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# Proceedings of the 29th National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.

*In Memoriam of Prof. Glauco Ambrosi*

November 15-16, 2019

*Bari University "Aldo Moro", School of Medicine*
*Bari, Italy*

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under the auspices of
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# *European Journal of Histochemistry*
## *a journal of functional cytology*

The *European Journal of Histochemistry* was founded in 1954 by Maffo Vialli and published till 1979 under the title of *Rivista di Istochimica Normale e Patologica*, from 1980 to 1990 as *Basic and Applied Histochemistry* and in 1991 as *European Journal of Basic and Applied Histochemistry*. It is now published under the auspices of the University of Pavia, Italy.
The *European Journal of Histochemistry* is the official organ of the Italian Society of Histochemistry and a member of the journal subcommittee of the International Federation of Societies for Histochemistry and Cytochemistry (IFSHC), and has been an influential cytology journal for over 60 years, publishing research articles on functional cytology and histology in animals and plants.

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- biology of cell development and senescence;
- nerve and muscle cell biology;
- cellular basis of diseases.



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# Proceedings of the 29th National Conference of the Italian Group for the Study of Neuromorphology "Gruppo Italiano per lo Studio della Neuromorfologia" G.I.S.N.



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## MAIN LECTURE

### EFFECTS OF ENDOCRINE DISRUPTORS ON NEURAL CIRCUITS AND BEHAVIOUR

G.C. Panzica, B. Bonaldo, A. Farinetti, M. Marraudino, G. Ponti, S. Gotti

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A large number of molecules of synthetic or even natural origin (*endocrine disruptors* or *endocrine disrupting chemicals*, EDCs)are able to bind hormone receptors thus potentially interfering with endocrine functions. In particular, EDCs may disrupt the development of the endocrine system with permanent effects. At first, studies on EDCs involved almost exclusively toxicological aspects, whereas the neuroendocrine and behavioural implications were less investigated.

During the last twenty years, cerebral effects of EDCs were investigated highlighting some important points:

- several behaviours and neural circuits related to their control are more sensitive endpoints than others applied in toxicological studies;
- neuropeptides and enzymes are major targets for the action of EDCs in the vertebrate brain, in particular, kisspeptin in rodents, vasotocin and vasopressin in birds and mammals, the hypothalamic NPY and POMC systems in rodents, as well as the enzyme aromatase in fish, or the enzyme NO-synthase in rodents appear the most sensitive to low levels of EDCs during early development;
- alterations of these circuits may induce profound effects on sexual behaviour, puberty, reproductive physiology. In addition, a large number of studies elucidated EDCs action on metabolic disorders and on neural circuits involved in the control of metabolism;
- the EDCs effects are generally through multiple receptors and their mechanisms of action needs to be more thoroughly explored.

A strong concern for human health has been raised by governments and the population, so an endless debate is underway for the regulation of these substances at the level of the European Commission and the European Parliament, but no definite rules have yet been promulgated.

# FIRST SESSION: NEUROIMAGING AND NERVOUS SYSTEM CIRCUITRY

## THE STRUCTURAL CONNECTIVITY OF THE DOPAMINERGIC MIDBRAIN IS TOPOGRAPHICALLY ALTERED IN SCHIZOPHRENIC PATIENTS

G.A. Basile[1], S. Bertino[1], A. Cacciola[1], D. Milardi[1,2], G.P. Anastasi[1]

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Dopamine plays a major role in several aspects of striatal and cortical functions both in healthy and pathological conditions. The most important dopaminergic (DA) structures of the human brain, namely pars compacta of Substantia Nigra (SNc) and Ventral Tegmental Area (VTA), are located in the ventral midbrain. Projections from the SNc/VTA complex to the striatum are topographically organized: a dorsomedial tier of DA neurons projects to the limbic striatum, an intermediate tier to the associative striatum and a ventrolateral tier to the motor striatum. However, evidences of a similar topographical organization in the human brain *in vivo* are relatively sparse. Schizophrenia (SZ) is a common mental illness, which symptoms are thought to emerge from the dysregulation of DA neurotransmission. It has been proposed that the DA imbalance in striatum and cortex may result from subtle structural alterations in the cortico-basal ganglia-mesencephalic circuitry, but the differential role of topographically organized regions within these structures has not been fully explored yet. In the first step of the present work, we reconstructed the cortical and striatal connections with the SNc/VTA in 100 healthy subjects. We were able to map the limbic, associative and motor territories within the main DA midbrain structures. We then reproduced our parcellation pipeline on a cohort of 31 SZ patients and 26 sex and age-matched healthy controls. We also reconstructed the connectivity profile of each functional territory *vs* both cortical and subcortical targets. We found widespread alterations in connectivity profiles of both SNc and VTA in SZ patients, mainly involving the limbic and associative subregions of SNc/VTA and their connections with prefrontal cortices and with the ventral striatum. Our results support the existence of a tripartite subdivision of the dopaminergic midbrain in the human brain and are in line with the hypothesis of SZ as a "brain dysconnectivity" disorder.

## UNRAVELLING THE BRAIN ANATOMY THROUGH THE LENS OF NETWORK SCIENCE

A. Cacciola[1], S. Bertino[1], A. Muscoloni[2], G.P.A. Basile[1], D. Milardi[1,3], C.V. Cannistraci[2,3], G.P. Anastasi[1]

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An increasing number of theoretical and empirical studies approach the structure and function of the human brain from a network perspective. Investigating the brain networks topology has been made feasible by the development of novel neuroimaging techniques as well as new tools from graph theory and network science. Although network topology seems to be somehow connected to network geometry, one of the most challenging issues of the current network science is to infer the hidden geometry from the mere topology of a complex network. Hence, understanding whether the latent geometry of the brain connectomes relates to the neuroanatomy is an interesting field of research. Here in, we apply innovative and advanced techniques that are able to map a given network in the latent geometrical space on different structural brain networks. We show that we can unsupervisedly reconstruct the intrinsic brain geometry with an incredible level of accuracy and that it strongly resembles the known brain anatomy. The first rule of organization of brain networks emerging in the latent space is their structural segregation into the left and right hemispheres. In addition, the intrinsic geometry of structural brain networks strongly relates to the lobes organization known in neuroanatomy. As a counterproof, we unveil the latent network organization of the cerebellum and its intracerebellar connectivity patterns embedded both in a structural and functional fashion, showing a clear distinction between the lobular organization and functional subdivisions of the cerebellum. The present findings bridge the gap between brain networks topology and geometry and open a completely new scenario in studying the brain, the cerebellum and their disorders from a network perspective.

## NEUROCHEMICAL DATA ON THE NON-TRADITIONAL LARGE NEURON TYPES OF THE GRANULAR LAYER OF THE HUMAN CEREBELLAR CORTEX

P. Flace[1]

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The circuitry of the cerebellar cortex is commonly composed by 5 neuron types: stellate, basket, Purkinje, granule and Golgi neurons. Studies evidenced the presence of large neuron types: neuron of Lugaro, candelabrum neuron, unipolar brush neuron, globular neuron, synarmotic neuron, perivascular neuron distributed in the three different zones of the granular layer. Although, the large neuron types are involved in specific circuitries of the cerebellar cortex and the presence of different subpopulations of large neuron types immunoreactive to GABA, glutamate, neuropeptides, cholinergic markers and calcium binding proteins were demonstrated, they continue to be neglected and called "non-traditional large neurons". The goal of this study was to ascertain the presence of monoaminergic and neurotensinergic non-traditional large neuron types in the granular layer of the human cerebellar cortex. The study was carried out on *post mortem* fragments of human cerebellum fixed in an aldehyde picric acid solution, embedded in paraffin, cut into 5 μm sections and subjected to light microscopic immunohistochemistry with specific rabbit polyclonal antibodies for serotonin (5-HT), dopamine transporter (DAT), dopamine receptor type 2 ($DRD_2$), neurotensin (NT), neurotensin receptor type 1 ($NTR_1$). For positive controls were used fragments of rat intestine subjected to the same experimental procedures. The immunoreactions were revealed by streptavidin-biotin technique and 3,3′-diaminobenzidine. The results demonstrate a strong positivity for all the antigens in neuronal cell bodies and processes of different non-traditional large neuron types distributed in the three zones of the granular layer. In particular, a widely presence of perivascular neurons positive to 5-HT, $DRD_2$, NT and $NTR_1$ in all zones of the granular layer were evidenced. In addition, the perivascular neuron, may be considered a new specific neuron type of the neurovascular unit involved in the regulatory mechanisms of the cerebellar blood flow and permeability of the blood-brain barrier. Finally, these findings evidenced which at least 11 different neuron types must be considered in the cerebellar cortex circuitries, which may play a considerable role in the motor and non-motor functions of the cerebellum and in its disorders.

# SECOND SESSION: NEUROPLASTICITY AND NEUROREGENERATION

## NERVE FIBROBLASTS COLONIZING TUBULAR CONDUITS EXPRESS SOLUBLE NEUREGULIN 1, A GROWTH FACTOR STRONGLY INVOLVED IN PERIPHERAL NERVE REGENERATION

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Tubular conduits for peripheral nerve repair have been proven to be an excellent alternative to autografts because they act as physical guidance for the regenerating axons. Autograft, being a transplanted nerve, yet contains most of the players involved in nerve regeneration, while an hollow tube requires to be colonized by Schwann cells (SCs), fibroblasts and so on.

In this study, we investigated the expression of different genes involved in nerve regeneration within an hollow tube. To this purpose, chitosan tubes were used to repair a 10mm nerve gap in rat median nerves and were analysed 7, 14 and 28 days after repair. In the early time points after repair, the expression of nerve fibroblast markers was observed in the hollow tube, while SC marker expression was barely detectable.

Soluble Neuregulin 1 (NRG1), which can be expressed by SCs and fibroblasts, was strongly expressed, while the NRG1 co-receptors ErbB2-ErbB3, usually expressed by SCs, were not expressed. NRG1 is a glial growth factor playing pivotal roles in the peripheral nervous system after injury; soluble isoforms are released after injury and promote SC survival and trans-differentiation. To better investigate the expression of NRG1 isoforms and ErbB in nerve fibroblasts, a primary culture of sciatic nerve fibroblasts was obtained and analyzed at mRNA and protein level. Our data show that fibroblasts express high levels of different NRG1 isoforms, while NRG1 receptors are not expressed, thus indicating that nerve fibroblasts signal in a paracrine (and not in an autocrine) manner.

In conclusion, the presence of different soluble NRG1 isoforms inside the tube in the early steps after injury, suggests that NRG1 released by nerve fibroblasts might play a key role in the following SC migration inside the tube.

## GHRELIN ENHANCES GLIAL CONDITIONED MEDIA EFFECTS ON NEURAL MARKER EXPRESSION OF ADIPOSE-DERIVED MESENCHYMAL STEM CELLS

C. Russo[1], D. Lo Furno[1], G. Mannino[1], S. Stanzani[1], A. Russo[1], M. Patané[1], R. Giuffrida[1], R. Pellitteri[2]

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Ghrelin (Ghre) is an orexigenic peptide playing an important role within the olfactory system and in some neuronal populations of the hypothalamus, amygdala and hippocampus. Recently, it attracted particular interest for its neuroprotective, antioxidant, anti-inflammatory and anti-apoptotic properties. Our previous study showed that conditioned medium (CM) from glial cells, such as Schwann cells (SCs) and Olfactory Ensheathing Cells (OECs) promotes a neural differentiation of Adipose-derived mesenchymal Stem Cells (ASCs). Here, we evaluated the effect of Ghre on ASCs isolated from human lipoaspirate. Results obtained in ASCs cultured either in the basal medium or in OEC-CM or SC-CM were considered as controls and compared with three other corresponding ASC culture samples, where Ghre (2 μM) was added. After 1, 3 and 6 days of growth, cells were tested by immunocytochemistry to detect the expression of some neural markers, such as Protein Gene Product (PGP) 9.5, Microtubule Associated Protein (MAP) 2, Glial Fibrillary Acidic Protein (GFAP), Neuron Specific Enolase (NSE). The expression of Ghre and its receptor was also evaluated. Results confirmed that both OEC-CM and SC-CM increased the expression of neural markers in ASCs. These increases, clearly visible already at day 1 and more evident at day 6, were more obvious when Ghre was added, although ASCs treated with Ghre alone exhibited weak modifications. The observed effects are likely due to interactions between Ghre and its receptor, whose expression was also increased. In conclusion, although Ghre alone was not capable of inducing pronounced effects, its addition facilitated neural ASC differentiation. This study highlights the synergic action of Ghre and glial CM on modulation of ASC growth and differentiation.

# THIRD SESSION: BRAIN METABOLISM AND BLOOD-BRAIN BARRIER

## EXOGENOUS ANNEXIN A1 EFFECTS ON BLOOD-BRAIN BARRIER BREAKDOWN AND NEUROINFLAMMATION IN METABOLIC IMBALANCE

A. d'Amati[1,3], M. Errede[1], F. Girolamo[1], M. Sheikh[4], D. Ferorelli[2], A. Dell'Erba[2], E. Maiorano[3], E. Solito[4], D. Virgintino[1]

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The central nervous system (CNS) microenvironment homeostasis is controlled by the blood-brain barrier (BBB), a complex structure located at brain microvessels, composed of cellular and non-cellular elements of the neurovascular unit, which are involved in regulation of the BBB integrity. Annexin A1 (AnxA1) is an anti-inflammatory protein with important roles in CNS, among them the control of BBB functions and neuroinflammation. It has been reported in literature that metabolic imbalance induces BBB breakdown and low-grade neuroinflammation, which have been correlated to mild cognitive impairment. The aims of this study were to examine the effects of metabolic imbalance on BBB structure and function and the possible remedial role of AnxA1. We investigated, by immunofluorescence confocal microscopy, the expression of endothelial tight junction proteins (claudin-5 and occludin), vascular basal lamina molecules (laminin-$\alpha$2 and -$\alpha$4), cell adhesion molecules (ICAM-1 and P-selectin) and neuroinflammation markers (Iba1 and CD45), in human brains from healthy and diabetic patients, and in mice brains from three experimental groups: mice fed with chow diet (CHOW), mice fed with high-fat high-sugar diet (HFHS) and mice fed with HFHS diet treated with human recombinant ANXA1 (HFHS + ANXA1). The results demonstrated tight junction strands disruption and laminins content reduction in brain microvessels of diabetic patients and HFHS mice, along with the presence of a low-grade neuroinflammation. The ANXA1 treatment in HFHS mice restored BBB integrity and switched off neuroinflammation, evoking a possible therapeutic function of ANXA1 in metabolic imbalance.

## BLOOD-BRAIN BARRIER DYSFUNCTION FOLLOWING ALCOHOL EXPOSURE

D. Carrino[1], J.J.V. Branca[1], G. Morucci[1], F. Paternostro[1], M. Gulisano[1], A. Pacini[1]

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The harmful use of alcohol is one of the leading risk factors for population health worldwide. Subjects with alcohol use disorder may be affected by the consequences of recurrent alcohol abuse on the body, including alcohol-related liver disease and alcohol-related brain injury.
Our laboratory recently investigated morphological and molecular alterations of rat brain microvascular endothelial cells (RBE4), an *in vitro* monolayer model of the blood-brain barrier (BBB), following alcohol exposure. To assess whether alcohol caused a concentration-related response, cells were treated with 35, 50, 75, or 100 mM ethyl alcohol at different times. These concentrations are equal or equivalent to two or three times the legal limits for blood alcohol concentration in Italy.
Some of the mechanisms involved in alcohol-induced pathologies including cellular changes, apoptosis and reactive oxygen species (ROS) generation were evaluated.
Our findings demonstrate that alcohol metabolism in RBE4 cells induces oxidative and endoplasmic reticulum (ER) stress by ROS production and GRP78 chaperone up-regulation respectively. Moreover, the morphological determinations performed on the RBE4 monolayer following alcohol exposure evidence a gradual transition from "dot-like" to "zipper-like" structures of ZO-1 staining as well as small gap formation indicating cytoskeleton rearrangements.
Better understanding of these processes will reveal additional potential target for therapy in brain injuries caused by alcohol abuse or in several CNS diseases involving BBB impairment.

## THE NEW ASTROCYTE ISOFORM AQP4EX IS ESSENTIAL FOR THE ANCHORING OF AQP4 WATER CHANNEL AT THE GLIAL PERIVASCULAR MICRODOMAIN

C. Palazzo, C. Buccoliero, P. Abbrescia, O.Valente, M. Trojano, A. Frigeri

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AQP4 plays a central role in CNS water homeostasis, fundamental for the maintenance of osmotic composition and volume within the glial and neuronal compartments. The recent discovery of the extended isoform of AQP4 (AQP4ex), generated by translational readthrough, revealed the importance of AQP4ex in the correct targeting of water channels at the blood-brain barrier interface and a potential new mechanism of water transport regulation.
CRISPR/Cas9 technology was employed to generate an AQP4ex-KO mouse model and evaluate the effect on the overall AQP4 expression, polarization, supramolecular organization in orthogonal arrays of particles (OAPs) and neuromyelitis optica (NMO-IgG) autoantibodies binding.
In WT mouse, AQP4ex, representing about 10% of all AQP4 isoforms, showed a polarized distribution in the cerebrum mostly confined to the pericapillary astrocyte endfeet. AQP4ex removal completely suppressed the specific location of AQP4 at the astrocyte endfeet and was compensated by an increased expression of the canonical isoforms (M1 and M23) indicating that the KI stop codons tightly work. Without AQP4ex, AQP4 was mislocalized in the brain parenchima, and $\alpha$-syntrophin expression, the selective partner for AQP4 localization, was partially altered. The supramolecular organization of AQP4 in OAPs was subtly altered. Indeed, the absence of AQP4ex slightly reduced the size of AQP4-OAPs but the number of AQP4-OAPs pools remained largely the same. The absence of AQP4 at the perivascular pole completely abolished the binding of pathogenic human NMO-IgG to the brain. This study provides the first direct evidence *in vivo* on the role of AQP4ex in perivascular OAP assembly and confinement, as well as its involvement as a structural component of the glial endfoot membrane protein functional unit.

### THE ROLE OF LACTATE ON METABOLIC REPROGRAMMING IN GLIOBLASTOMA MULTIFORME

R. Avola[1], L. Longhitano[1], G. Giallongo[2], G. Camiolo[1], M.R. Spampinato[1], A. Distefano[1], G. Li Volti[1], D. Tibullo[1]

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Glioblastoma multiforme (GBM) is the most malignant type of primary brain tumor in humans and it is often associated with a poor prognosis. The Warburg effect is a dominant phenotype of most cancers, which in responsible of excessive conversion of glucose to lactate, and most tumor cells use glycolysis rather than oxidative phosphorylation (OXPHOS) as the main energy metabolic pathway to produce ATP. Although glycolysis is far less efficient than OXPHOS for ATP generation, tumor cells display abnormally high glycolytic rates in order to preserve high ATP levels. Within the glioblastoma tumor microenvironment (TME), tumor cells, stromal cells, and infiltrating immune cells continuously interact and exchange signals through various secreted factors including cytokines, chemokines, growth factors, and metabolites. Glioma cells in the TME transform immune cells to suppress anti-tumor immune cells and evade immune surveillance. In a number of malignancies such as glioma, myeloid-derived suppressor cells (MDSCs) have been shown to infiltrate malignant tissues having critical role in the network.
The aim of the present study was to evaluated the role of Lactate on metabolic reprogramming in an *in vitro* model of glioblastoma multiforme. Our results suggested that Lactate (5 mM) induces a significant increase in cell proliferation, migration and invasion and was able to regulate positively mitochondrial biogenesis and increased OXPHOS genes, showing that it is involved in metabolic switch of GMB cell line.
In addition, we observed that Lactate induce a significant expansion of Treg and M-MDSCs in Healthy control PBMCs, confirming that it is involved in immune-escape mechanisms. In conclusion, the Lactate pathway may be a therapeutic target in Glioblastoma.

## FOURTH SESSION: SEXUAL BEHAVIOR AND DIMORPHISM

### SEXUALLY DIMORPHIC EFFECT OF GENISTEIN ON HYPOTHALAMIC NEURONAL DIFFERENTIATION *IN VITRO*

M. Marraudino[1,2], A. Farinetti[1,2], M.A. Arevalo[3,4], S. Gotti[1,2], G.C. Panzica[1,2], L.-M. Garcia-Segura[3,4]

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Developmental actions of estradiol in the hypothalamus are well characterized. This hormone generates sex differences in the development of hypothalamic neuronal circuits controlling neuroendocrine events, feeding, growth, reproduction and behavior. *In vitro*, estradiol promotes sexually dimorphic effects on hypothalamic neuritogenesis. Previous studies have shown that developmental actions of the phytoestrogen genistein result in permanent sexually dimorphic effects in some behaviors and neural circuits *in vivo*. In the present study, we have explored if genistein, like estradiol, affects neuritogenesis in primary hypothalamic neurons and investigated the estrogen receptors implicated in this action. Hypothalamic neuronal cultures, obtained from male or female embryonic day 14 (E14) CD1 mice, were treated with genistein (0.1 μM, 0.5 μM or 1 μM) or vehicle. Under basal conditions, female neurons had longer primary neurites, higher number of secondary neurites and higher neuritic arborization compared to male neurons. The treatment with genistein increased neuritic arborization and the number of primary neurites and decreased the number of secondary neurites in female neurons, but not in male neurons. In contrast, genistein resulted in a significant increase in primary neuritic length in male neurons, but not in female neurons. The use of selective estrogen receptor antagonists suggests that estrogen receptor α, estrogen receptor β and G-protein-coupled estrogen receptors are involved in the neuritogenic action of genistein. In summary, these findings indicate that genistein exerts sexually dimorphicactions on the development of hypothalamic neurons, altering the normal pattern of sex differences in neuritogenesis.

### TRIBUTYLTIN ALTERS THE DEVELOPMENT OF BRAIN CIRCUITS CONTROLLING FOOD INTAKE

G. Ponti[1,2], E. Bo[3], A. Farinetti[2,3], M. Marraudino[2,3], B. Bonaldo[2,3], G.C. Panzica[2,3], S. Gotti[2,3]

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Spreading of metabolic syndrome is a raising concern. Recent findings highlight the possible involvement of environmental metabolic disruptors or obesogens (*i.e.*, compounds which may interfere with neuroendocrine system impairing the control of energetic balance) in this multifactorial disease. Organotins, as

tributyltin (TBT), are highly diffused environmental pollutants, acting as obesogens. In a recent study performed in adult mice chronically exposed to TBT we demonstrated alterations of the hypothalamic neuropeptide Y (NPY) expression in the paraventricular (PVN), in the arcuate and in the dorsomedial nuclei of males, whereas no changes have been observed in females. Also the pro-opio-melanocortin is affected but only in females. In the present study, we tested different doses of TBT (0.25-2.5-25 μg/Kg body weight/day) diluted in olive oil, administered orally to C57/BL6 dams from gestational day 8 to postnatal day 21, and we evaluated the long term effects in the adult offspring (33 male and 41 females perfused at 2 months of age). The selected doses are particularly interesting because the higher one corresponds to the "no observed adverse effect level" (NOEL) and the lower one to the "tolerable daily intake" (TDI). We have observed that indirect TBT exposure permanently alters feed efficiency, in particular at the intermediate dose in male and at the lower dose in females. Immunohistochemical analysis showed significant changes in NPY expression in females only in the PVN, but not in other hypothalamic areas, at all the tested doses. These results confirm that the NPY system is particularly vulnerable to the action of TBT, even if the effects are different depending on the period of exposure. Alarmingly, TBT doses defined as TDI had a deep and sex specific persistent effect.

## MATERNAL SEPARATION IN ABA RATS PROMOTES CELL PROLIFERATION IN THE DENTATE GYRUS OF THE HIPPOCAMPUS

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Anorexia nervosa (AN) is a serious eating disorder characterized by self-starvation and excessive weight loss. Several studies support the idea that altered maternal care during the postnatal period could play a pivotal role in the pathogenesis of AN, highlighting a multifactorial etiology for this disorder. The activity-based anorexia (ABA) animal model mimics core features of the psychiatric disorder, including severe food restriction, weight loss, and hyperactivity. Previous results in rodents from our lab, obtained through the ABA model, showed that Maternal Separation (MS) induces behavioral changes in anorexic rats in a sexually dimorphic way: in females, the MS promoted hyperactivity and a less anxious-like phenotype in ABA animals; in males, instead, the MS attenuated the anxiolytic effect of the ABA protocol. These results led us to investigate the effect of the MS on brain areas involved in the control of the anxiety-like behavior. We focused our attention on the hippocampal neurogenesis, a process involved in the response to environmental stimuli and stressful condition. We analyzed the volume of the whole hippocampus and the proliferation rate in the dentate gyrus (DG), by quantifying Ki67 density and characterizing neuronal phenotype cells (DCX) and glial cells (GFAP) with double-fluorescence technique. Results obtained showed that only in maternally separated anorexic rats there is an increase of proliferation in DG, underlying the presence of a synergic effect of MS and ABA, that promoted the proliferation of new neurons and glia progenitors in the DG in a more evident way in females in comparison to males.

## MARKERS OF NEURAL PLASTICITY AND ACTIVATION IN THE HIPPOCAMPUS OF MALE ROMAN HIGH- AND LOW-AVOIDANCE RATS THAT SHOW DIFFERENCES IN SEXUAL BEHAVIOR

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**Equal contribution*

The Roman High- (RHA) and Low-Avoidance (RLA) rat lines, displaying divergent biobehavioral traits and significant differences in sexual behavior (RHA rats exhibit higher sexual motivation and better copulatory performance than RLA rats), have been used in this study to characterize the neural plasticity processes induced by the sexual experience and underlying the adaptive modifications of behavior. These differences are very evident in sexually naïve rats, and persist, though reduced, after five copulatory tests, when sexual experience has been acquired. Since sexual activity is a natural reward that induces a wide range of neuroplastic changes in the limbic brain, we extended our previous data by studying whether the differences in sexual activity between the Roman lines are related to changes in the expression of Brain-Derived Neurotrophic Factor (BDNF) and its tyrosine kinase receptor B (trkB), c-Fos, FosB, and Activity regulated cytoskeleton-associated (Arc) protein in the dorsal (dHC) and ventral hippocampus (vHC) of sexually naïve and experienced RHA and RLA rats by Western Blot and/or immunohistochemistry. The results showed that, after sexual activity, the selected markers changed differentially in the dHC *vs* vHC of RHA and RLA rats. In both Roman lines, the changes were usually more evident in naïve rats, diminished in experienced rats and were higher in RHA than RLA rats. Our findings confirm that sexual activity induces a different neural activation in the dHC *vs* vHC, hippocampal divisions respectively involved in the processing of sensory signals into memories and in the emotional salience of memories, and leads to changes in synaptic plasticity with sexual experience acquisition, that depend upon the animals' genotypic/phenotypic characteristics.

# FIFTH SESSION: PERIPHERAL AND ENTERIC NERVOUS SYSTEMS, AND GUT-BRAIN AXIS

## VALIDATION AND INTER-RATER RELIABILITY OF THE VAGUS NERVE NEURODYNAMIC TEST AMONG HEALTHY SUBJECTS

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A growing body of evidence have shown that the Vagus Nerve (VN) is not only the main anatomical structure responsible for the brain and guts communication but is also a target for many interventions in which drugs or classic treatments have failed. The VN cervical tract stimulation have reported positive results for high social burden problems like acute and chronic pain, psychiatric diseases, disturbs of consciousness and epilepsy. Also it is well known that the selective tension of the Peripheral Nervous System, or neurodynamic test (NDT), is useful for diagnosis and treatment of neuropathic diseases and pain. Over the last 30 years NDTs were validated for upper and lower limbs nerves but nowadays a VN-NDT is lacking and could be a potential alternative in diagnosis and treatment for critical or neglected conditions.
30 healthy participants (16 Females) completed a questionnaire on vagal symptoms and a neurological assessment of the cervical tract was performed before the test. The VN-NDT was administered through a standardized sequence of physiological neck movements. Symptoms and heart rate (HR) were monitored. Validity of NDT was tested with ultrasonography assessment (C6-VN distance) during the tests administered by an expert and a novice. Motion of both testers and subjects was tracked through an optoelectronic system. Inter-examiner accordance was obtained comparing assessors' positive and negative tests due to symptoms provocation and reduction (Cohen's Kappa).
Reduction of the C6-VN distance during the test was significantly related to 13-10% HR reduction ($p<000.1$) and neck ipsilateral sub-occipital tension. The inter-examiner accordance was 0.6.
The NDT is a valid and reliable measure for evaluating VN performance.

## QUANTITATIVE ANALYSIS AND NEUROCHEMICAL PROFILE OF ENTERIC NEURONS IN THE SUBMUCOSAL AND MYENTERIC PLEXUS OF THE PIG COLON

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The enteric nervous system (ENS) controls and modulates gastrointestinal functions. Here we characterized enteric neurons in the ascending (AC) and descending (DC) porcine colon using multiple labeling immunohistochemistry, confocal imaging and IMARIS software for quantification with antibodies to HuC/D as pan-neuronal marker, and choline acetyltransferase (ChAT) and nitric oxide synthase (NOS). In the pig, as in humans, the enteric plexus in the submucosa is multilayered with an outer submucous plexus (OSP) near the circular muscle and an inner submucous plexus (ISP) near the mucosa. The ISP of the AC and DC contains the highest number of Hu neurons per $mm^2$ (1183 ± 129 and 578 ± 110), followed by the OSP (326 ± 80 and 325 ± 76) and the myenteric plexus (MP) (223 ± 43 and 270 ± 32). In the MP, ChAT+ neurons were 50% of enteric neurons in the DC *vs* 43% in the AC ($p<0.05$) and NOS+ neurons were ~50% in AC and DC. In the ISP, ChAT+ neurons were 34% in AC and 30% in DC, whereas NOS+ neurons were more abundant in DC than AC (42% *vs* 15%, $p<0.001$). By contrast, in the OSP, ChAT+ and NOS+ neurons were of similar density in AC and DC (ChAT+ 39% *vs* 44%; NOS+ 44% *vs* 38%). ChAT+/NOS+ neurons were more abundant in DC *vs* AC in both MP (16% *vs* 12%, $p<0.05$) and ISP (9% *vs* 5%), whereas in the OSP, ChAT+/NOS+ neurons were ~ 10% of enteric neurons in AC, but only a few in the DC. We identified ChAT+ excitatory and NOS+ inhibitory neurons innervating the muscle and the mucosa, ChAT+/NOS+ interneurons and ChAT- and NOS- neurons. Our results show structural and neurochemical similarities between the porcine and human ENS supporting the suitability of porcine colon as a model of translational research to study neuronal control of colonic functions in humans.

## INTESTINAL BARRIER AND CONSTIPATED PARKINSON'S DISEASE PATIENTS: A PILOT STUDY

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Parkinson's disease (PD) patients commonly complain of gastrointestinal disorders, which often precede the onset of the neurological motor symptoms. An intriguing theory suggests a link between PD and intestinal barrier that may represents a doorfor pathogens towards the central nervous system. Therefore, the characterization of early markers and histological patterns associated with digestive disturbances in PD patients are highly expected. The aim of the present work was to evaluate constipated PD patients for possible changes of blood and faecal inflammatory markers and/or morphological remodelling of the colonic mucosal tissues. Ten constipated PD patients and 10 constipated, sex and age matched patients (Rome IV) were enrolled. Blood (CBC, TSH, CRP, TNF, IL-1β, LBP) and stool (Hp antigen, fecal calprotectin, IL-1β, TNF) tests were carried out. All subjects underwent a colonoscopy with biopsies (descending colon), which were processed for markers of tight junctions and acid mucin expression; inflammatory and glial cells; collagen deposition. The onset of constipation was documented in 7/10 PD patients before the appearance of neurological symptoms. Faecal IL-1β levels were significantly higher in PD patients as compared with controls. The colonic biopsies of PD patients showed the following significant changes: a decrease in claudin-1

expression and eosinophils, an increase in epithelial acid mucins, collagen fibers and mucosal glia. On the basis of the results in our hands we can conclude that the constipated PD patients display higher index of intestinal inflammation, an impairment of the enteric mucosal barrier with a fibrotic remodelling compared to constipated patients without PD.

## EFFICACY EVALUATION OF PERIPHERAL NERVE DECELLULARIZATION PROTOCOLS

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When a peripheral nerve injury with a large defect occurs, end-to-end suture is not possible and conduit is not enough to obtain good results. Allograft could be an alternative, but nerves from donors frequently cause immunogenic response. Several authors are looking for the correct way to decellularize nerves preserving both the extracellular matrix (ECM) and basal lamina to improve nerve regeneration. Over the past years, the decellularization of peripheral nerves has been used to provide a natural substrate composed of nerve ECM without the resident cells to prevent the host immune response when transplanted in patients. Despite the existence of other protocols, as the Hudson and Sondell, they are either complex to prepare or the nerve ECM is slightly affected by the chemicals used, our aim is to find a new efficient decellularization protocol that is both easy to prepare and conserves well the nerve ECM. In this study rat sciatic nerves and human nerves were decellularized following 2 different protocols: P1) already described in the literature for nerve decellularization; P2) described in the literature for tendon decellularization and here applied on nerves.
Preliminary analysis in light and electron microscopy demonstrated that both protocols act differently on rat and human nerves. Nevertheless, P2 lead to better results maintaining the organization of the ECM and eliminating a huge amount of cells. The combination of chemicals (several detergents at low concentration) with physical forces (agitation) can be a promising technique for nerve decellularization that is effective in removing cells and preserving ECM.

## OBESITY AND PERIPHERAL NEUROPATHY RISK: COMPARISON OF SCIATIC NERVE ALTERATIONS IN RATS FED WITH A HIGH-FAT DIET AND IN OBESE ZUCKER RATS

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Obesity, diabetes and metabolic syndrome are connected to neuropathy onset risk. However, studies that have investigated the contribution of the single component revealed mixed results.
We aimed to compare the nerve fiber changes of the sciatic nerve in a diet-induced obese (DIO) rats and leptin receptor-deficient obese Zucker rats (OZRs). After five weeks with a high-fat diet (HFD), *ad libitum*, DIO rats developed obesity. The rats were studied for the other 12 weeks of HFD. Animals fed with standard diet were used as controls (CHOW rats). Both the OZRs and DIO rats had a significant increase in body weight compared to the lean Zucker rats (LZRs) and CHOW, respectively. Morphological, immunochemical, and immunohistochemical techniques were performed. Blood pressure, glycemia, and insulin levels were higher in both DIO rats and OZRs in comparison to CHOW and LZRs. No difference in total cholesterol and triglycerides levels was observed in DIO rats. On the contrary, the OZRs were characterized by hyperlipidemia. Morphometric results did not reveal differences in myelin thickness and axonal area of the nerve fibers. Immunohistochemical analysis of the sciatic nerve in obese rats compared to the controls evidenced reduced expression of neurofilament (NF). Moreover, a lower expression of the myelin basic protein (MBP) was observed in the sciatic nerve of obese animals. Our data showed an increased interleukin-1 beta (IL-1β) expression in both obese animal models. Besides, increased levels of oxidized proteins were found in obesity.
These findings suggest that the concomitant presence of hypertension, inflammation and oxidative stress in DIO rats and OZRs significantly increases the risk for peripheral neuropathy.

## PRECLINICAL AND CLINICAL EVALUATIONS OF THE EFFICACY OF RACEMIC AND DEXTROROTATORY FORMS OF THIOCTIC ACID IN NEUROPATHIC PAIN

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Compressive pain, radiculopathy, low back pain are heterogeneous disorder including patients with dominant nociceptive, inflammatory and neuropathic pain. Selected antioxidants have been proposed as potential therapeutic agents in the treatment or prevention of these pathologies strongly related to redox unbalance. Thioctic acid is an antioxidant existing in nature and expressed in two optical isomers. The present study assessed in preclinical model of compression of sciatic nerve, induced by loose ligation, and in a clinical trial, the possible neuroprotective role of racemic and dextro-rotatory forms of thioctic acid. Loose ligation of the right sciatic nerve was performed in spontaneously hypertensive rats (SHR), used as a model of increased oxidative stress, and in normotensive Wistar-Kyoto rats (WKY) used as a control group. Animals with sciatic nerve ligation were left untreated or were treated intraperitoneally for 14 days with intraperitoneal injection of different dose of racemic (rac) and two enantiomers form [R(+) and S(-)] of thioctic acid. Control SHR and WKY rats received the same amounts of vehicle. Treatment with thioctic acid preserves the structure of the distal portion of sciatic nerve. In the spinal cord, antioxidant treatment reduced oxidative stress and astrogliosis developed following loose ligation. R(+)-thioctic acid was more active than rac or S(-). The clinical trial has showed a greater influence on painful symptomatology, a quicker recovery and a better impact on quality of life of R(+) *vs* rac. The results of preclinical and clinical studies suggest that thioctic acid, with particular reference to its R(+) enantiomer, may have a place in the treatment of neuropathies.

# SIXTH SESSION: NEURAL DISORDERS

## A MODEL OF BRAIN MICROVESSEL ISOLATION APPLIED TO THE STUDY OF NEUROVASCULAR UNIT DYSFUNCTIONS

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In the mouse model of experimental autoimmune encephalomyelitis (EAE), EAE-affected wild type (WT) mice show a high number of oligodendrocytes precursors cells (OPCs) in contact with cerebral cortex microvessels, characterized by a damaged blood-brain barrier (BBB). Vessel-associated OPCs have been hypothesized to belong to the neurovascular unit (NVU) components and to be, therefore, involved in regulation of BBB function. In EAE-WT mice, OPCs proliferate and express higher level of the proteoglycan Nerve/Glial antigen 2 (NG2), compared with naïve WT mice. On the contrary, when EAE is induced NG2 knock-out (NG2KO) mice, the cortex microvessels show a preserved BBB. In order to better understand the effects of NG2 on OPC recruitment, the NG2-mediated role of these cells on BBB dysfunction, and the regulative factors involved in NVU cellular and molecular composition, a biofunctional analysis has been carried out on three ligand/receptor systems. Vascular endothelia growth factor A (VEFG-A), platelet-derived growth factor -AA (PDGF-AA), and transforming growth factor-β (TGF-β) have been analyzed by immunofluorescence confocal microscopy, dual RNAscope immunohistochemistry/hybridization *in situ* (IHC/ISH), and real time-PCR assays on both brain sections and isolated brain microvessels, in WT and NG2KO mice, naïve and EAE-affected. Overall, the results confirm that OPCs are integral cell components of the NVU, which are involved in BBB dysfunction and appear regulated by specific regulative pathways.

## FRIEDREICH ATAXIA: EFFECTS OF THE TREATMENT WITH NUTRACEUTICALS ON THE NEURONAL AND GLIAL PHENOTYPE IN CEREBELLAR CORTEX AND SPINAL CORD

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In this study the phenotype of animal models of the inherited spinocerebellar disorder known as the Friedreich Ataxia are explored. Transgenic mice (M12 line) carrying the corresponding mutation within the gene encoding the mitochondrial protein Frataxin, (the M12 line), were used and further transgenic lines were obtained by crossing such line with mutant mice expressing the human Frataxin gene, the Pook transgene, which generated the M02 line, resulting into an attenuated phenotype of mutant mice, allowing them to survive until the sixth month of age, when a detailed morphological and morphometric analysis was performed. In addition, the effects were tested of therapeutic treatment with nutraceuticals, in particular the polyphenol Epigallocatechingallate (EGCG). Phenotype analysis in regions characterized by differential profiles of neurogenesis, including the cerebellar cortex and the spinal cord, by using neuronal (β-tubulin and Heavy Chain Neurofilament) as well as glial (Glial Fibrillary Protein, GFAP) markers indicated that their neural phenotype was significantly affected by the mutation, although it underwent a consistent recovery upon EGCG administration, indicating that treatment with antioxidants represents an appropriate tool for counteracting the associated neurodevelopmental delay. Besides on neuronal markers expression an effect was also demonstrated on the GFAP glial marker: a positive effect on its expression indicating that a "glia upregulation", potentially involved in neural repair mechanisms, contributed to the phenotype of the disorder. In addition, the expression of the Contactin1 adhesive glycoprotein, revealed an early downregulation, which was also efficiently counteracted by the EGCG treatment. This indicated that changes in Contactin 1 gene activation also contributed to the phenotype of the disorder and that the protective effects of antioxidant administration on the neural phenotype could imply the function of such molecule.

## EXPRESSION OF NUCLEOLIN IN THE NEUROVASCULAR UNIT DURING NORMAL AND GLIOBLASTOMA NEOVASCULARIZATION

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Since malignant gliomas are among the most vascularized tumors, one of the most important aspect for the development of malignancy is the interaction of tumor cells with the cells of the neurovascular unit (NVU). We investigated the role of Nucleolin (NCL), a multifunctional phosphoprotein ubiquitously distributed in cells of different tissues and with multiple roles in normal cell growth and metabolism. NCL has been demonstrated to be overexpressed in highly proliferative cells as well as in tumor cells. The analysis of NCL expression in high grade gliomas, compared to normal adult and developing brain, reveals differential expression and subcellular localization. During normal development, NCL shows a nucleoplasm and nucleolar localization in the NVU cells involved in angiogenesis whereas in tumor cells and in the tumor NVU cells, NCL is overexpressed and presents specific localization in the nucleoplasm as well as on plasma membrane and cytoplasm. These specific patterns of NCL expression could be considered the hallmark of different cell populations that may play different roles in glioblastoma growth and neovascularization and thus can represents a potential new molecular target for both anti-angiogenic and anti-proliferative therapy.

## REAC (RADIO ELECTRIC ASYMMETRIC CONVEYER) TECHNOLOGY MODULATES NEUROINFLAMMATION IN A MOUSE MODEL OF NEURODEGENERATION

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In this work were investigated the effects of Radio Electric Asymmetric Conveyer (REAC), a non-invasive physical treatment, on neuroinflammatory responses in a mouse model of parkinsonism induced by intoxication with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). We found that the REAC tissue optimization treatment specific for neuro-regenerative purposes (REAC TO-RGN-N) attenuated the inflammatory picture evoked by MPTP-induced nigro-striatal damage in mice, decreasing the levels of pro-inflammatory molecules and increasing anti-inflammatory mediators. Besides, there was a significant reduction of both astrocyte and microglial activation in MPTP-treated mice exposed to REAC TO-RGN-N. These results indicated that REAC TO-RGN-N treatment modulates the pro-inflammatory responses with potential beneficial effects on neuronal damage in MPTP-induced parkinsonism.

## NURR1 AND ERR1 MODULATE THE EXPRESSION OF GENES OF A *DRD2* CO-EXPRESSION NETWORK ENRICHED FOR SCHIZOPHRENIA RISK

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Multiple schizophrenia (SCZ) risk loci may be involved in gene co-regulation mechanisms, and analysis of co-expressed gene networks may help to clarify SCZ molecular basis. We have previously identified a dopamine D2 receptor (*DRD2*) co-expression module enriched for SCZ risk genes and associated with cognitive and neuroimaging phenotypes of SCZ, as well as with response to treatment with antipsychotics. Here we aimed to identify regulatory factors modulating this co-expression module and their relevance to SCZ.

We performed motif enrichment analysis to identify transcription factor (TF) binding sites in human promoters of genes co-expressed with *DRD2*. Then, we measured transcript levels of a group of these genes in primary mouse cortical neurons in basal conditions and upon overexpression and knockdown of predicted TFs. Finally, we analyzed expression levels of these TFs in dorsolateral prefrontal cortex (DLPFC) of SCZ patients.

Our *in silico* analysis revealed enrichment for NURR1 and ERR1 binding sites. In neuronal cultures, the expression of genes either relevant to SCZ risk (*Drd2, Gatad2a*, *Slc28a1*, *Cnr1*) or indexing co-expression in our module (*Btg4*, *Chit1*, *Osr1*, *Gpld1*) was significantly modified by gain and loss of Nurr1 and Err1. *Post mortem* DLPFC expression data analysis showed decreased expression levels of NURR1 and ERR1 in patients with SCZ. For NURR1 such decreased expression is associated with treatment with antipsychotics.

Our results show that NURR1 and ERR1 modulate the transcription of *DRD2* co-expression partners and support the hypothesis that NURR1 is involved in the response to SCZ treatment.