleonora Vannini¹, Laura Baroncelli¹, Mario Costa¹, Matteo Caleo¹

¹CNR Neuroscience Institute, Pisa, Italy; ²University of Pisa, Italy

Glioblastoma (GBM) is the most aggressive type of brain tumor, with a median survival expectancy of 15-18 months since the first diagnosis. Typically, many gliomas become symptomatic with focal neurological deficits (i.e. motor weakness or seizures). In this context, animal models represent an excellent tool for the early detection and longitudinal mapping of neuronal dysfunction, that are critical for the preclinical validation of new therapeutic strategies. For this reason, we exploited a well-accepted mouse model of glioma to develop sensitive behavioral readouts that allow early recognizing and following neurological symptoms.

Briefly, we injected GL261 cells into the primary motor cortex of syngenic mice and we used a battery of behavioral tests to longitudinally monitor the dysfunction induced by tumor growth. Grip strength test revealed an early onset of functional deficit associated to the glioma growth, with a significant forelimb weakness appearing 9 days after tumor inoculation. A later deficit was observed in the rotarod and in the grid walk tasks. Our data provide a detailed and precise examination of how tumor growth reverberates on the behavioral functions of glioma-bearing mice, providing normative data for the study of therapeutic strategies for glioma treatment.

It is now under debate how and if neuronal activity might impact on glioma growth. A recent article by Venkatesh et al. showed a frightening link between synaptic activity and glioma proliferation, whereas another group displayed a reduced tumor growth in glioma-bearing mice that were exposed to EE, suggesting that neuronal activity might act as an anti-neoplastic agent in gliomas (Venkatesh et al., 2015; Garofalo et al., 2014). At the moment we are trying to assess whether physiological levels of activity can control glioma proliferation. In order to address this issue, glioma-bearing mice were allowed to perform voluntary exercise through running wheels. Experiments are still ongoing, but our preliminary data indicate that running could decrease both tumor cells proliferation and tumor volume.

INHIBITION OF N-ACYLETHANOLAMINE-HYDROLYZING ACID AMIDASE SUPPRESSES NICOTINE-INDUCED ACTIVATION OF MESOLIMBIC DOPAMINE TRANSMISSION

Mauro Congiu¹, Claudia Sagheddu¹, Sonia Aroni¹, Marta De Felice¹, Maria Scherma¹, Paola Fadda¹,
Alexandros Makriannis², Michael Malamas², Marco Pistis^{1,3}

¹*Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Monserrato (CA), Italy;* ²*Center for Drug Discovery, Northeastern University, Boston, MA;* ³*CNR Neuroscience Institute, Cagliari, Italy*

Not accessible because of intellectual property rights

CHRONIC SYNTHETIC CANNABINOID WIN 55,212-2 ADMINISTRATION INDUCES NEUROMOLECULAR CHANGES AND CROSS-SENSITIZATION BEHAVIORAL EFFECTS TO COCAINE IN ADOLESCENT RATS

Matteo Deidda¹, Philippe A. Melas^{3,4}, Maria Scherma¹, Denise B. Kandel³, Eric R. Kandel³, Walter Fratta^{1,2}, Paola Fadda^{1,2}

¹Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Centre of Excellence "Neurobiology of Dependence" University of Cagliari; ³Columbia University, New York, USA; ⁴Karolinska Institutet, Stockholm, Sweden

Nowadays cannabis and cocaine are two of the most commonly used illicit drugs, their use start often in adolescence and frequently users consume them together (EMCDDA, 2017; DPA, 2016). Noteworthy, epidemiological studies about adolescent drug use have shown a specific pattern of progressive use of different substances that is described in the "gateway hypothesis" (Kandel, 1975). Since the endocannabinoid system plays a central role in adolescence brain development (Galve-Roperh et al., 2009), early exposure to cannabinoids could cause neurobiological changes which affect brain maturation increasing the risk of vulnerability to abuse other drugs (Chadwick et al., 2013). In this study we investigate the prospective gateway effect of the synthetic cannabinoid receptor agonist WIN55,212-2 (WIN) evaluating drug's cross-sensitizing behavioural and neurobiological effects to cocaine in both adolescence and adulthood. Adolescents (PND 40) and adults (PND 70) male Sprague Dawley rats received systemic intraperitoneal (i.p.) administration of increasing doses of WIN (2, 4 e 8 mg·kg⁻¹), or its vehicle, twice-daily for 11 consecutive days. After a washout period of 7 day rats were treated i.p. with cocaine (10 mg·kg⁻¹) for 4 consecutive days and tested for locomotor activity on day 1 and day 4. On day 1 adolescent WIN-pretreated rats showed a significant increase to the motor-activating effects of cocaine compared to vehicle-pretreated rats, whereas on day 4 they displayed a convergence of sensitization values reducing the gap. In adulthood on day 1 no difference in behavioural response to cocaine were found between groups, while on day 4 WIN-pretreated rats showed a reduction of locomotor sensitization to the drug. In keeping with this, in a different groups of adolescent rats, we investigated the possible WIN-mediated brain's molecular changes. We found that at the "baseline" time point (brain dissections were performed 24 hours after the last WIN administration), WIN led to an increase of Δ FosB in the in dorsal striatum and nucleus accumbens and a reduction in the amygdala. Moreover, in the nucleus accumbens, there was also a significant upregulation of p-ERK1/2 and a trend upregulation of p-CREB. In conclusion, the results reveal that chronic exposure to cannabinoids in adolescence, but not in adulthood, can lead to an enhanced sensitization to the motor-activating effects of cocaine that were accompanied by significant neuromolecular modifications, emphasizing the importance of the developmental stages in mechanisms underlying substance abuse.

References

- Chadwick B, Miller ML, Hurd YL (2013) Front Psychiatry 4:129.
 DPA (2016). Dipartimento Politiche Antidroga, Presidenza del Consiglio dei Ministri, *Relazione annuale al Parlamento sullo stato delle Tossicodipendenze in Italia*, 2017.
 EMCDDA (2017). European Monitoring Centre for Drug and Addiction: *European Drug Report*, 2017.
 Galve-Roperh I, Palazuelos, Aguado T, Guzmán M (2009) Eur. Arch. Psychiatry Clin. Neurosci, 259:371.
 Kandel DB (1975) Science 190(4217):912.

EFFECT OF EXPOSURE TO THE ACTIVITY-BASED MODEL OF ANOREXIA NERVOSA ON LIVER OF FEMALE RATS

Elisa Giunti¹, Gianfranca Carta², Andrea Demurtas², Roberto Collu¹, Matteo Deidda¹,
Maria Scherma¹, Walter Fratta^{1,3}, Sebastiano Banni², Paola Fadda^{1,3}

¹Division of Neuroscience and Clinical Pharmacology and ²Division of Physiology,
Department of Biomedical Sciences, University of Cagliari, Monserrato (CA) Italy; ³Centre of Excellence
"Neurobiology of Dependence", University of Cagliari

As a consequence of severe malnourishment patients affected by anorexia nervosa (AN) typically present a huge range of medical disturbances which involve all systems and peripheral organs (Sharp *et al.*, 1993). Several lines of evidence show that hepatic dysfunctions are among the most frequent metabolic complications displayed by anorexic patients (Furuta *et al.*, 1999). On these basis, the aim of the present study was to evaluate in rats the effect of exposure to "early" and "late" stages of the activity-based anorexia (ABA) model of AN on behavior, liver and visceral adipose tissue (VAT). For this purpose, female rats were subjected to ABA induction, during which they had a daily time restricted access to food and free access to a running wheel. We consider as early stage the first three days of ABA induction, while late stage is referred to all the six days of the ABA induction protocol. Our results show that both in early and late stages, ABA rats presented the typical anorexic-like phenotype with a marked body weight loss and an increased running wheel activity (RWA). Moreover, in both stages, the liver weight of ABA rats was significantly decreased compared to those of control rats. VAT of ABA rats was markedly reduced, especially in the late stage of the ABA induction phase. There was also a decrease in the ratio between liver or VAT weight and body weight gain or loss. Taken together, our preliminary results demonstrate the impact of the ABA paradigm on liver and VAT of female rats emphasizing the need to study health risks arising from these alterations, further analysis will be carried out in order to evaluate the metabolic changes associated to this model.

References

- Furuta S, Ozawa Y, Maejima K, Tashiro H, Kitahora T, Hasegawa K, Kuroda S, Ikuta N (1999). *Intern Med*, 38:575.
Sharp CW, Freeman CP (1993). *Br J Psychiatry*, 162:452.

MATERNAL Δ^9 -TETRAHYDROCANNABINOL BIASES DOPAMINE SYSTEM TOWARDS BEHAVIORAL METAPLASTICITY

Claudia Sagheddu¹, Vivien Miczan^{2,3}, Sonia Aroni¹, Mauro Congiu¹, Roberto Frau¹, István Katona², Miriam Melis¹

¹Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary.

³Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary

Early life adverse events can persistently affect brain functions, and contribute to the development of psychiatric trajectories. Hence, exposure to drugs of abuse early in life produces long-lasting cognitive deficits in humans. Particularly, prenatal Cannabis exposure (PCE) has negative impact in humans on cognitive processing, such as reduced attention processing and impairments in motor and language skills as well as associative and discrimination learning. Despite the high prevalence of Cannabis use among pregnant women, its impact on the developing brain remains largely elusive. In the present study, we sought to gain insights into how PCE affects reward-related brain areas such as the ventral tegmental area (VTA) in the offspring by using in a multi-disciplinary approach including behavioral, electrophysiological and molecular techniques.

Rat dams were administered THC (2 mg/kg), or its vehicle, once per day from gestational day 5 to 20. Offspring were maintained without any further manipulation, on normal light/dark cycle with ad libitum access to food and water, until their third postnatal week.

Behavioral experiments unveiled increased reward function in the PCE offspring, underpinning a vulnerable endophenotype to THC. *Ex vivo* whole cell patch-clamp recordings from VTA dopaminergic cells showed that PCE induces an intrinsic plasticity associated with increased postsynaptic response to excitatory stimuli, as well as a reduced GABA release. Moreover, correlated confocal/STochastic Optical Reconstruction Microscopy (STORM) showed significant decrease in the density of the cytomatrix active zone protein bassoon in excitatory axon terminals impinging onto VTA dopaminergic cells, whereas substantial increase of bassoon density was found at inhibitory synapses.

These findings demonstrate that PCE induces molecular reorganization in the active zone of excitatory and inhibitory axon terminals, which may contribute to the enhanced excitation and reduced inhibition of dopaminergic neurons, respectively. Altogether, our results show that PCE perturbs molecular and cellular mechanisms in brain reward-related areas, which might result into increased vulnerability toward Cannabis use disorder, later in life.

OXYTOCIN INDUCES PENILE ERECTION AND YAWNING WHEN INJECTED INTO THE BED NUCLEUS OF THE STRIA TERMINALIS: INVOLVEMENT OF GLUTAMIC ACID, DOPAMINE AND NITRIC OXIDE

Jessica Bratzu¹, Fabrizio Sanna¹, Rahul Bharatiya¹, Antonio Argiolas^{1,2,3}, Maria Rosaria Melis^{1,2}

¹*Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy;* ²*Centre of Excellence for the Neurobiology of Dependence, University of Cagliari, Italy;* ³*CNR Neuroscience Institute, Cagliari Section, Italy*

The bed nucleus of the stria terminalis (BNST) is a complex forebrain structure divided in different subregions, which plays an important role in autonomic, neuroendocrine and behavioral responses such as anxiety, fear, stress and sexual behavior. The control of physiological and behavioral activities exerted by the BNST is mediated through the action of various neurotransmitters including GABA, glutamic acid, dopamine, serotonin, nitric oxide (NO), and neuropeptides as corticotrophin releasing factor (CRF) and oxytocin [1].

The presence of oxytocin and oxytocin receptors in the BNST together with the fact that all oxytocin neurons present in the central nervous system originate from the paraventricular nucleus of the hypothalamus and that some of these neurons exert a facilitatory role on penile erection and sexual behavior and on yawning as well [2,3], raise the possibility that oxytocin may play a role in the control of penile erection, sexual behavior and yawning at the level of this brain area.

In order to verify such possibility, we studied the effect of the injection of oxytocin into the BNST on penile erection and yawning and its possible interaction with other BNST neurotransmitter systems in inducing these effects. Briefly, male Sprague Dawley rats were stereotactically implanted unilaterally with a stainless-steel guide cannula (22 gauge) aimed at the BNST under isoflurane anaesthesia. After surgery, rats were given 7 days for recovery. Microinjections of oxytocin and other compounds usually dissolved in saline or appropriate vehicles were performed through an internal cannula (28 gauge) extending 6 mm below tip of the guide cannula (Paxinos & Watson, The stereotaxic atlas of the rat brain, 2004) in a volume of 0.3 µL in 2 min. Rats injected with saline or vehicle alone were used as controls.

Oxytocin (5-100 ng) but not vasopressin (100 ng) injected into the BNST, dose-dependently induced penile erection and yawning. The minimal effective dose of oxytocin was 20 ng for penile erection and 5 ng for yawning, while the maximum effect was found for both responses with the dose of 100 ng. Oxytocin responses were completely abolished by the oxytocin receptor antagonist d(CH₂)₅Tyr(Me)²-Orn⁸-vasotocin (1 µg) injected into the BNST 15 min before oxytocin. Penile erection and yawning were also abolished by the prior injection into the BNST of (+) MK-801 (1 µg) and CNQX (1 µg), excitatory amino acid receptor antagonists, the first of the NMDA and the second of the AMPA receptor subtype, respectively, by SCH 23390 (1 µg), a D1 receptor antagonist and by SMTc (40 µg), an inhibitor of neuronal nitric oxide synthase. In contrast, no effect on oxytocin-induced penile erection and yawning was found when haloperidol, a D2 receptor antagonist, (1 µg), bicuculline (20 ng) and phaclophen (5 µg), a GABA_A and GABA_B receptor antagonist, respectively, and CP 376395 (5 µg) and astressin 2B (150 ng), CRF receptor antagonists, the first of the CRF-1 and the second of the CRF-2 receptor subtype, were given into the BNST 15 min prior oxytocin. Finally, the excitatory amino acid N-methyl-D-aspartic acid (NMDA) (100 ng) per sé was able to induce penile erection and yawning when injected into the BNST, and these effects were antagonized by the prior injection of (+) MK-801 (1 µg) into the BNST.

Taken together, the results of this study show that oxytocin injected into the BNST induces penile erection and yawning in a dose dependent manner and that this effect can be attributed to the interaction of BNST oxytocin neurons with BNST dopaminergic, glutamatergic and nitric oxide pathways and not through CRF and/or GABAergic pathways. The involvement of glutamic acid and NMDA receptors in the pro-erectile and pro-yawning effects of oxytocin at the level of the BNST is also supported by the ability of NMDA given into the BNST to induce both penile erection and yawning.

References

- [1] Crestani et al., *Curr Neuropharm*, 11: 141-159, 2013
- [2] Melis & Argiolas, *Neurosci Biobehav Rev*, 35: 939-955, 2011
- [3] Argiolas & Melis, *Prog Neurobiol*, 108: 80-107, 2013

ADOLESCENT ALCOHOL EXPOSURE INCREASES NICOTINE AND COCAINE REWARD ONLY IN RATS GENETICALLY VULNERABLE TO DRUGS OF ABUSE

Cristina Cadoni^{1,3}, Silvia Corongiu², Elena Espa, Sandro Fenu^{2,3}

¹CNR Neuroscience Institute, Cagliari, Italy; ²University of Cagliari, Dept. Biomedical Sciences, Neuropsychopharmacology section, Cagliari, Italy; ³Centre of Excellence “Neurobiology of Dependence”, University of Cagliari, Cagliari, Italy

Since its theorization the Gateway Hypothesis (Kandel, 1975), that posits that initial exposure to legal drugs causes subsequent addiction to illicit drugs, is still debated on the basis of contrasting findings in pre-clinical and clinical studies. On the other hand, the Common liability theory (Vanyukov et al, 2012), that hypothesizes a shared addiction vulnerability to legal and illegal drugs, is gaining attention and interest of clinicians and researchers working with animal models. Human studies cannot test experimentally the gateway hypothesis for obvious reasons, while animal models offer a unique opportunity to test it in controlled experimental conditions.

Two critical factors influencing the development of drug addiction are the age of first exposure to drug and the genetic background of the individual. Thus, these factors might be critical also in testing a gateway effect of a drug toward the use and abuse of another one. While the role of age is becoming increasingly clear, in the light of recent evidence on adolescent brain development, the role of genetic factors is often neglected.

For these reasons we investigated the role of genetic influence on the postulated gateway effect of adolescent alcohol exposure toward nicotine and cocaine abuse.

To this aim we used 3 strains of rats, 1 outbred strain (Sprague-Dawley rats) and 2 inbred rat strains (Lewis and Fischer 344 rats) known to be addiction prone and addiction resistant respectively (Cadoni, 2016).

At the age of 6 weeks (mid-adolescence) rats were exposed to an intermittent access to 20 % alcohol (two bottle choice) in their homecage for 9 weeks (three 24h-session/week). Control animals were kept in the same conditions but the only liquid available was water. After 10 days of withdrawal animals were conditioned, in a conditioned place preference (CPP) apparatus, with 3 injections of 0.2 mg/kg s.c. of nicotine paired to the less preferred compartment. After 10 days from the nicotine CPP test animals were conditioned with 3 injections of cocaine (10 mg/kg i.p.) paired always to the less preferred compartment.

While Sprague-Dawley and Fischer 344 rats did not show any preference for the compartment previously paired to nicotine or cocaine, either control or alcohol group, Lewis rats displayed a significant preference for the nicotine paired compartment and this preference was significantly potentiated by adolescent exposure to ethanol. Following cocaine conditioning while control Lewis rats did not display any preference ethanol pre-exposed group showed a significant CPP for the cocaine paired compartment.

These results suggest that adolescent exposure to alcohol might increase the rewarding effects of nicotine and cocaine but only in genetically vulnerable individuals and therefore support the hypothesis of a common liability to addiction, likely due to genetic makeup, rather than a gateway effect of alcohol toward use and abuse of other licit or illicit drugs.

References

- Cadoni C (2016) *Front Neurosci.* 9;10-13.
- Kandel D (1975) *Science* 190: 912-914.
- Vanyukov MM et al (2012) *Drug Alcohol Depend* 123: S3-17.

IMPAIRED FEAR RESPONSE IN PAIN INSENSITIVITY CAUSED BY NGF MUTATION

Giovanna Testa¹, Laura Pancrazi², Francesco Olimpico¹, Chiara Morelli³, Paul Heppenstall³, Mario Costa², Antonino Cattaneo^{1,4*}, Simona Capsoni^{1,5*}

¹Laboratory of Biology, Scuola Normale Superiore, Pisa, Italy; ²CNR Institute of Neuroscience, Pisa, Italy;

³EMBL, Monterotondo, Italy; ⁴EBRI, Rome, Italy; ⁵University of Ferrara, Ferrara, Italy

* Co-senior authors

Hereditary Sensory and Autonomic Neuropathies (HSAN) are rare autosomal recessive disorders characterized by the inability to detect painful stimuli. This family of diseases is composed of seven members and, in particular, HSAN V is linked to defects in Nerve Growth Factor (NGF) signaling. HSAN V is clinically characterized by absence of pain sensation leading to painless fractures, bone necrosis, osteochondritis, and neuropathic joint destructions (Capsoni, 2014). The genetic mutation responsible for the disease is the R100W point mutation (661C>T) in the NGF gene (Einarsdottir et al., 2004). HNGF R100W is a preferential TrkA-biased agonist (Covaceuszach et al., 2010; Capsoni et al., 2011). We confirmed the finding by Larsson et al. (2009) that the R100W mutation inhibits processing of proNGF to mature NGF, with an accumulation of proNGF in cultured cells. To understand the mechanisms at the basis of this disease, we have generated two knock-in mouse lines in which the murine NGF coding sequence is replaced by the coding sequence of human NGF, either in its wild type or R100W forms. Haploinsufficiency of mature NGF could be the cause of death of homozygous HSAN V mice occurring during the first month of life. To continue the characterization of the effects of HSAN V mutation during adulthood, we performed a combination of molecular, neuroanatomical and behavioral analyses in heterozygous mice. Due to the known effects of NGF on learning and memory, we analyzed heterozygous mice for the presence of deficits in object recognition and spatial memory tests and found no impairment in both tasks. At two months, heterozygous mice show no deficits in thermal perception, anxiety and “cage behavior”, which, however, are impaired in 6-month-old heterozygous HSAN V mice. At all ages, the R100W mutation causes less sensitivity to pain induced by capsaicin injection, in dosage and sex-dependent manner. The insensitivity to pain might interfere with learning and memory of painful events or with their interpretation as painful. Thus, in the fear conditioning test, we increased the potency of the aversive stimulus with respect to wild type mice and found that, despite the perception of the aversive stimulus, HSAN V mice do not show a fear response. Interestingly, the late onset of the phenotype and the inability to acquire aversive responses to pain accurately model the clinical phenotype of heterozygous patients (Minde et al., 2009). In conclusion, we were able to reproduce in mice the main clinical HSAN V phenotype. Further study of this model will allow to expand our knowledge on the pro-nociceptive actions of NGF, from peripheral sensory system to central mechanisms of pain perception and will provide new insights into the central consequences of growing without feeling pain.

Funding: Telethon grant n. GGP11179 and FP7 PAINCAGE project n. 603191

THE 5 α -REDUCTASE INHIBITOR FINASTERIDE REDUCES DYSKINESIA IN A RAT MODEL OF PARKINSON'S DISEASE

Silvia Fanni¹, Roberto Frau¹, Paola Savoia⁴, Chiara Fiorentini⁴, Francesca Rossi³, Elisabetta Tronci³, Francesco Traccis¹, Roberto Stancampiano³, Mario Meloni², Antonio Cannas², Francesco Marrosu², Marco Bortolato⁵, Pierluigi Saba¹, Camino Fidalgo⁶, Alessandra Pardu¹, Cristina Missale⁴, Paola Devoto¹, Manolo Carta³

¹Dept. of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Movement Disorders Center, Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Italy; ³Dept. of Biomedical Sciences, Section of Physiology, University of Cagliari, Italy; ⁴Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Italy; ⁵Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Utah, USA; ⁶Dept. of Psychology and Sociology, University of Zaragoza, Spain

Long-term administration of 1-3,4-dihydroxyphenylalanine (L-DOPA), the mainstay therapy for Parkinson's disease (PD) patients, is accompanied by the development of dyskinesia, a disabling motor complication that dramatically affects patients' quality of life. Although its etiology remains unclear, there is accumulating evidence that L-DOPA induced dyskinesia (LID) relies on an excessive dopamine receptor transmission, particularly at the downstream signaling of D1 receptors.

Over the last years, we have reported that the inhibition of 5 α -reductase (5AR), that is the rate-limiting enzyme in neurosteroidogenesis, elicits antidopaminergic effect. Specifically, we found that in rodents, finasteride (FIN), the prototypical inhibitor of 5AR, is able to restore the PPI deficits, hyperactivity and stereotyped responses induced by dopaminomimetic agents. Interestingly, all these behavioral effects were mediated by post-synaptic DAergic regulation in the striatum and were not associated to extrapyramidal symptoms.

Since LID is closely related to dysregulation of dopamine signaling in the striatum and finasteride elicited its antidopaminergic effects in this area, the aim of the present study was to investigate whether the 5AR inhibition may counteract the development and expression of dyskinesia.

First, we assessed the acute and chronic effect of different doses of FIN (30-60 mg/kg) on LID, in male 6-OHDA-lesioned L-DOPA primed rats. Afterwards, to fully characterize the therapeutic potential of FIN on LID, we assessed the abnormal involuntary movements in hemiparkinsonian male rats chronically injected with FIN (30-60mg/kg/24days) either prior to- or concomitant with L-DOPA administration. Furthermore, to investigate whether the impact of FIN on LID may be mediated by D1- or -D2/D3-receptor pathway, dyskinesias were assessed in L-DOPA-primed 6-OHDA-lesioned rats that received FIN in combination with selective direct dopaminergic agonists. Finally, due to the critical role of 5AR in androgen and estrogen synthesis, we investigated whether FIN may produce similar antidyskinetic effect shown in male, also in hemiparkinsonian female rats.

The results indicated that FIN administrations significantly reduced development and expression of dyskinesia induced by both L-DOPA and dopamine-mimetic drugs, in all treatment regimens. Moreover, FIN produced significant antidyskinetic effect also in parkinsonian female rats, although only at the highest tested dose.

To our knowledge, this is the first study that highlights a possible role of 5AR and its related neurosteroids in the pathophysiology of LID, and suggests FIN as a promising tool for the treatment of dyskinesia in Parkinson's patients.

COMPARISON OF THE EFFICACY OF 5 α -REDUCTASE INHIBITORS ON L-DOPA INDUCED DYSKINESIA IN A MODEL OF PD

Francesca Rossi¹, Roberto Frau², Silvia Fanni², Elisabetta Tronci¹, Francesco Traccis², Roberto Stancampiano¹, Marco Bortolato⁴, Pierluigi Saba², Camino Fidalgo³, Alessandra Pardu², Paola Devoto², Manolo Carta¹

¹Dept. of Biomedical Sciences, Section of Physiology, University of Cagliari, Italy; ²Dept. of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy;

³Dept. of Psychology and Sociology, University of Zaragoza, Spain;

⁴Dept. of Pharmacology and Toxicology, College of Pharmacy, University of Utah, USA

L-DOPA induced dyskinesia (LID) remain a major burden for Parkinson's disease (PD) patients. LID has consistently been related to abnormal dopaminergic transmission, with dysfunction of down-stream signaling of the striatal D1 receptors as the most characterizing features. We previously reported that the pharmacological blockade of 5-alpha reductase (5AR), the rate-limiting enzyme in neurosteroid synthesis, rescued a number of behavioral aberrations induced by D1 receptor-selective and non-selective agonists, without inducing extrapyramidal symptoms. Accordingly, we have recently shown that the inhibition of 5AR by finasteride (FIN) induced significant antidyskinetic effect in 6-OHDA lesioned rats under different treatment regimens.

Since FIN is approved for human use for the treatment of androgenetic alopecia and benign prostatic hyperplasia, these results suggest a potential therapeutic value of this drug; however, while the clinical effects of FIN are mostly mediated by the isoform 2 of 5AR in humans, this drug has also high affinity for the isoform 1 in rats. To further consolidate the notion that 5AR inhibition may be a viable therapeutic strategy for LIDs, the present study was designed to investigate whether the antidyskinetic effects of FIN could be mimicked by dutasteride (DUTA), another 5AR inhibitor used in benign prostatic hyperplasia treatment that has comparable affinities for both 5AR isoforms.

Thus, a side-by-side comparison of DUTA and FIN was carried out to investigate both their ability to dampen development of LID and the impact on L-DOPA- induced therapeutic effect on the rat forelimb use in the stepping test and general locomotor activation. We found that FIN (30 and 60 mg/kg doses) and DUTA (15 and 30 mg/kg doses) treatment elicited similar extent of AIMs reduction. However, DUTA appeared more effective than FIN at a lower dose. Furthermore, the improvement of the forelimb use induced by L-DOPA was not affected by DUTA, while FIN produced a forelimb use reduction. Interestingly, these results were corroborated by the locomotor measurement, which indicated a partial reduction in the therapeutic effect of L-DOPA induced by FIN but not by DUTA.

The present data further support 5AR enzyme as an intriguing target for the treatment of dyskinesia in PD. Moreover, the preservation of the therapeutic efficacy of L-DOPA shown by DUTA, indicates that this drug may be the preferred compound to be tested in clinical trials.

METABOLIC ALTERATIONS, CELLULAR STRESS AND NEURODEGENERATIVE PROCESSES

Silvia Penati^{1,2}, Nicole Colombo¹, Michela Matteoli^{1,2}, Patrizia Rosa¹, Maria Luisa Malosio^{1,2}

¹CNR Institute of Neuroscience, Milano, Italy;

²Laboratory of Pharmacology and Brain Pathology, & Neuro Center, Humanitas Clinical and Research Center, Rozzano (MI), Italy

Dementia is a clinical condition, caused by neurodegeneration, associated with a decline in cognitive skills, such as memory, thinking and the associated ability to perform everyday's activities. After age 65, the likelihood of developing dementia roughly doubles every five years (www.england.nhs.uk/mental-health/dementia). Due to the lack of early diagnosis and effective preventive and therapeutic strategies, these neurodegenerative diseases are believed to be a health emergency in the coming years. The most common type of dementia is Alzheimer's disease (AD), other types include vascular, Lewy bodies and frontotemporal dementia. Increasing evidence suggests an association between metabolic disorders, notably insulin-resistance (IR), type 2 diabetes (T2D), and Alzheimer's Disease (AD) [PMID:17430239; PMID:24236899; PMID:23485579]. Clinical and epidemiological studies indicate that diabetic patients have increased risk of developing AD [PMID:10599761; PMID:27385744; PMID:28088029] and vice versa.. Moreover AD brains exhibit defective insulin signaling [PMID:18479783; PMID:22476196; PMID:22710630; PMID:22476197] and insulin resistance. Recent studies have shown that diet-induced changes in peripheral insulin sensitivity contribute to alterations in brain insulin signaling and cognitive functions [PMID:27771511]. IR could in fact be the common pathogenetic mechanism underlying AD and T2D affecting glucose metabolism in different organs, including the brain. In addition, chronic low grade inflammation is a condition accompanying AD, T2D and the condition of IR, typical of pre-diabetes. Moreover, elevated fatty acids levels [PMID:15255786] in combination with hyperglycemia determine prolonged exposure of cells to gluco-lipotoxicity, which has been shown to induce pancreatic beta cell functional impairment and death. The biological framework for explaining this connection is very complex as we are faced with a multifactorial process where it is difficult to identify the contribution of individual factors.

The aims of this study are:

1. to set-up an *in vitro* model for studying the cellular and molecular mechanism leading to cognitive impairment associated with metabolic disorders;
2. to determine how high fat diet-induced insulin resistance can lead to cognitive impairment prodromal to Alzheimer's disease;
3. investigate the effect of insulin-resistance on Central Nervous System myelination

In order to mimic the metabolic condition determined by high fat diet on neuronal, astrocyte and microglia cell cultures the treatment with palmitic acid has been chosen. Palmitic acid (PA) is a saturated fatty acid, which is a major component of the palm trees oils. It mimics the effects of elevated plasma free fatty acids (FFA), a condition usually seen in obesity and which has been shown to cause insulin-resistance [PMID:21297467]. As a control oleic acid (OA), a monounsaturated acid, was used. We will show the results of acute and chronic PA treatment on cell cultures of neurons, astrocytes and microglia. Hippocampal and cortical neurons treated with different concentrations of PA and OA show that higher concentrations of PA induce significant cell death, while the same concentrations of OA seem to be innocuous. An interesting observation is that the same concentrations of PA that induce significant neuronal cell death do not seem to be toxic for astrocytes. The mechanisms underlying PA toxicity are currently under investigation. Myelination defects have been proposed to be involved in peripheral neuropathy, a long-term complication of diabetes. Central myelination might also be affected by elevated FFA. The effect of PA and OA on oligodendrocyte precursor cells and their ability to differentiate *in vitro* will be presented.

IMPACT OF ACUTE-PHASE COMPLICATIONS AND INTERVENTIONS ON 6-MONTH SURVIVAL AFTER STROKE. A PROSPECTIVE STUDY

Antonio Di Carlo¹, Marzia Baldereschi¹, Maria Lamassa², Benedetta Piccardi^{2,1}, Marco Franceschini³, Francesca Bovis², Lorenzo Cecconi⁴, Annibale Biggeri⁴, Domenico Inzitari^{1,2}, Salvatore Ferro⁵

¹CNR Institute of Neuroscience, Florence, Italy; ²NEUROFARBA Department, University of Florence, Italy; ³IRCCS San Raffaele Pisana, Rome, Italy; ⁴DISIA Department, University of Florence, Italy; ⁵Emilia-Romagna Region Health Authority, Bologna, Italy

Background and objective.

The outcome of stroke patients is complex and multidimensional. We evaluated the impact of acute-phase variables on 6-month survival after first-ever stroke, taking into account baseline conditions exerting a possible effect on outcome.

Methods.

As part of a National Research Program, we performed a prospective observational study of acute stroke patients in four Italian Regions. Consecutive patients admitted for a period of 3 months to the emergency rooms of participating hospitals were included.

Results.

A total of 1030 patients were enrolled (median age 76.0 years, 52.1% males). At 6 months, 816 (79.2%) were alive, and 164 (15.9%) deceased. Survival status was missing for 50 (4.9%). Neurological state in the acute phase was significantly worse in patients deceased at 6 months, who showed also higher frequency of acute-phase complications. Cox regression analysis adjusted for demographics, pre-stroke function, diseases and risk factors, indicated as significant predictors of 6-month death altered consciousness (HR, 1.70; 95% CI, 1.14-2.53), total anterior circulation infarct (HR, 2.13; 95% CI, 1.44-3.15), hyperthermia (HR, 1.70; 95% CI, 1.18-2.45), pneumonia (HR, 1.76; 95% CI, 1.18-2.61), heart failure (HR, 2.87; 95% CI, 1.34-6.13) and nasogastric feeding (HR, 2.35; 95% CI, 1.53-3.60), while antiplatelet therapy (HR, 0.56; 95% CI, 0.39-0.79), and early mobilization (HR, 0.55; 95% CI, 0.36-0.84) significantly increased 6-month survival.

Conclusions.

In a prospective survey, stroke severity and some acute-phase complications, potentially modifiable, significantly increased the risk of 6-month death, independently of baseline variables. Early mobilization positively affected survival, highlighting the role of early rehabilitation after stroke.

GABAERGIC NETWORK ACTIVITY AND POTASSIUM ELEVATIONS AT THE ONSET OF SEIZURE-LIKE EVENTS IN ENTORHINAL CORTEX SLICES

Gabriele Losi^{1,2}, Laura Librizzi³, Iacopo Marcon^{1,2}, Michele Sessolo^{1,2},
Paolo Scalmani³, Marco de Curtis³ and Giorgio Carmignoto^{1,2}

¹CNR Neuroscience Institute, Padova and ²Department of Biomedical Sciences,
University of Padova, Italy; ³Unit of Epileptology and Unit of Clinical Neurophysiology,
Fondazione Istituto Neurologico Carlo Besta, Milano, Italy

Focal seizures characterized by low-voltage fast activity represent the most common pattern of seizure-onset in human focal epilepsies. In humans and in different animal models, focal seizures initiate with a reduction of principal neuron firing and enhanced interneuronal network activity (de Curtis and Avoli, 2016). In parallel increased extracellular potassium levels ($[K^+]_o$) are observed during seizures but it is not clear whether and how they contribute to seizure initiation. We here utilized entorhinal cortex (ECx) slices perfused with 4-aminopyridine (4-AP) to evaluate the correlation between interneuronal GABAergic network activity, $[K^+]_o$ and spontaneous seizure-like events (SLEs). We performed patch-clamp recordings from pyramidal neurons and parvalbumin expressing interneurons and simultaneous $[K^+]_o$ measurements to monitor SLE onset in layer V-VI of the ECx. We observed that 90% of SLEs recorded in principal cells were preceded by rhythmic large GABAergic events coupled with $[K^+]_o$ elevations. Of note, we found that the speed of $[K^+]_o$ elevations during GABAergic events correlated with subsequent seizure onset. Blocking glutamatergic transmission prevented SLE generation but not GABAergic and K^+ events. In these conditions, adding also a GABA_A receptors antagonist prevented $[K^+]_o$ elevations but not PV-INs firing activity, suggesting that $[K^+]_o$ derives from postsynaptic cells rather than from interneuron repolarizations during firing activity. Potassium elevations associated to GABAergic population events were confirmed in the entorhinal cortex of the *in vitro* isolated whole guinea pig brain. Our data show that in the 4-AP model SLE onset is sustained by inhibitory network activity associated with a fast and prolonged $[K^+]_o$ increase. In a similar model of evoked SLEs in brain slices we previously showed that SLEs initiate after a period of intense parvalbumin (PV) GABAergic interneuron firing followed by a sudden depolarization-block that coincides with full seizure generation, when all nearby principal neurons are activated (Cammarota et al 2013). We also revealed that rhythmic optogenetic activation of PV-interneurons may favor seizure initiation (Sessolo et al 2015) by increasing principal neurons synchronous activity as soon as PV-interneurons cease firing, due to a rebound spiking. The increased $[K^+]_o$ may thus favor both interneuron depolarization-block and the consequent principal neurons rebound spiking that finally triggers seizure onset. Further studies are needed to clarify the postsynaptic source of K^+ and its effect on interneuron firing activity.

CALCITONIN GENE-RELATED PEPTIDE (CGRP) ACTS AS AN HOMEOSTATIC FACTOR ON MICROGLIAL CELLS IN EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Stefano Morara^{1,2}, Ilaria Rossetti^{1,2}, Annamaria Finardi³, Laura Zambusi^{1,2}, Luciano Provini², Roberto Furlan³

¹CNR Neuroscience Institute, Milano, Italy; ²University of Milano, Dip. Bio.Me.Tra., Milano, Italy;

³Inst. Exp.I Neurol., San Raffaele Hospital, Milano, Italy

Multiple sclerosis (MS) is a complex neuroinflammatory disease whose pathogenic mechanisms involve an autoimmune activation against central nervous system (CNS) antigens. Peripheral activation is followed by immune cell infiltration in the CNS and biased interaction with resident CNS cells, including microglia and astrocytes. We decided to investigate molecular mechanisms that occur during the induction phase of MS, in order to evaluate molecules that, released inside CNS, would be able to 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.

To analyze the effect of CGRP in the development of experimental autoimmune encephalomyelitis (EAE) in mice, we released the peptide intrathecally by osmotic minipumps in a chronic fashion during the induction phase: two days after EAE induction with MOG35-55 in C57BL/6 mice, we implanted a catheter (attached to an osmotic minipump) in a subdural location of the spinal canal (at the level of the VI vertebra). CGRP was delivered at 50 pmol/h and peptide 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 Iba1 or CD11b (microglia markers), GFAP (astrocyte markers), iNOS (marker that in macrophage/microglia has been associated to a so-called inflammatory activation), Ym1 (marker that in macrophage/microglia has been associated to a so-called protective activation), CD206 or CD68 (macrophage/microglia activation markers), phosphorylated ERK1/2, phosphorylated p38.

We had already shown that the neuropeptide dampens the disease course and changes the 2D shape of microglial cells (indicative of inhibition of activation; Sardi et al., 2014). In order to increase the sensitivity of the morphological analysis of microglia activation we carried out a 3D analysis of microglia ramifications: the results 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-1 β 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- α 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 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.

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 opposite activities (so-called inflammatory and protective) rather than to a switch from inflammatory to protective function.

THE ITALIAN STROKE-APP: ICTUS 3R

Marzia Baldereschi¹, Antonio Di Carlo¹, Benedetta Piccardi^{2,1}, Domenico Inzitari^{2,1}

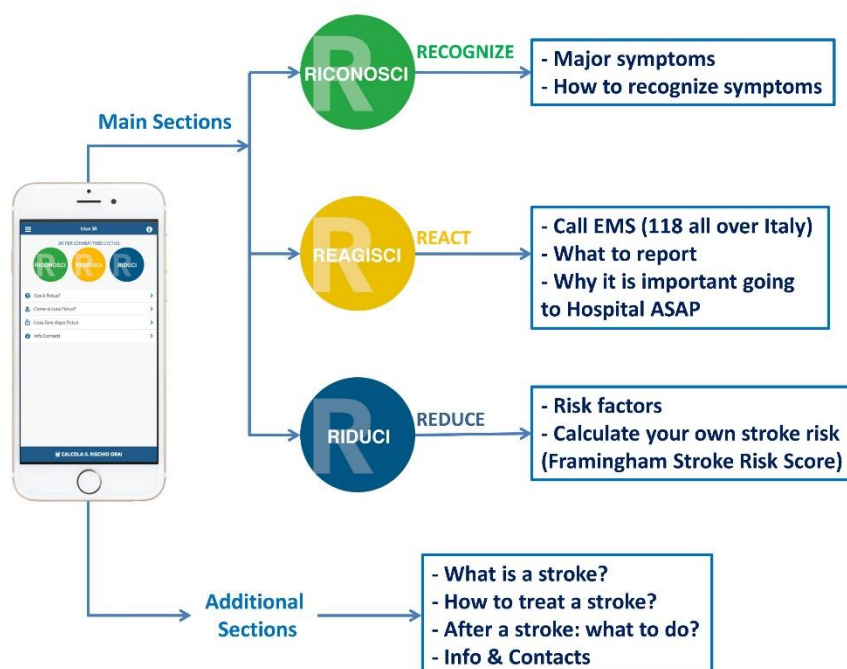
¹CNR Neuroscience Institute, Florence, Italy; ²NEUROFARBA Department, University of Florence, Italy

Objective. Most stroke-related applications currently available for patients and general population lack scientific validity in terms of adherence to current stroke literature and guidelines all of them are in English only. We developed a user-friendly, free-of-cost application in Italian, available at www.ictus3r.it as a web-app as well as native app at Apple Store and Google Play, to contribute filling the gaps in stroke knowledge among Italian people. Here we report on the use of this application.

Methods. The application was developed in collaboration with communication experts, stroke leaders and web producers. ICTUS3R was pilot tested in terms of number and distribution of downloads. Data on usage were anonymously collected during the first 14 months following release (October 29, 2014).

Results. ICTUS3R was designed to include comprehensive stroke information primarily accessed via a scrollable index. Three major sections were dedicated to stroke symptoms recognition, reaction, and prevention. Other sections included stroke information and calculation of personal stroke risk according to the Framingham Stroke Risk Score. The application provides individual's relative risk, compared with someone of the same age and sex free of modifiable risk factor for stroke. Contact information of Italian patients' support organizations are made available as well. The application was downloaded a total of 37500 times, of which 87% was by individuals in Italy. The age-group mostly interested in the applications was 25-34 years of age (33,5%) but also the 12% of the downloads were from people 55 and over. Geographical distribution shows a north-to-south gradient. More than 1600 downloads were done in US. The most interesting (most times visited) page was the stroke risk calculator. Visitors picked in relation to national newspaper articles or national television broadcasting where the ICTUS3R application had been mentioned and described.

Conclusions. ICTUS3R for smartphone, tablet, and PC is a well-received application for dissemination of stroke information among Italians. Application widespread and download are strongly correlated with specific mass-media promotional events.



MCU KNOCK-DOWN IMPAIRS SKELETAL MUSCLE AND MOTOR NEURON DEVELOPMENT IN ZEBRAFISH EMBRYOS

Elisa Lidron^{1,2}, Sofia Zanin^{1,2}, Enrico Moro³, Francesco Argenton⁴,
Rosario Rizzuto², Giorgia Pallafacchina^{1,2}

¹CNR Neuroscience Institute, Padova, Italy; ²Department of Biomedical Sciences,
University of Padova, Italy; ³Department of Molecular Medicine, University of Padova, Italy;
⁴Department of Biology, University of Padova, Italy

Ca²⁺ signals regulate a plethora of biological processes, from egg fertilization to organogenesis, contraction in muscle and neuron firing in brain. Inside the cell, mitochondria are one of the most important targets and regulators of Ca²⁺ signalling. Their strategic localization close to the site of ER Ca²⁺ release allows them to efficiently accumulate the cation upon cytosolic Ca²⁺ increase. Mitochondrial Ca²⁺ uptake is functional to buffer cytosolic Ca²⁺ elevation but it is also crucial for the stimulation of mitochondrial respiration and ATP production. Moreover, dysregulation of mitochondrial Ca²⁺ level is one of the major triggers of apoptosis.

Since the identification of the molecules responsible for the entry of Ca²⁺ in mitochondria, the mitochondrial Ca²⁺ uniporter (MCU) and its regulators, the biochemical and molecular characterization of the mechanisms underlying mitochondrial Ca²⁺ contribution to cell homeostasis made a great step forward. The MCU^{-/-} mouse model was first described in 2013 having an unexpected mild phenotype, the severity of which, however, depends on the genetic background. This underlies still a role for MCU during embryogenesis. In line with this, our work aims to explore the contribution of MCU and mitochondrial Ca²⁺ to the regulation of vertebrate development and organogenesis using zebrafish (*Danio rerio*) as a model organism. To this purpose, we adopt a knock-down strategy to reduce MCU expression during zebrafish development by injecting morpholino antisense oligonucleotides in fertilized eggs. Western blot analysis reveals an efficient MCU knock-down starting from 48 hours post fertilization, which is accompanied by a reduced mitochondrial Ca²⁺ uptake in isolated skeletal muscle fibers of injected embryos. MCU morphant embryos develop without gross morphological abnormalities. However, to a deep analysis, they display alterations in several tissues, in particular they show: 1) impaired locomotor activity, as assessed by touch-evoked escape response test; 2) disorganized skeletal muscle structure, as assayed by birefringence, phalloidin staining and immunofluorescence analysis; 3) impaired motor neuron development, as revealed by imaging motor neuron branching in *Tg(HB9-mGFP)* embryos at different time points.

Skeletal muscle differentiation and motor neuron pathfinding are intimately connected processes during zebrafish embryogenesis. A population of muscle pioneer cells, called adaxial cells, is required for driving motor neuron axon growth, while neuron innervation and growth factor release are mandatory for muscle fiber morphological and functional maturation. Thus, we explored the distribution of this adaxial cell population in MCU morphant embryos. Our preliminary results indicate a remarkable mislocalization of these cells and a strong reduction in their number in embryos injected with MCU morpholino. This deficit may be responsible for the defective skeletal muscle-motor neuron developmental axis seen in our morphants.

In conclusion, our data indicate an important role of MCU and mitochondrial Ca²⁺ in zebrafish embryo development. In particular, we highlight their fundamental contribution to the differentiation and maturation of both skeletal muscle fibers and motor neurons.

INDUCTION OF AUTOPHAGY BY THE PLANT PHENOL PTEROSTILBENE: MECHANISMS AND BIOMEDICAL POTENTIAL

Martina La Spina^{1,2}, Michele Azzolini^{1,2}, Marco Schiavone², Giulietta Di Benedetto¹, Mario Zoratti^{1,2}, Lucia Biasutto^{1,2}

¹CNR Institute of Neuroscience, Padova, Italy; ²Department of Biomedical Sciences, University of Padova, Italy

Pterostilbene (Pt), a phenolic component of blueberries, has been proposed to be beneficial in many human pathologies such as the metabolic syndrome, neurodegenerative diseases and cancer. However, the molecular mechanisms underlying its effects have been poorly characterized. Several papers suggest that Pt may induce autophagy. This process is mainly regulated by Transcription Factor EB (TFEB), whose action is controlled by inhibitory phosphorylation by mTORC1, which retains it in the cytosol, and dephosphorylation by Ca²⁺-dependent Calcineurin, which promotes its nuclear translocation and thus its transcriptional activity.

We confirmed that Pt at physiologically relevant (low μ M) levels promotes autophagy *in vitro* by inducing TFEB nuclear migration, and traced back the upstream signaling cascade to ROS generation by mitochondria. Indeed, pre-treatment with an antioxidant reduced the TFEB nucleus/cytosol ratio. In agreement, we observed inhibition of mitochondrial respiratory chain complex I by Pt. ROS seem to be connected to an increase of cytoplasmic Ca²⁺ which activates Calcineurin. Cyclosporin A, which blocks Calcineurin, reduced Pt-induced nuclear translocation of TFEB as well.

Both ROS and Ca²⁺ are involved in AMPK activation. AMPK in turn inhibits mTORC1, facilitating TFEB migration. This metabolic sensor is known to play an important role in promoting mitochondrial biogenesis through Pgc-1 α . Accordingly, we found that Pt both up-regulates Pgc-1 α by acting on AMPK, and increases the mitochondrial content of cells.

These results led us to test the biomedical potential of Pt in a zebrafish model of Collagen VI myopathies characterized by a defective autophagy and accumulation of damaged mitochondria. Strikingly, this molecule partially rescued both motor and structural abnormalities in these fish at sub- μ M concentrations. Preliminary experiments suggest that these effects are indeed associated with the activation of autophagy and mitochondrial biogenesis. We have shown that pterostilbene can readily reach μ M levels in the brain of rodents after oral administration of pharmacological doses. Induction of autophagy might therefore also account, at least in part, for the reported beneficial effects of stilbenoids in animal models of cognitive impairment associated with aging or neurodegeneration.

PGE1 DELIVERED BY LIPOSOMES ANTAGONIZED DENDRITIC SPINE LOSS AND REDUCTION OF VEGF AND VEGF-R2 IN FRONTAL CORTEX AND HIPPOCAMPUS OF DIABETIC RATS

Francesco Cuccu¹, Francesca Biggio¹, Valentina Locci², Laura Boi¹, Maria Letizia Manca¹, Anna Maria Fadda¹, Giovanni Biggio^{1,2}, Maria Cristina Mostallino³

¹*Department. of Life and Environmental Sciences, section Neuroscience, University of Cagliari, Italy;*

²*Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, USA;*

³*CNR Neuroscience Institute, Cagliari, Italy*

Diabetes (type 1 and 2) is a common metabolic disorder that can lead to functional and structural neurological complications such as cognitive impairment, dementia and reduced volume of hippocampus and frontal cortex. Diabetes related impairments in cognition and neural structure and function could be the consequence of a progressive damage at vascular level. Given that prostaglandins (cyclic oxygenated fatty acids) exert a potent positive action on vascular endothelium in many tissues, we used one of these molecules (PGE1) to prevent or ameliorate the negative effects of diabetes in vascular district in a rat model of diabetes (streptozotocin treated rats). Since prostaglandins are rapidly metabolized by different enzymes we included PGE1 into liposomes made with phosphatidylcholine and Poly-L-lysine. These liposomes (1µg/kg) were intraperitoneally administered (twice a week for three months) to streptozotocin (70 mg/Kg) treated rats. Healthy control rats and diabetics rats treated with saline have been used as control; all rats were sacrificed after three months. The glycemia was checked one time a week and 1 UI insulin retard administered once a week. In diabetes rats the dendritic spines density, VEGF (vascular endothelial growth factor) and VEGF-R2 (tyrosine kinase receptor of VEGF) expression levels were markedly reduced in the hippocampus and frontal cortex. As expected, the morphology and some molecular parameters of gastrocnemius muscle, lungs and kidneys showed a dramatic alteration effects almost completely reversed by PGE1 treatment. The results suggest that PGE1 treatment has great efficacy in antagonizing the neurochemical and molecular consequences elicited by experimental diabetes both in brain and periphery. The reduced expression of dendritic spines densities, associated to the impairment of learning and memory present in this pathology, are consistent with the structural brain damage and functional decline present in diabetes patients.

PCDH19 C-TERMINUS IS CLEAVED IN RESPONSE TO NMDAR ACTIVATION AND HOMEOSTATICALLY REGULATES IEGs

Laura Gerosa¹, Francesco Rusconi², Luca Murru¹, Alessandra Longaretti², Elena Battaglioli², Maria Passafaro¹, Silvia Bassani¹

¹CNR Neuroscience Institute, Milano Italy; ²BIOMETRA, Department of Medical Biotechnologies and Translational Medicine, University of Milano, Italy

PCDH19 gene (Xq22) encodes protocadherin-19 (PCDH19), a transmembrane protein that belongs to the cadherin superfamily of calcium-dependent cell-adhesion molecules. *PCDH19* mutations cause female epilepsy (PCDH19-FE), a pathology characterized by early-onset epilepsy, intellectual disability and autism. PCDH19 consists of 6 extracellular cadherin domains, a transmembrane region and a cytoplasmic C-terminal tail (CT) harboring a nuclear localization signal. By biochemical and immunofluorescence assays in primary rat hippocampal neurons we showed that PCDH19 is cleaved by the gamma-secretase upon sustained NMDA receptor (NMDAR) activation and that the resulting fragment corresponding to the CT enters the nucleus. We found that CT overexpression in cultured neurons decreases immediate early genes (IEGs) expression levels. Conversely, PCDH19 shRNA-mediated downregulation increases IEGs transcripts.

IEGs activation represents the first and most relevant transcriptional event in response to stimuli that modulate neuronal excitability and synaptic plasticity. Among the epigenetic regulators of IEGs there is the CoREST complex and we showed that PCDH19 CT is able to associate with key components of this corepressor complex, namely LSD1, CoREST and SRF. Notably, PCDH19 cleavage occurs also *in vivo* upon epileptogenic stimuli, as demonstrated by CT generation in hippocampal homogenates from mice that experienced pilocarpine induced-seizures. We therefore hypothesize that PCDH19 cleavage might represent a homeostatic mechanism in response to strong neuronal activation that prevents IEGs overactivation, possibly via the modulation of the CoREST complex by interaction with PCDH19 CT.

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

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS: NEW THERAPEUTIC TARGETS OF GLIOMA AND GLIOBLASTOMA CELLS?

Francesca Fasoli¹, Susanna Pucci¹, Vanessa Mucchietto¹, Roberta Benfante¹, Dilascio Simona¹, Pallavicini Marco², Bolchi Cistiano², Francesco Clementi¹, Cecilia Gotti¹

¹CNR Neuroscience Institute, Milano, Italy; ² Department of Pharmaceutical Science, University of Milano, Milano, Italy

One of the major controversies of contemporary medicine is created by the increased consumption of nicotine and growing evidence of its connection to cancer. Nicotine, the addictive and most active component of tobacco smoke, does not initiate tumorigenesis in humans or rodents but it can alter the physiology of various cells by inducing the secretion of growth factors, neurotransmitters and cytokines, and promotes tumour growth and metastasis by inducing cell-cycle progression, migration, invasion, angiogenesis, and the evasion of apoptosis. The tumour-promoting effects of nicotine, its cotinine metabolite, and nitrosamine derivatives are due to their binding to neuronal acetylcholine nicotinic receptors (nAChRs).

Gliomas and glioblastomas (GBMs), which account for about 80% of all malignant brain tumours, originate from different glial elements, and are characterized by substantial radio-resistance, rapid cell proliferation, highly invasive growth, and local recurrences after surgical treatment.

Cigarette smoking plausibly influences the development of gliomas, but the role of smoking in the etiology of malignant glioma remains controversial. As nAChRs are expressed in glial cells, but very little is known about their signalling mechanisms, the aim of this study was to investigate the role of these receptors in glioma and GBM cells.

We molecularly established which nAChR subtypes are expressed in the U87MG glioma cell line and GBM primary cultures, and pharmacologically characterised the effects of nicotine, nicotine nitrosamine metabolites, and nAChR-selective antagonists on cell proliferation. It was found that nicotine and nitrosamines increase cell proliferation by activating $\alpha 7/\alpha 9$ -containing nAChRs. This activation for 5-30 minutes increased pAKT levels, and could be prevented by selective and non-selective nAChR antagonists. The results of these pharmacological and biochemical studies were confirmed by silencing experiments in which we found that knocking down $\alpha 7$, $\alpha 9$ and $\alpha 5$ subunits prevents nicotine-induced cell proliferation and signalling. We also found that long-term chronic treatment (six days) with $\alpha 7$ and $\alpha 9$ nAChR antagonists reduces glioma cell proliferation under basal conditions.

Magnetic resonance spectroscopy studies have shown that high-grade gliomas have higher total choline levels than low-grade gliomas. In particular, phosphocholine (PC) is abundant in high-grade gliomas, whereas glycerophosphocholine and free choline are predominant in low-grade gliomas. In order to identify possible endogenous ligands of nAChRs, we analyzed the supernatants of cultured glioma cells and, together with other neurotransmitters, detected the presence of choline (a selective agonist of native $\alpha 7$ -containing nAChRs and heterologously expressed $\alpha 9$ - $\alpha 10$ nAChRs). Ongoing studies are investigating the effects of choline and the neurotransmitters on glioma proliferation and signalling.

Taken together, these findings highlight the pathophysiological role of $\alpha 7$, $\alpha 9$ and $\alpha 5$ -containing nAChRs in promoting glioma cell growth and intracellular signalling, thus making them possible targets for new therapeutic strategies.

GLIOBLASTOMA EXOSOMES SHAPE TUMOR ENVIRONMENT ENHANCING CANCER CELL INVASIVENESS

Matteo Tamborini^{*1,2}, Eleonora Vannini^{*1}, Lorenzo Bello³, Giulio Maira³, Maurizio Fornari³, Elisa Setten⁴, Massimo Locati⁴, Daisy Sproviero⁵, Cristina Cereda⁵, Lorena Passoni¹, Michela Matteoli^{1,2}

¹Laboratory of Pharmacology and Brain Pathology, Humanitas Research Hospital, Rozzano (MI), Italy;

²CNR Neuroscience Institute, Milano, Italy; ³Neurosurgery Unit, Humanitas Research Hospital, Rozzano (MI), Italy, ⁴BIOMETRA, University of Milano, Italy; ⁵C. Mondino National Institute of Neurology Foundation, Pavia, Italy

** equally contributed to this work*

Glioblastoma multiforme (GBM) is the most aggressive and common primary brain tumor, highly proliferative and invasive, characterized by remarkable biological heterogeneity. The standard of care consists in surgical removal of the tumor mass followed by cycles of radio- and chemotherapy. Unfortunately, this treatment isn't effective, mostly due to recidives. Indeed, the median survival expectancy for GBM is 15-18 months since the first diagnosis, with a five-year survival rates <10%.

Exosomes are a subtype of extracellular vesicles which are released by all cells, during normal development and in response to pathogenic conditions. It is now clearly established that exosomes, whose composition varies with respect to the cells of origin and their physiologic status, can carry signalling molecules, which are able to modify the behaviour of neighbouring cells. Exosomes are largely detectable in the tumor and in biofluids of cancer patients, where they might act as carriers of molecular and oncogenic signatures involved in the regulation of oncogenic signalling between tumor cells and stroma, thus possibly representing the targets for new therapeutic strategies.

Our studies focused on the molecular features and functional effects of exosomes in the brain immune-microenvironment modulation and GBM cell invasiveness.

Exosomes were extracted from three patient-derived GBM stem-like cell (GSC) cultures established from GBM patient surgical samples and subsequently characterized in terms of quantity and dimensions. When hGBM cells were incubated with GSCs' exosomes, an increased speed and migration of recipient GBM cells was observed. Also, GSCs' exosomes caused a specific induction of TNF α and IL8 in microglia and human macrophages. Interestingly, pre-treating GSCs' exosomes with Gap27, a connexin-43 (Cx43) mimetic peptide which inhibits gap-junction mediated communication, prevented these effects. Overall, these data provide new insights into the role of exosomes in GBM progression. In particular, we demonstrated that GSCs' exosomes are able to shape the surrounding environment, and make it more favourable for tumoral growth.

LIPOSOMES TREATMENT ANTAGONIZED DENDRITIC SPINE LOSS AND REDUCTION OF NEUROGENESIS IN HIPPOCAMPUS OF CHRONICALLY STRESSED RATS

Clara Porcedda¹, Francesca Biggio¹, Laura Boi¹, Valentina Locci², Gino Toffano³; Giovanni Biggio^{1,2}; Maria Cristina Mostallino⁴

¹*Department of Life and Environmental Sciences, section Neuroscience, University of Cagliari, Italy;*

²*Department of Psychiatry, University of Illinois at Chicago, Chicago, IL, USA;* ³*FIDIA Farmaceutici, Abano Terme, Padova, Italy;* ⁴*CNR Neuroscience Institute, Cagliari, Italy*

Phosphatidylserine is a naturally occurring phospholipid which is found in the cell membranes of a wide variety of organism from bacteria to man. The presence of phosphatidylserine in the neuronal membranes is not limited to a static structural function but it also important to the regulation of many metabolic processes, indicating that this phospholipid may play a role in regulating crucial cerebral functions such as neuronal excitability, message transduction, neurotransmitter activity and neuronal plasticity.

Given that treatment with phospholipids improves brain neuron activity while pathological processes and/or natural aging reduce the renewal of the phospholipids membrane component, we used phospholipids liposomes, containing phosphatidylserine and phosphatidylcholine to prevent or ameliorate the negative effects of stress in neuronal plasticity. Neurogenesis and dendritic spine density were evaluated in stressed rats treated with liposomes. Liposomes were intraperitoneally administered (once of day) for 4 weeks in rats exposed to chronic unpredictable stress for 5 weeks. As expected the neurogenesis and dendritic spine density were decreased in rats exposed to chronic stress. On the contrary, liposomes treatment abolished the reduction of neurogenesis and dendritic spine density elicited by chronic stress. Moreover, treatment with liposomes increased the density of dendritic spine in control not stressed rats. These results demonstrate that liposomes treatment has great efficacy in antagonizing the neurochemical and molecular consequences elicited by chronic exposure to stress in the brain. The mechanisms underlying the beneficial effects of liposomes might be mediated through actions exerted by phospholipids on neuronal membranes, neurotransmitters and/or interaction with trophic factors (NGF, BDNF). These mechanisms in turn might increase the efficacy of such treatment in people with impairment of cognitive function.

Founded by Fidia Farmaceutici

GENDER DIFFERENCES IN A NEW POTENT 5HT2A AGONIST EFFECTS: NEUROCHEMICAL AND BEHAVIORAL STUDIES AFTER 25I-NBOME ADMINISTRATION

Nicholas Pintori¹, Cristina Miliano¹, Giulia Margiani¹, Andrea Ossato³, Sabine Bilel³, Matteo Marti³,
Maria Antonietta De Luca^{1,2}

¹*Department of Biomedical Sciences, Neuropsychopharmacology Section, University of Cagliari, Italy;*

²*National Institute of Neuroscience, University of Cagliari, Italy;* ³*Department of Life Sciences and
biotechnology (SveB), University of Ferrara, Italy*

25I-NBOMe, commonly called “N-Bomb”, is a new synthetic compound, recently abused for its psychedelic and entactogenic effects; it is available on internet as a legal alternative to LSD, and as a surrogate of methamphetamine as well. It acts as full agonist of 5-HT_{2A} receptor with high affinity on human and rat 5-HT_{2A} receptors ($K_i = 0.044$ nM and $K_i = 0.087$ nM, respectively). Users are often unaware of ingesting fake LSD, and several intoxication cases and some fatalities have been reported after the ingestion. Overdoses of “N-Bomb” can cause several effects such as tachycardia, hypertension, seizures, and agitation persisting for up to three days. We decided to test 25I-NBOMe in both males and females to evaluate if there were gender differences in the pharmacological effects. In the current literature, there are no data about the abuse liability of this compound and its pharmacological effects.

By *in vivo* microdialysis studies, we evaluate the effects of 25I-NBOMe (0.3-1mg/kg/ip) on dopamine (DA) and serotonin (5-HT) transmissions, both in male and female rats, moreover, sensorimotor studies, body temperature evaluation and nociception tests, were performed in both genders.

Our results showed that the phenethylamine 25I-NBOMe is more active in females, compared to males, in increasing DA transmission in NAc shell and in the mPFC; behavioural data showed that this compound caused visual alterations in both sexes, whereas core temperature in females is heavily affected, compared to males; indeed, the highest dose tested exerts an analgesic effect prominent in male rats, compared to female rats.

Taken together these results suggest that 25I-NBOMe affects DA and 5-HT transmissions in male and females in a different way, highlighting gender differences that can influence the frequency of ingestion, as well as the psychoactive effects, and the long-term effects. Further investigations are necessary to examine in depth the reason of these gender differences.

GAMMA-HYDROXYBUTYRIC ACID INDUCES CHANGES IN RELATIVE CEREBRAL BLOOD VOLUME

Anna Lisa Muntoni¹, Hanbing Lu², Clara A. Scholl^{2,3}, Elliot A. Stein²

¹CNR Neuroscience Institute, Cagliari, Italy; ²Neuroimaging Research Branch, National Institute on Drug Abuse, National Institutes of Health, Baltimore, USA

Functional magnetic resonance imaging (fMRI) provides a powerful means to map changes in specific brain regions in response to pharmacological agents. Here we have utilized the cerebral blood volume (CBV) fMRI technique to investigate the acute effects of the recreational drug gamma-hydroxybutyric acid (GHB), also known as “liquid ecstasy”, on the rat brain. Indeed, CBV fMRI employing monocrystalline iron oxide nanoparticle (MION) contrast agent, has been shown to yield a better spatial localization of the active brain regions, an increase in contrast-to-noise ratio and a higher statistical power compared with experimental blood oxygenation level dependent contrast (BOLD) studies. Systemic GHB administration produced dose-dependent and region-specific fMRI signal changes manifested either as CBV signal increases or decreases. Signal increases were located predominantly in cortical regions such as the anterior cingulate and prelimbic cortex. In contrast, CBV signal decreases were seen in several subcortical regions, including the nucleus accumbens, caudate putamen/striatum, hippocampus and thalamus. This study demonstrates that GHB-induced regional brain activation and inhibition can be detected by using pharmacological fMRI methods.

BEHAVIORAL EFFECTS OF THE NOVEL PSYCHOACTIVE SUBSTANCE (NPS) METHOXETAMINE IN RATS

Mary Tresa Zanda¹, Silvia Antinori¹, Anna Mutti², Laura Mancini², Laura Padovani², Paola Fadda¹, Walter Fratta¹, Cristiano Chiamulera², Liana Fattore³

¹Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Neuropsychopharmacology Laboratory, Division of Pharmacology, University of Verona, Italy; ³CNR Institute of Neuroscience, Cagliari, Italy

Methoxetamine (MXE) is one of the new psychoactive substances that in the last decade emerged at an impressive rate via the Internet. Although sold as safer than ketamine, fatal and non fatal intoxication has been reported after MXE use (Zanda et al., 2016). Only very few studies have started recently to investigate the toxicological and pharmacological effects of MXE. The aim of our study was to evaluate the effect of acute MXE administration on motor, analgesic and emotional behaviour in rats and its rewarding effects in the drug discrimination and self-administration substitution test.

Findings showed that MXE (0.5-5 mg/kg) significantly affects motor activity in a dose- and time-dependent manner, with low and high doses inducing hyper- and hypo-motility, respectively. A similar biphasic effect of MXE was also observed in test measuring analgesia and emotional responses (Zanda et al., 2017). In particular, at low/intermediate doses (0.5 and 1 mg/kg) MXE induces anxious and/or obsessive-compulsive traits as shown in the marble burying test and slightly (but not significantly) increases sociability in the social interaction test, although it does not induce spatial anxiety in the elevated plus maze test. At the dose of 2.5 mg/kg, MXE significantly decreased the time spent in social interactions, while at the highest dose tested (5 mg/kg) MXE significantly i) induces transient analgesia, as revealed in the hot plate but not in the tail flick test, ii) increases the time spent in closed arms in the elevated plus maze and iii) reduced immobility time while increasing swimming activity in the forced swim test, suggesting an antidepressant effect. Moreover, MXE fully generalizes to ketamine interoceptive stimulus in a two-lever operant drug discrimination paradigm in rats trained to discriminate ketamine from saline (Chiamulera et al., 2016) and substitutes for ketamine in a drug self-administration substitution study (Mutti et al., 2016), thus showing to own ketamine-like discriminative stimulus properties. Altogether, our data indicate that MXE differentially affects motor activity, behaviour and emotional states in rats depending on the dose tested, and possesses discriminative and reinforcing effects which could explain its increasing abuse worldwide.

References

- Chiamulera C, Armani F, Mutti A, Fattore L (2016) *Behav Pharmacol* 27(2-3 Spec Issue):204-10.
 Mutti A, Aroni S, Fadda P, Padovani L, Mancini L, Collu R, Muntoni AL, Fattore L, Chiamulera C (2016) *Psychopharmacology* 233:2241-51.
 Zanda MT, Fadda P, Chiamulera C, Fratta W, Fattore L (2016) *Behav Pharmacol* 27:489-96.
 Zanda MT, Fadda P, Antinori S, di Chio M, Fratta W, Chiamulera C, Fattore L (2017) *Br J Pharmacol*, in press.

ANXIOLYTIC EFFECT OF A STANDARDIZED EXTRACT OF *SALVIA MILTIORRHIZA* IN RATS

Carla Lobina¹, Mauro A.M. Carai², Gian Luigi Gessa¹, Giancarlo Colombo¹

¹CNR Neuroscience Institute, Section of Cagliari, Italy;

²Cagliari Pharmacological Research, Cagliari, Italy

Background – Preparations from roots of *Salvia miltiorrhiza*, an herb widely used in traditional Chinese medicine, have been reported to induce a series of central effects, including sedation and amelioration of different alcohol-related behaviors. Additional ethnopharmacological information suggests that preparations from roots of *Salvia miltiorrhiza* possess “calming” properties.

Aims – The present study was designed to assess the anxiolytic (or “calmness”-inducing) effects of a standardized extract of *Salvia miltiorrhiza* roots using two validated rat models of “anxiety”: Elevated Plus Maze (EPM; a test based on the innate unconditioned fear of rodents for open, heightened, and unprotected environments and their preference for sheltered, enclosed, and dark spaces) and Stress-Induced Hyperthermia (SIH; a physiological response to anxiogenic and stressful events).

Methods – Male, adult Wistar rats were treated, acutely and intragastrically, with *Salvia miltiorrhiza* extract (0, 50, and 100 mg/kg; i.g.); 30 min later, rats were exposed to the EPM. SIH was evaluated as the difference in rat body temperature before and after exposure to the EPM (rat exposure to the EPM represented indeed the anxiogenic and stressful event inducing SIH).

Results – Treatment with 100 mg/kg *Salvia miltiorrhiza* produced robust anxiolytic effects at the EPM test; specifically, it increased (a) percent of entries into open arms, (b) percent of time spent in open arms, (c) total number of head dips, (d) number of unprotected head dips, and (e) number of end-arm explorations in open arms, without any alteration in spontaneous locomotor activity (indicative of the lack of sedative and motor-incoordinating effects). Treatment with 100 mg/kg *Salvia miltiorrhiza* extract also suppressed SIH response.

Conclusions – These data demonstrate the ability of an extract of *Salvia miltiorrhiza* roots to produce anxiolysis in two different rodent models of “anxiety”. Additionally, these data generalize to roots of *Salvia miltiorrhiza* recent literature data demonstrating that an essential oil obtained from the aerial parts of *Salvia miltiorrhiza* exerted robust anxiolytic effects in rats. All together, these data suggest that *Salvia miltiorrhiza* is likely rich in active compounds with anxiolytic potential.

BEHAVIOURAL AND NEUROCHEMICAL CHANGES INDUCED BY Δ^9 -TETRAHYDROCANNABINOL IN MICE PRE-EXPOSED TO NICOTINE

L. Ponzoni¹, D. Braidà², M. Moretti^{2,3}, F. Clementi^{2,3}, M. Sala³, C. Gotti³

¹Fondazione Umberto Veronesi, Milano, Italia; ²BIOMETRA, Università degli Studi di Milano, Italia;

³CNR Istituto di Neuroscienze, Milano, Italia

Marijuana and tobacco are substances frequently used by adolescents. Among adolescent tobacco smokers who also smoke marijuana, the frequency of marijuana use is associated with greater levels of nicotine addiction [1]. Many smokers have recently switched from standard to electronic cigarettes (e-cigs) as an alternative means of nicotine delivery or smoking cessation aids despite the unfavourable opinion of the World Health Organisation and the lack of scientific evidence.

The first aim of this study was to test whether seven weeks' exposure to e-cigs or standard cigs reinforces the subsequent effects of Δ^9 -tetrahydrocannabinol (THC) using a model that has recently been validated in our laboratory [2]: two or 60 days after nicotine withdrawal, mice are intraperitoneally injected with a low rewarding dose of THC (0.01 mg/kg) or vehicle for five days and submitted to the conditioned place preference (CPP) task. As cig and e-cig smoke cessation may be involved in the pathogenesis of major psychiatric disorders such as depression, the second aim was to evaluate depressive-like behaviour 60 and 90 days after withdrawal using the tail suspension and sucrose preference tasks. The third aim was to correlate the behavioural findings with the possible neurochemical and neurobiological changes involved in reward and depression (variations in glutamatergic and cannabinoid subtype receptor levels and functions).

The mice exposed to e-cig or cig were more sensitive to THC than control mice both two and 60 days after withdrawal as shown by the increased time spent in the drug-associated compartment. They also showed depressive-like behaviour starting from 60 days as shown by an increased duration of immobility and anhedonia. The mice that performed the CPP test two days after nicotine withdrawal did not show any difference in the activity of CB1 receptors in the nucleus accumbens (a brain area that is important for the rewarding properties of drugs of abuse); however, the mice that performed the CPP test 60 days after withdrawal showed less GTP γ S activity than the control mice, and a significant increase in GluR1 receptor density. Our results show that e-cig and cig exposure induces altered responses to THC-induced CPP and depressive-like behaviour probably because of the involvement of multiple neurotransmitters.

References

1. Rubinstein et al. 2014 Drug and Alcohol Dependence 141:159–162.
2. Ponzoni et al. 2015 Eur Neuropsychopharmacol 25(10):1775-86.

MECHANISMS UNDERLYING THE SUPPRESSING EFFECT OF COR659 ON ALCOHOL AND CHOCOLATE SELF-ADMINISTRATION

Irene Lorrain¹, Giancarlo Colombo¹, Mauro A.M. Carai², Gian Luigi Gessa¹, Paola Maccioni¹

¹CNR Neuroscience Institute, Section of Cagliari, Italy;

²Cagliari Pharmacological Research, Cagliari, Italy

Background – COR659 is a new, positive allosteric modulator (PAM) of the GABA_B receptor. Previous studies demonstrated that COR659, similarly to all GABA_B PAMs tested to date, reduced operant alcohol self-administration in rats. Quite unexpectedly however, a series of additional experiments revealed that treatment with COR659 also suppressed operant self-administration of highly palatable foods (sucrose and chocolate solutions) in rats. Literature data indicating that GABA_B PAMs do not affect behaviors motivated by palatable foods suggested that the latter effects of COR659 were likely mediated by receptor system(s) other than the positive allosteric modulation of the GABA_B receptor.

Aims – The present study was designed to assess the contribution of the GABA_B and cannabinoid CB₁ receptors in the mediation of the suppressing effect of COR659 on alcohol and chocolate self-administration in rats. Involvement of the cannabinoid CB₁ receptor was suggested by the results of preliminary *in vitro* experiments indicating that micromolar concentrations of COR659 (i) displaced [³H]CP55940 from the cannabinoid CB₁ receptor binding site and (ii) inhibited WIN 55,212-2-induced stimulation of [³⁵S]GTPγS binding to cannabinoid CB₁ receptors.

Methods – Male, selectively bred Sardinian alcohol-preferring (sP) rats were trained to lever-respond for alcohol (15% v/v) under a fixed ratio (FR) 4 (FR4) schedule of reinforcement. Male Wistar rats were trained to lever-respond for chocolate [5% (w/v) Nesquik® in water] under a FR10 schedule of reinforcement. Once lever-responding had stabilized, rats of both groups (alcohol and chocolate) were exposed to self-administration sessions after treatment with the combination of the GABA_B receptor antagonist, SCH50911 (0 and 2 mg/kg, i.p.), or the cannabinoid CB₁ receptor antagonist, AM4113 (3 mg/kg, i.p.), and COR659 (5 mg/kg, i.p.). SCH50911 and AM4113 doses were chosen as to be totally ineffective, when given alone, on alcohol and chocolate self-administration.

Results – Pretreatment with SCH50911 only partially blocked COR659-induced reduction of alcohol self-administration, being totally ineffective on reduction of chocolate self-administration. Conversely, pretreatment with AM4113 fully blocked COR659-induced reduction of chocolate self-administration, being totally ineffective on reduction of alcohol self-administration.

Conclusions – COR659 apparently exerts its behavioral effects *via* a composite mechanism: (i) positive allosteric modulation of the GABA_B receptor, responsible for a large proportion of reduction of alcohol self-administration; (ii) an action at other receptor system(s), including the cannabinoid CB₁ receptor, through which COR659 likely affects seeking and consumption of highly palatable foods.

DIFFERENTIAL RESPONSIVENESS OF NUCLEUS ACCUMBENS DOPAMINE TO STIMULI INSTRUMENTALLY CONDITIONED TO CONVENTIONAL OR DRUG REINFORCERS

Augusta Pisanu¹, Daniele Lecca², Valentina Bassareo², Annesha Sil², Roberto Frau², Andrea Scifo², Gaetano Di Chiara^{1,2}

¹CNR Neuroscience Institute, Cagliari, Italy; ²Department of Biomedical Sciences, University of Cagliari, Italy

Nose-poking (NP) and lever-pressing (LP) represent two different response modalities which have been utilized in the self-administration (SA) paradigm. NP is part of a rodent's natural exploratory repertoire, whereas LP requires the animal to learn the action of pressing a lever in order to obtain a reward.

The objective of these experiments were to study differences in the mesolimbic dopaminergic responsiveness induced by heroin or sucrose self-administration (SA) using LP and NP as operant responses.

Male Sprague-Dawley rats were trained for 10 days on an FR1 schedule to acquire heroin (0.05 mg/kg) or sucrose SA using NP or LP. After acquisition of SA behaviour, microdialysis was carried across three consecutive days, in which dialysate dopamine (DA) was measured in the nucleus accumbens (NAc) shell and core of animals, under a heroin or sucrose SA session in the first day; under an extinction session on the second day and under a passive heroin or sucrose administration on the third day. Results show that during active SA, dialysate DA preferentially increased in the shell only in the NP groups using either reinforcers, while DA increased both in the shell and core in LP groups. During the extinction DA did not change from basal values in both LP and NP groups using drug reinforcers under FR1 schedule, while it did change using conventional reinforcers and, under FR5 LP paradigm, limited to the core, for drug reinforcers too. Finally, DA was observed to increase both in the shell and core during passive non-contingent presentation of either reinforcers.

These results add to the growing body of evidence about the differential involvement of the NAc shell and core in different aspects of reinforcement and incentive learning. Further, they show that the specific operant response utilised for modelling SA behaviour is able to determine the pattern of activation of DA transmission in the NAc core and shell.

INVOLVEMENT OF CORE CLOCK GENES IN LITHIUM RESPONSE

Claudia Pisanu¹, Donatella Congiu¹, Carla Melis¹, Andrea Angius², Giovanni Severino¹, Raffaella Ardau³, Caterina Chillotti³, Maria Del Zompo^{1,3}, Alessio Squassina¹

¹Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Istituto di Ricerca Genetica e Biomedica, CNR, Cagliari, Italy; ³Unit of Clinical Pharmacology of the University Hospital of Cagliari, Italy

Background: In a recent paper, Geoffroy et al. (2017) reported an effect of in vitro lithium treatment (1 mM) on the expression of circadian genes in lymphoblastoid cell lines (LCL) derived from patients with bipolar disorder (BD) characterized for lithium response. Here, we present data on the effect of lithium in vitro on the same gene-set using our dataset of microarray data from LCLs of BD patients with different response to lithium (excellent responders, ER; non-responders, NR) and healthy controls (HC).

Methods: Patients were selected retrospectively from a larger sample of BD patients characterized for lithium response using the Alda scale as in Geoffroy et al. The selected sample included 10 ER, 10 NR and 10 HC. All patients were taking lithium at the time of blood collection. LCLs from each subject were split in two lines: one was treated with LiCl 1mM for 7 days, while the other was cultured in regular medium. Total RNA was collected at day 7 and used for genome wide expression analysis with GeneChip® Human Gene 1.0 ST Arrays, (Affymetrix, CA, USA). The 17 clock genes analysed by Geoffroy were tested for differential expression using the linear model implemented in limma (R). The hypergeometric test was used to assess over-representation of clock genes among significant ($p < 0.05$) genes in our dataset. P-values were corrected for multiple comparisons using Bonferroni based on the number of genes-of-interest ($n=17$). Among the three time points analysed by Geoffroy et al. (day 2, 4 and 8), day 8 was the most comparable with our treatment protocol of one week, which is generally considered as the standard time point for in vitro lithium chronic treatment.

Results: Among the 17 genes, lithium only affected the expression of PER3 in ER, though the effect did not survive Bonferroni correction ($FC_{Li+/Li-}=1.19$, uncorrected $p=0.0048$; corrected $p = 0.08$;). This gene was upregulated by lithium in the paper by Geoffroy and colleagues but at day 4. In our dataset, no gene was affected by lithium in NR. To gather more insights into the role of clock genes in lithium response and BD, we extended the analysis to untreated LCLs (baseline) from ER, NR and HC. Comparison of baseline expression levels showed four underexpressed (ARNTL, $FC=0.72$, $p=0.02$; ARNTL2, $FC=0.75$, $p=0.01$; CRY2, $FC=0.91$, $p=0.046$ and TIMELESS, $FC=0.82$, $p=0.02$) and one overexpressed (BHLHE40, $FC=1.58$, $p=1.9E-05$) genes in ER compared to NR. However, only BHLHE40 was significant after Bonferroni correction ($p=0.00032$), suggesting this gene could be involved in modulating lithium response in BD despite not representing a lithium target. BHLHE40 encodes a protein that can repress CLOCK/ARNTL's transactivation of PER1. Since excellent lithium responders have been previously suggested to represent the core phenotype of BD, we compared expression levels of ER with HC. We observed significant different expression for 9 out of 17 genes in untreated LCLs from ER, with 6 genes showing statistically significant corrected p values. The hypergeometric test showed an over-representation of circadian genes in the microarray data from ER compared with HC (total =5615; $p = 0.016$), while the test was not significant in NR versus HC.

Discussion: We suggest that clock genes could be only marginally involved in the mechanism of action of lithium, and that BHLHE40 could be an important player in modulating clinical response to lithium. Our findings also support the hypothesis that ER to lithium may be characterized by a different genetic architecture compared to non-responders.

INTEGRATED ANALYSIS OF CONVERGING GENOME-WIDE GENOTYPING AND TRANSCRIPTOMIC DATA TO IDENTIFY GENES ASSOCIATED WITH LITHIUM RESPONSE IN BIPOLAR DISORDER

Claudia Pisanu¹, Donatella Congiu¹, Marta Costa², Caterina Chillotti³, Raffaella Ardu³, Giovanni Severino¹, Andrea Angius⁴, Urs Heilbronner⁵, Liping Hou⁶, Francis J. McMahon⁶, Thomas G. Schulze^{5,6}, Maria Del Zompo^{1,3}, Alessio Squassina¹

¹Department of Biomedical Sciences, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²The Francis Crick Institute, London, UK; ³Unit of Clinical Pharmacology, University Hospital of Cagliari, Italy; ⁴Istituto di Ricerca Genetica e Biomedica, CNR, Cagliari, Italy; ⁵Institute of Psychiatric Phenomics and Genomics (IPPG), Medical Center University of Munich, Campus Innenstadt, Germany; ⁶Intramural Research Program, National Institute of Mental Health, NIH, Department of Health & Human Services, Bethesda, MD, USA

Lithium is the mainstay treatment in bipolar disorder (BD) for its effectiveness in the acute phases of illness and in prevention of recurrences. Response to lithium has a strong genetic background, but findings from pharmacogenetic studies have only marginally succeeded in identifying lithium response genes. Moreover, lithium's mechanism of action is complex, and while it modulates the expression and function of hundreds of molecular players, most of these effects have been shown to be unspecific and not relevant for its clinical efficacy. It is therefore of crucial importance to conduct studies aimed at identifying lithium-modulated genes that are most likely responsible for predisposing patients to respond to the treatment. To this regard, approaches exploiting data from different omic platforms can constitute powerful tools to identify genes and pathways involved in lithium's response with a potentially higher translational value.

In the present study we applied an integrated analytical approach using genome-wide expression and genome-wide genotyping data from BD patients characterized for lithium response aiming at identifying, through convergent findings, lithium-responsive genes that may serve as biomarkers of its clinical efficacy.

We tested the effect of *in vitro* treatment with lithium chloride 1mM for one week on the transcriptome of lymphoblastoid cell lines (LCL) from 10 full responders (FR) and 10 non-responders (NR) patients and identified genes significantly influenced by the treatment. Genes were tested for differential expression after *in vitro* lithium treatment in both FR and NR using the paired t-test implemented in limma in R (v. 3.3.3). We focused on genes altered by lithium exclusively in FR, as these genes could be involved in modulating clinical efficacy of this drug. Findings from this approach were integrated with findings from a gene-based analysis performed with MAGMA (de Leeuw et al., 2015) using genome-wide genotyping from an extended sample of 205 BD patients characterized for lithium response (Alda et al., 2002; Manchia et al., 2013).

The expression of 29 genes was significantly changed by lithium in FR but not in NR. Two of these genes, zinc finger protein 429 (ZNF429; $p = 0.0003$) and zinc finger protein 493 (ZNF493; $p = 0.0005$), were respectively the fourth and the fifth most significant genes in the gene-based analysis. Validation with quantitative real-time PCR confirmed the under-expression of ZNF493 in FR after lithium treatment [fold change (FC) = 0.71; $p = 0.036$], while ZNF429 showed a trend for downregulation (FC = 0.82, $p = 0.06$).

Using convergent analyses of data from genome wide genotyping and gene expression studies, we identified two zinc finger protein genes as lithium-responsive targets that may be involved in modulating lithium efficacy in BD. These genes codify for zinc finger proteins, a large family of functional domains involved in several functions comprising transcriptional activation, regulation of apoptosis and protein folding. To our knowledge, this is the first evidence supporting the involvement of zinc finger proteins in lithium mechanism of action and response.

References

- de Leeuw et al. (2015). *PLoS Comput Biol.* 11, e1004219.
 Grof et al. (2002). *J Clin Psychiatry* 63, 942-947.
 Manchia et al. (2013). *PLoS One* 8, e65636.

HEROIN ADDICTION AND LEUKOCYTE TELOMERE LENGTH

Carla Melis¹, Claudia Pisanu¹, Donatella Congiu¹, Eleni Merkouri Papadima¹, Alessio Squassina¹, Caterina Chillotti², Maria Erminia Stochino², Maria Del Zompo^{1,2}

¹Department of Biomedical Science, Section of Neuroscience and Clinical Pharmacology, University of Cagliari, Italy; ²Unit of Clinical Pharmacology, University Hospital of Cagliari, Italy

Background - Telomeres are repetitive sequences at the end of chromosomes that play a key role in the maintenance of genomic stability. In general, during each cellular division, the telomeres shorten but also other external factor, like various mental states, depressed mood, drug and alcohol addiction can lead to an accelerated erosion of the chromosome terminal portion. To this end, the present study sought to investigate the correlation between heroin abuse and leukocyte telomere length (LTL). For a subgroup of heroin-dependent patients and healthy controls, we also analysed the association between LTL and the 5-HTTLPR polymorphism. The 5-HTTLPR polymorphism is a 44 bp insert / deletion located in the promoter region of the gene encoding for the serotonin transporter (hSERT). The short variant (S\S) is associated with a lower expression of 5-HTT and, as recently shown in a Chinese population, with shorter LTL.

Methods - The study was conducted on a sample of 99 heroin-dependent patients, in methadone maintenance therapy, and 99 healthy controls. The patients were diagnosed by the Drug Addiction Service in Cagliari (ASL8), according to the criteria of DSM-IV. The genomic DNA extraction was performed by conventional salting-out method and the relative quantification for LTL was carried out with qPCR by SYBR Green Assay using Step One Plus Instrument (ThermoFisher).

The LTL was calculated using the $2^{-\Delta\Delta CT}$ method ($\Delta\Delta CT = \Delta CT \text{ sample} - \Delta CT \text{ calibrator}$; $\Delta CT \text{ sample} = CT \text{ Tel gene} - CT \text{ Hgb gene}$). The Polymerase Chain Reaction (PCR) was used to identify genotypes for the 5-HTTLPR polymorphism. The association between LTL and quantitative (e.g., age at sampling) and dichotomous variables (e.g., sex, diagnosis for heroin addiction) was evaluated using the Spearman correlation test and the U test of Mann-Whitney, respectively. To evaluate the association between LTL and clinical variables, correcting for age at sampling, a linear regression model was constructed using LTL as the dependent variable, clinical variables as predictors and age as covariate. Finally, the association between LTL and the 5-HTTLPR polymorphism genotype was also evaluated using a linear regression model with LTL as the dependent variable, the genotype as a predictor, and the age of withdrawal as a covariate.

Results - We found a negative correlation between LTL and age at sampling (Spearman rho = -0.17, p = 0.015) and no association with sex. The Mann-Whitney's U test did not highlight a difference in mean LTL between heroin-dependent patients and healthy controls.

No clinical variable analysed was associated with LTL. Finally, the linear regression model did not reveal a significant association between LTL and the genotype for 5-HTTLPR polymorphism.

Discussion - Our study didn't show a significant correlation between LTL, heroin addiction and the genotype for 5-HTTLPR polymorphism. This finding does not support the hypothesis of heroin addiction as an illness associated with accelerated telomere shortening. However, more studies on larger independent samples controlling for potential variables suggested to be able to exert an impact on LTL are necessary.

A NOVEL THERAPEUTIC STRATEGY FOR THE PREVENTION OF THE ONSET OF DYSKINESIA IN THE THERAPY OF PARKINSON'S

Annalisa Pinna¹, Giulia Costa², Liliana Contu², Marcello Serra², Nicola Simola², Manolo Carta², Micaela Morelli^{1,2}

¹CNR Neuroscience Institute, Cagliari, Italy; ²Department of Biomedical Sciences, University of Cagliari, Cagliari, Italy

The mixed serotonin 5-HT_{1A/1B} receptor agonist eltoprazine suppressed dyskinetic-like behavior in animal models of Parkinson's disease (PD), but simultaneously reduced L-dopa-induced motility. Moreover, adenosine A_{2A} receptor antagonists, as preladenant, significantly increase L-dopa efficacy in PD without exacerbating dyskinetic-like behavior. Our previous report demonstrated that combination of eltoprazine, with preladenant produces prevention and reduction of L-dopa-induced dyskinesia, without impairing the efficacy of L-dopa in relieving motor symptoms.

On this basis, we hypothesize that the early combined administration of eltoprazine and preladenant may produce prevention of the onset of L-dopa-induced-dyskinesia in a rodent model of PD.

Unilateral 6-OHDA-lesioned L-dopa-non primed rats, were treated for two weeks with eltoprazine (0.6 mg/kg) and/or preladenant (0.3 mg/kg), singularly or in combination with L-dopa (4mg/kg), and abnormal involuntary movements (AIMs) as index of dyskinesia, were evaluated. Four days after the last administrations all rats were treated with L-dopa. Moreover, induction of immediate-early gene *zif-268* (an index of long-term changes correlated with dyskinesia), and microglia and astroglia markers (indexes of neuroinflammation) were evaluated.

Results show that combined administration of L-dopa plus eltoprazine plus preladenant significantly prevented and delayed the onset of dyskinetic-like behaviors induced by L-dopa.

Preliminar results showed that *zif-268* was increased in striatum of rats treated with L-dopa and L-dopa plus preladenant compared with vehicle. In contrast, rats treated with eltoprazine (with or without preladenant) had lower *zif-268* activation after treatment with L-dopa.

Results suggest that combination of L-dopa with eltoprazine and preladenant may be a promising therapeutic strategy for treating motor symptoms, delaying, at the same time, the onset of dyskinesia in PD.

LYS49 PHOSPHOLIPASE A2 FROM *BOTHROPS ASPER* (Mt-II) FORMS CELL SURFACE AMYLOID-LIKE STRUCTURES THAT COLOCALIZE WITH NUCLEOLIN: A NEW FUNCTIONAL AMYLOID?

Fiorella Tonello¹, Maria Lina Massimino¹, Morena Simonato¹

CNR Neuroscience Institute, Padova, Italy

Snake venoms contain a high percentage of phospholipase A2 (PLA2) and PLA2 like proteins (natural mutant deprived of catalytic activity) homologues to the mammalian group IIA secreted PLA2. These proteins have myotoxic and cytotoxic activity but their mechanism of action is still not clear (1, 2). We recently demonstrated that myotoxin II of *Bothrops asper* (Mt-II), a Lys 49 PLA2 like protein, is internalized in myotubes and macrophages by interaction with nucleolin present on cell surface. The internalized protein is consequently localized in perinuclear and nuclear zone (3).

Here we show that, at low temperature (to inhibit the internalization process), nucleolin and *B. asper* Mt-II colocalize on surface cell structures. As human PLA2G2a has been reported to form amyloid-like structures on lipid membranes (4) and nucleolin is known to interact with, and internalize oligomeric form of beta-amyloid 42 peptide (5), we decided to enquire if the Mt-II cell surface structures are sensitive to Congo red staining. We found that Mt-II forms polymers on cell surface that, after staining with Congo red, are visible in fluorescence by excitation at 543 nm and show green birefringence in polarized light, indicating that these structures have an amyloid-like behavior. Nucleolin contains a 50 % of low complexity and disordered sequence and has a C-terminal domain rich in (R/F)GG repeats, a domain similar to that of yeast chaperons involved in interactions with and propagation of prion-like structures (6). We demonstrated, by pull-down experiments in presence of different competitors, that the (R/F)GG domain of nucleolin is involved in the interaction with Mt-II.

Moreover, by analysing the primary and tertiary structure of Mt-II, we observed that this protein possesses amyloid-like domains in strategically exposed loops.

We speculate that the amyloid-like polymerization of *B. asper* Mt-II on cell surface is functional to the interaction with nucleolin and to trigger the internalization process. Our hypothesis is that this mechanism can be implicated in the internalization of other proteins and secreted PLA2s.

References

1. Tonello and Rigoni, Cellular Mechanisms of Action of Snake Phospholipase A2 Toxins, Snake Venoms, Toxinology series 2015.
2. Tonello et al. A Lys49-PLA2 myotoxin of *Bothrops asper* triggers a rapid death of macrophages that involves autocrine purinergic receptor signaling, Cell death & disease, 3:e343, 2012.
3. Massimino et. al. Nucleolin is a receptor of *Bothrops asper* Lys49 phospholipase A2. In preparation.
4. Code et al. Amyloid-type fiber formation in control of enzyme action: interfacial activation of phospholipase A2. Biophys J. 95(1):215-24, 2008.
5. Ozawa et al., Shuttling protein nucleolin is a microglia receptor for amyloid beta peptide 1-42. Biol Pharm Bull. 36(10):1587-93, 2013.
6. Sporn and Hines, Hsp40 function in yeast prion propagation: Amyloid diversity necessitates chaperone functional complexity. 9(2):80-9, Prion. 2015.

DEVELOPMENT OF A MULTIPARAMETRIC PROGNOSTIC INDEX FOR DETERMINING REHABILITATION OUTCOME IN STROKE PATIENTS

Maria Luisa Malosio^{1,2}, Francesca Davi², Chiara Adriana Elia², Laura Straffi³, Sara Ghirmai⁴, Simona Marcheselli³, Lucia Fontana⁶, Bruno Bernardini⁴, Leonardo Tenori⁶, Alfonso Mastropietro⁵, Claudio Luchinat⁶, Michela Matteoli², Giovanna Rizzo⁵, Marco Grimaldi⁷

¹CNR Neuroscience Institute, Milano, Italy; ²Laboratory of Pharmacology and Brain Pathology, & Neuro Center, Humanitas Clinical and Research Center, Rozzano (MI), Italy; ³Stroke Unit & Neuro Center, Humanitas Clinical and Research Center, Rozzano (MI), Italy; ⁴Neurorehabilitation Unit & Neuro Center, Humanitas Clinical and Research Center, Rozzano (MI), Italy; ⁵Istituto di Bioimmagini e Fisiologia Molecolare (IBFM), CNR, Segrate (MI), Italy; ⁶Magnetic Resonance Center (CERM), University of Florence, Sesto Fiorentino (FI), Italy; ⁷Neuroradiology Unit & Neuro Center, Humanitas Clinical and Research Center, Rozzano (MI), Italy

Stroke is a main health concern with a high incidence in Italy (100,000 person/years) and a prevalence of 6.5% in the elderly population (65-84 years). Stroke in Italy is the main cause of disability, the second cause of dementia and the third cause of death. One third of stroke survivors has a high degree of disability at 1 year after the event. This determines social and psychological problems to the relatives and high health care costs. Objective of the study is to generate a multiparametric profile of a cohort of 30 stroke patients (age 40-85) hospitalized in the Stroke Unit of Humanitas Hospital in order to identify the profile associated with the initial damage, the damage at 6-12 months and the functional outcome.

The study involves

- 1) analysis of lesional and contra-lesional areas of the brain by means of functional Magnetic Resonance Imaging at 3T, allowing to assess brain functionality (fMRI), structural integrity and post stroke remodeling (DTI) at an early time after the stroke (10-15 days) and at 6-12 months;
- 2) collection of serum and plasma samples early (5 days) and at two later stages (30 days and 6-12 months) in order to investigate
 - a.) the metabolomic fingerprint of stroke patients and to evaluate its evolution at the transition from acute to chronic phase of the disease
 - b.) the inflammatory profile, investigating multiple markers (cytokines, chemokines and growth factors)
 - c.) the biochemical profile of multiple markers.

The long-term goal is to identify which parameters correlate with a better rehabilitation outcome and identify a therapeutic perspective for those who have a bad rehabilitation score.

A NOVEL CAUSATIVE GENE FOR AUTOSOMAL DOMINANT LATERAL TEMPORAL EPILEPSY (ADLTE)

Emanuela Dazzo¹, Roberto Michelucci², Valeria Vianello Dri³, Clementina Boniver⁴, Pasquale Striano⁵, Carlo Nobile¹

¹CNR Neuroscience Institute and Department of Biomedical Sciences, University of Padova, Italy;

²IRCCS-Institute of Neurological Sciences, Bologna, Italy; ³APSS Trento, Trento, Italy; ⁴University of Padova, Italy; ⁵University of Genoa, Genova, Italy

Autosomal dominant lateral temporal epilepsy (ADLTE; OMIM 600512) is one of the genetic epileptic syndromes more intensely studied. It is clinically characterized by focal seizures with auditory or aphasic symptoms, likely originated from the lateral region of the temporal lobe, and absence of detectable structural brain abnormalities. ADLTE is genetically heterogeneous and inherited with an autosomal dominant pattern with reduced penetrance (around 70%). It has been estimated that prevalence of ADLTE may account for up to 19% of familial focal epilepsies. Earlier, we identified mutations causing ADLTE in the leucine rich, glioma inactivated 1 (LGI1) and Reelin (RELN) genes. Altogether, mutations in these two genes account for the disease in about 50% of ADLTE families. LGI1 and RELN are expressed in the brain and encode secreted proteins which exert multiple important functions in brain development and functioning. The relationship between LGI1 and Reelin and the pathogenic mechanism underlying ADLTE are unknown.

Using a genomic approach based on a combination of genome-wide linkage and whole exome sequencing, we identified two variants in a novel gene, here named EPTP3, which segregated with ADLTE in the corresponding families. The variants, one missense and one indel, were validated by Sanger sequencing, were not found in 240 Italian healthy controls, are extremely rare in EVS and ExAC population databases, and are predicted to be deleterious by computational prediction tools. EPTP3 encodes an intracellular protein of known function expressed in brain and other tissues. Cell-based functional analysis of EPTP3 mutant proteins showed a significant alteration of protein activity compared to wild-type control, strongly supporting a causal role for EPTP3 in ADLTE. Future studies of the epilepsy-related function of EPTP3 and of the relationship of its protein product with LGI1 and Reelin will shed light on the pathogenesis of ADLTE.