



Dipartimento
di **NEUROSCIENZE,
BIOMEDICINA E MOVIMENTO**



XXXIII Congresso Nazionale del Gruppo Italiano per lo Studio della Neuromorfologia

24 e 25 Novembre 2023
VERONA



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Università di Verona

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PROGRAMMA

24 novembre

9:00-9:15 Apertura dei lavori e saluti istituzionali

Saluti del Direttore del Dipartimento di Neuroscienze, Biomedicina e Movimento Università degli Studi di Verona, Prof. Corrado Barbui

Saluti del Presidente GISN, Prof. Fabrizio Michetti

9:15-10:00 Lettura Magistrale “Golgi e le Neuroscienze”

Prof. Paolo Mazzarello (Università di Pavia) (Moderatore: Fabrizio Michetti)

10:00-11:45 Sessione I: New findings and Techniques in Neuroanatomy and Behavioral studies (Moderatori: Michele Papa, Graziella Cappelletti)

10:00-10:15 THE NIGRO-THALAMIC DOPAMINERGIC PATHWAY IN THE HUMAN BRAIN: A MULTI-SCALE AND INTEGRATED STUDY

Cirillo G, Caiazza G, Cirillo M, Esposito F, De Luca C, Papa M

10:15-10:30 LIGHT SHEET IMAGING OF LARGE CNS SAMPLES: OPPORTUNITIES AND PITFALLS

Zanella L, Baldassarro VA, Lorenzini L, Quadalti C, Giardino L, Calzà L

10:30-10:45 THE MULTIMODAL GRADIENT ARCHITECTURE OF THE HUMAN PULVINAR

Basile GA, Rizzo G, Anastasi GP and Cacciola A

10:45-11:15 Pausa caffè + poster session

11:15-11:30 IMMATURE NEURON DISTRIBUTION AND DENSITY IN DOGS

Pattaro A, Ghibaudi M, Corrente C, Bonfanti L

11:30-11:45 SYNAPTIC DYSFUNCTION IN BRAIN DISORDERS: AN INTEGRATED STUDY ON PROTEIN ARCHITECTURE AND INTERACTION AT THE SYNAPSE

Amadeo A, Mazzetti S, Marroccoli V, Invernizzi C, Ascagni M, Alini M, Zeynali A and Cappelletti G

11:45-12:15 Presentazione sponsorizzata: (Presentatore: Raffaella Mariotti)

Video Killed the Imaging Star

Girardi F

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12:15-13:15 Sessione II: New findings and Techniques in Neuroanatomy and Behavioral studies 2 (Moderatori: Roberto De Giorgio, Stefano Gotti)

12:15-12:30 COMPARATIVE EFFECTS OF FORCED SWIM AND TAIL PINCH ON THE LEVELS OF BDNF AND TRKB IN THE MESOCORTICOLIMBIC SYSTEM OF ROMAN RATS
Serra MP, Sanna F, Boi M , Trucas M, Lai Y, Giorgi O, Corda MG, Quartu M

12:30-12:45 ROLE OF SYNAPTIC PLASTICITY IN EPILEPTIC ENCEPHALOPATHIES DURING SLOW-WAVE SLEEP.
Salari V, Del Gallo F, Corghi F, Spagnoli G, Mengoni F, Fabene P, and Bertini G

12:45-13:00 CHRONIC CONSTIPATION IN PARKINSON: MOLECULAR TRAIT
Costanzini A, Ioannou A, Giancola F, Cabanillas L, Lungaro L, Manza F, Caio G, Torresan F, Sternini C, De Giorgio R

13:00- 13:15 POSTNATAL GENISTEIN DIET ACTS ON ANXIOUS BEHAVIORS AND 5-HT SYSTEM IN MICE
Marraudino M, Ricci E, Casile A, Maggio R, Bonaldo B, Gotti S

13:15-14:30 Pausa pranzo

14:30-15:15 Sessione III: Brain tumors (Moderatore: Velia D'Agata)

14:30-14:45 TARGETING HUMAN HIGH-GRADE GLIOMAS INVASIVENESS
Virtuoso A, Milior G, Rouach N and Papa M

14:45-15:00 HEME OXYGENASE-1 INHIBITION INTERFERES WITH GLIOBLASTOMA PROGRESSION
D'Amico AG, Maugeri G, Magri B, Vanella L, Sorrenti V, Saccone S, Federico C, Fallica AN, Pittalà V and D'Agata V

15:00-15:15 ROLE OF c-KIT RECEPTOR IN FETAL BRAIN AND GLIOBLASTOMA VASCULARIZATION
Errede M, d'Amati A, Annese T, Bizzoca A, Lorusso L, de Trizio I, Girolamo F, Virgintino D

15:15-15:30 Saluti del Presidente SII, Prof.ssa Roberta Di Pietro

**15:30-16:15 Lettura Magistrale "A 50-year Odyssey through neuropeptide country"
Tomas Hokfelt (Karolinska Institutet) (moderatore: Raffaella Mariotti)**

16:15-16:45 Pausa caffè + poster session

16:45-18:00 Session IV Neurodegeneration and neuroinflammation (Moderatori: Daniele Tomassoni e Marina Quartu)

16:45-17:00 CLUSTERIN IN HUMAN BRAINS AFFECTED BY PARKINSON'S DISEASE
Pizzi S, Zanchi G, Mazzetti S, Calogero AM, Rolando C, Pezzoli G, Ramazzina I, Russo I, Cappelletti G

17:00-17:15 NEUROPROTECTIVE EFFECTS INDUCED BY BACOPA MONNIERI AGAINST METHAMPHETAMINE AND MPP+ TOXICITY IN CATECHOLAMINE CELLS "IN VITRO"

Lazzeri G, Ferrucci M, Busceti CL, Biagioni F, Puglisi-Allegra S, Frati A and Fornai F

17:15-17:30 ADIPOSE STEM CELLS FOR BLOOD-RETINAL BARRIER REPAIR

Mannino G, Romano IR, D'Angeli F, Giurdanella G, Giuffrida R and Lo Furno D

17:30-17:45 THIOCTIC ACID AND CDP-CHOLINE MODULATE THE NEUROINFLAMMATION IN LPS-STIMULATED MICROGLIA CELLS AND HIPPOCAMPUS OF HYPERTENSIVE RATS

Martinelli I, Tayebati SK, Bellitto V, Roy P, Amenta F and Tomassoni D

17:45-18:00 COMPARATIVE ANTI-INFLAMMATORY EFFECT OF EPA AND DHA DERIVATIVES ON OECs EXPOSED TO LPS.

Pellitteri R, La Cognata V, Patti A, Sanfilippo C

18:00-19:00 Assemblea Soci

20:30 Cena Sociale

25 novembre

9:30-10:15 Lettura magistrale “The involvement of olfactory neural cells in neurodegenerative disorders”

Prof. Gianluigi Zanusso (Università di Verona) (Moderatori: Fabrizio Michetti, Khosrow Tayebati)

10:15-11:00 Sessione V: Neurodegeneration and neuroinflammation 2 (Moderatori: Daniela Virgintino, Alessandra Pacini)

10:15-10:30 SYNUCLEINOPATHIES PREDICTORS AND WHERE TO FIND THEM.

Basellini MJ, Luppino A, Novello C, Calogero AM, Emanuele Cereda⁴, Elena Contaldi⁵, Isaias IU, Gianni Pezzoli, Mazzetti S, and Cappelletti G.

10:30-10:45 ROLE OF PACAP-ADNP AXIS ON SOD1-G93A MOTOR NEURONS

Magri B, D’Amico AG, Maugeri G, Morello G, La Cognata V, Saccone S, Federico C, Cavallaro S, D’Agata V

10:45-11:00 LABELLED NANOVESICLES FROM ADIPOSE MESENCHYMAL STEM CELLS TARGET INFLAMED LYMPH NODES IN CHRONIC EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS.

Turano E, Scambi I, Angelini G, Rossi B, Marostica G, Furlan R, Constantin G, Mariotti R and Bonetti B.

11:00-11:30 Pausa caffè

11:30-13:00 Sessione VI: Peripheral system and gut-brain axis (Moderatori: Roberto De Giorgio, Khosrow Tayebati)

11:30-11:45 TARGETING GUT-BRAIN AXIS IN ALZHEIMER’S DISEASE

D’Antongiovanni V, Segnani C, Pierucci C, Di Salvo C, D’Amati A, Errede M, Virgintino D, Antonioli L, Pellegrini C and Bernardini N

11:45-12:00 MORPHOLOGICAL MODULATION OF COLONIC ENTERIC PLEXI IN MICE MODEL OF COLITIS AND THE POSSIBLE EFFECTS OF BACTERIAL STRAIN SUPPLEMENTATION.

Bellitto V, Gabrielli MG, Martinelli I, Roy P, Salvesi C, Silvi S, Galosi L, Miceli C, Tayebati SK and Tomassoni D

12:00-12:15 PERIPHERAL NERVE STIMULATION PRESERVES PERIPHERAL NERVE INJURY INDUCED MORPHOLOGICAL CHANGES IN THE TRIGEMINAL SYSTEM

Korai SA, Panetsos F, Papa M and Cirillo G

12:15-12:30 CIPN AND ITS MECHANISM OF DEVELOPMENT

Tarasiuk O, Pozzi E, Canta A, Chiorazzi A, Ballarini E, Rodriguez-Menendez V, Foti M, Cavaletti G, Meregalli C

12:30-12:45 ACELLULAR PIG NERVE GRAFT TO REPAIR MEDIAN NERVE INJURY: A PRELIMINARY STUDY ON RAT.

Muratori L, Lovati A, Crosio A, Molinaro D, Raimondo S

12:45-13:00 OXALIPLATIN NEUROTOXICITY: MORPHO-FUNCTIONAL APPROACH

Alberti P, Pozzi E, Serra MP, Trucas M, Boi M, Capelli C, Invernizzi C, Rodriguez-Menendez V, Quartu M, Cavaletti G.

13:00-13:30 Conclusioni del Direttivo GISN e premiazioni

SESSIONE POSTER
24 novembre 9:00-18:00

- IN VITRO ANALYSIS OF AN INNOVATIVE BIOMATERIAL

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-SATELLITE GLIAL CELLS IN CHEMOTHERAPY NEUROTOXICITY IPN

Pozzi E, *Rodriguez-Menendez V, Ballarini E, Argentini A, Canta A, Chiorazzi A, Alberti P, Meregalli C, Cavaletti G, Carozzi VA*

-THE ROLE OF PACAP IN AN IN VITRO MODEL OF ALS

Mauqeri G, *D'Amico AG, Magrì B and D'Agata V*

-BBB PERMEABILITY: HOW MUCH SHEAR STRESS AFFECT IT

Branca JJV, *Guarnieri G, Morelli A, Paternostro F, Gulisano M, Pacini A*

-FUNCTIONALLY INDEPENDENT SUBUNITS OF THE ARCUATE FASCICULUS AND THEIR CONTRIBUTION TO HIGHER-ORDER PROCESSING

*Basile GA, Milardi D, Anastasi GP and **Cacciola A***

-POSTNATAL ESTROGEN RECEPTOR ANTAGONIZATION HAS ORGANIZATIONAL EFFECT ON KISS SYSTEM

Marraudino M, *Paiano M, Bonaldo B, Tanese G, Ostuni MT, Pinos H, Collado P, Gotti S*

-NEURAL-LIKE DIFFERENTIATION OF ADIPOSE STEM CELLS

Romano IR, *Mannino G, Pellitteri R, Denaro S, Giuffrida R, D'Angeli F, Vicario N, Parenti R and Lo Furno D*

-NEUROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM ADIPOSE STEM CELLS DIFFUSED THROUGH AN EPITHELIUM ON INJURED NEURONAL CELLS

Caprioli M, *Virla F, Marostica G, Scambi I, Furlan R, Mariotti R, Turano E.*

-EXTRACELLULAR VESICLES FROM ADIPOSE STEM CELLS: THEIR EFFECT ON A MICROGLIAL MODEL OF ALS

Virla F, *Dabrowska S, Scambi I, Nodari A, Galiè M, Turano E and Raffaella Mariotti R*

-EFFECTS OF mTOR INHIBITION IN iPSC-DERIVED MOTOR NEURONS FROM PATIENTS WITH C9orf72 REPEAT EXPANSION

*Lenzi P, Ratti A, Biagioni F, **Scotto M**, Casiraghi V, Ferese R, Silani V, and Fornai F*

- γ -PGA PROTECTIVE EFFECT IN A CELLULAR PD MODEL

Novello C, *Parati M, Cascio A, Rolando C, Calogero A, Mazzetti S, Moda F, Radecka I, Cappelletti G*

-PATHOLOGICAL REMODELING OF GUT BARRIER IN OBESITY

Pellegrini C, *D'Antongiovanni V, Pierucci C, Segnani C, Ippolito C, Antonioli L, Colucci R, Fornai M, Bernardini N*

INFORMAZIONI GENERALI

Comunicazioni orali: le comunicazioni orali saranno tenute in italiano e dovranno avere una durata di 10 minuti (+5 discussione).

Poster: i poster dovranno avere come dimensioni massime 70 cm x 100 cm (portrait).

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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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**33rd National Conference of the Italian Group
for the Study of Neuromorphology
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November 24-25, 2023

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MAIN LECTURES

GOLGI AND THE NEUROSCIENCES

Paolo Mazzarello

Department of Brain and Behavioral Sciences and Museum System, University of Pavia, Italy

Camillo Golgi was born in Corteno (today Corteno Golgi in his honour), a tiny village in an Alpine village of the upper Valcamonica, in the eastern-northern extremities of Austrian Lombardy, on 7 July 1843. In 1873 he published an article that contained the description of entire nerve cells stained in black with a new histological procedure, the black reaction. Using this method, Golgi was able to highlight the fine structure of the central nervous system. Moreover, his name is linked to many other important scientific discoveries and innovations such as: the discovery of the Golgi apparatus or complex, one of the fundamental components of the cell; the discovery of the perineural net (an extracellular matrix meshwork that wrap around some neurons with important physiological functions), the identification of the Golgi tendon organ (a proprioceptor that sense tension from the muscle); the description of the malaria plasmodium cycle in the “tertian” and “quartan” forms of the disease with the identification of the correspondence between the multiplication of the parasite and febrile access (Golgi law) and the relationship between the vascular pole of the Malpighian glomerulus and the distal tubule. On the basis of the studies on the structure of the brain, Golgi developed a physiological model of the brain that was influenced by a holistic conception he had in mind. He named this theory diffuse nervous network, assuming that the axonal prolongations were fused (or intimately interlaced) in a diffuse web along which the nervous impulse propagated. One of the scientists who quickly understood the importance of Golgi’s results was the Spanish anatomist Santiago Ramón y Cajal. However, when he studied the brain with the black reaction, he had in mind the idea of the nerve cells as independent “units” (named neurons by Waldeyer, 1891). Thus Ramón y Cajal quickly became the champion of the neuron theory that paradoxically developed thanks to the same black reaction used by Golgi for the formulation of the opposite diffuse nervous network theory. The controversy between Golgi and Ramón y Cajal represents a dramatic instance of a theory-driven perception of the same morphological evidence.

A 50-YEAR ODYSSEY THROUGH NEUROPEPTIDE COUNTRY

Tomas Hokfelt

Department of Neuroscience, Karolinska Institutet, Biomedicum, Stockholm, Sweden

In 1962 Nils-Åke Hillarp came to our Histology Department at Karolinska Institutet in Stockholm, introducing research on monoamine neurotransmitters based on the Falck-Hillarp formaldehyde fluorescence method. Later we employed, thanks to Dr. Menek Goldstein of NYU, immunohistochemistry to study catecholamine synthesizing enzymes, and this method then became the inroad to our work on neuropeptides. These molecules had already been studied at Karolinska by Ulf von Euler, Viktor Mutt and others, and antibodies originally generated for radioimmunoassay could also be used for immunohistochemistry. We first focused on substance P and somatostatin, both in brain and periphery, especially dorsal root ganglia. This line was then followed up by analysis of two newly peptides discovered in the Mutt laboratory, galanin and NPY, and their possible role in pain signaling (collaboration with Zsuzsanna Wiesenfeld-Hallin) and also in mood behavior. After having worked with these and several other peptides in rodents for many decades, I raised the question to myself: what is the significance of our work on rodents for understanding the functional role of neuropeptides in the human nervous system? Thus, we embarked on studies of human postmortem spinal cord, dorsal root ganglia and brains. That work included, e.g., analysis of (i) multiple subregions of the prefrontal cortex, (ii) single noradrenergic locus coeruleus neurons and (iii) brains from depressed subjects who died from suicide. It is hoped that the results will help understand peptidergic mechanisms possibly underlying disease and will provide a basis for drug development.

THE INVOLVEMENT OF OLFACTORY NEURAL CELLS IN NEURODEGENERATIVE DISORDERS

Gianluigi Zanusso

Department of Neuroscience, Biomedicine and Movement Sciences, University of Verona, Italy

In neurodegenerative disorders such as AD or PD, different studies have shown the presence of neurofibrillary tangles, β -amyloid deposits, or Lewy neurites in the olfactory sensory neurons (ONs) suggesting that abnormally conformed proteins may be transported from ONs to the glomeruli of the olfactory bulb where they accumulate, aggregate, and assemble into fibrils. The olfactory neuroepithelium (OE) is a neural epithelium mainly located in the upper nasal cavity and it is directly exposed to the external environment and it is vulnerable to physical and chemical injuries. OE is composed of a variety of cell types including olfactory sensory neurons, supporting glial-like cells, microvillar cells, and basal stem cells. Although olfactory neurons undergo constant recycling every 2-3 months it is still unknown the half-life the other glial-like neural cells. To investigate the involvement of olfactory neuroepithelium in neurodegeneration, a non-invasive and painless olfactory brushing procedure to collect olfactory neuroepithelium was set up. This procedure allowed to detect the pathologic prion protein in olfactory mucosa samples (OM) from patients with sporadic Creutzfeldt-Jakob disease, using the Real-Time Quaking-

Induced Conversion (RT-QuIC) assay. In OM of patients with Parkinson's disease (PD), Dementia with Lewy bodies and in patients with idiopathic REM sleep behavioral disorder (a prodromal stage of PD) α -synuclein aggregates were detected by RT-QuIC and α -syn and phospho- α -syn deposits were observed in OM from PD patients but not in controls. In OM of patients with genetic FTLD-TDP, carrying TARDBP, PRGN and C9orf72 mutations, TDP-43 aggregates were detected by RT-QuIC for TDP-43. In addition, phospho-TDP-43 was mainly found in the cytoplasm of Beta-III tubulin positive cells (*i.e.* sustentacular and microvesicular cells) but sporadically in ONs. These studies indicate that in the OE the pathological forms of α -syn in clinically affected patients but also during a prodromal stage and of TDP-43 in patients with FTLD-TDP are detected. An interesting finding is that both phosphorylated α -syn and TDP-43 are found in supportive neural cells rather than in ONs. Ongoing studies are investigating why protein aggregation occurs in these glial-like cells and less in ONs and we speculate on a shorter half-life of ONs.

SESSION I
NEW FINDINGS AND TECHNIQUES
IN NEUROANATOMY AND BEHAVIORAL STUDIES

THE NIGRO-THALAMIC DOPAMINERGIC PATHWAY IN THE HUMAN BRAIN: A MULTI-SCALE AND INTEGRATED STUDY

Cirillo G¹, Caiazzo G², Cirillo M², Esposito F², De Luca C¹, Papa M¹

¹Division of Human Anatomy, Neuronal Network Morphology & Systems Biology Lab, University of Campania "Luigi Vanvitelli", Italy; ²Department of Advanced Medical and Surgical Sciences, University of Campania "Luigi Vanvitelli", Italy

The subcortical network of the substantia nigra pars compacta (SNc), one of the main dopaminergic nuclei of the brain, is well known and mainly represented by the nigro-striatal pathway that exerts a regulatory function on the basal ganglia circuitry. However, dopaminergic innervation of other brain centers has been only investigated in non-human primates and never characterized in humans. The impossibility of tract-tracing studies in humans has boosted advanced magnetic resonance imaging (MRI) techniques that have shed new light on the whole brain connectivity, in particular diffusion tensor imaging (DTI).

We aim to dissect the dopaminergic innervation of the human thalamus using multi-scale and integrated analyses.

First, consecutive human thalamic sections will be processed for immunohistochemistry (IHC) and stained for morphological analysis (Nissl, Haematoxylin and eosin) and for the main dopaminergic markers (TH, VMAT-2, DAT, AADC).

Second, high-resolution MRI segmentation of the SN (that allows the identification of the postero-medial SNc from the gabaergic anterolateral SN pars reticulata - SNr) and multi-shell high-angular resolution diffusion MRI (MS-HARDI) (that allows the tractographic reconstruction of the SNc) will be performed in a group of 10 healthy subjects (age 25-30, sex-matched, 5M, 5F). Finally, PET-FDOPA data coming from healthy subjects (n=20) will be evaluated for both qualitative and quantitative analysis of the thalamic region.

Our preliminary MS-HARDI results performed with two previously validated diffusion MRI schemes on 10 healthy subjects demonstrate a reproducible structural connectivity between the SNc and the thalamus, with up to an average of ~19% of the total number of streamlines encompassing the SNc and the thalamus, with no other major subcortical structures involved (and without necessarily reaching the cortex). To the best of our knowledge, this is the first report of a direct nigro-thalamic dopaminergic projection, with a multi-scale and integrated approach. The significance and the characterization of these connections, however, is still under investigation. Understanding of these new pathways will lead to the optimization of the treatments for dopaminergic-related disorders, paving the way for targeted and personalized therapies.

LIGHT SHEET IMAGING OF LARGE CNS SAMPLES: OPPORTUNITIES AND PITFALLS

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Light sheet fluorescence microscopy (LSFM) coupled to tissue clarification is an emerging tool offering the opportunity to visualize large volumes at micrometer resolution. The fluorescence signals can be generated by viral vector-GFP-tagged genes, but also via antibody staining. 3D voxel-based image analysis software based on machine learning and artificial intelligence algorithms capable to handle multi-GB dataset could be used not just as visualization, but also as calculation tool. In front of the extraordinary potentiality of 3D whole brain microscopy, the challenge is to verify feasibility of quantitative imaging until single cell-resolution. To approach such goal, our lab considered two main methodological issues: (i) imaging of antibody-stained section; (ii) the use of non-toxic clearing protocols. We then used the Miltenyi MACS® Clearing Kit, anti CD31 VioB515 - and anti NF- VioR667 antibodies to visualize endothelial cells and neurons, respectively, and Miltenyi UltraMicroscope Blaze™. We set preliminary experiments aimed to evaluate antibodies penetration and imaging in mouse and rat hemibrains and spinal cord, two CNS area having different lipid/protein/water ratio, therefore a different refractive index. Signal penetration was evaluated acquiring adjacent three-dimensional images (442×442×500 μm for the hemibrain, 442×442×250 μm for the spinal cord) from the lateral to the medial side. The images were acquired with a fixed z interval and y position, moving the x axis through the tissue. This images set was also used to produce a stitched image (20% overlap) processed by the Miltenyi stitcher software.

Three-dimensional images were then analyzed by IMARIS software (v. 9.6.2; Oxford instruments). We used the algorithm “filament” and “surface” to construct the three-dimensional microvascular net, based on the antibody staining. These preliminary tests indicates that: i) the technology can label and detect 3– 5μm diameter microvessels; ii) in the cerebral cortex, the fluorescence signal intensity constantly decreases moving inside the tissue and consequently the detected immunoreactive volume; iii) using the stitching method is possible to balance the fluorescence intensity decay along the investigated tissue depth.

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THE MULTIMODAL GRADIENT ARCHITECTURE OF THE HUMAN PULVINAR

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The pulvinar, the largest nucleus in the human thalamus, is a complex, highly interconnected structure. Through a dense, organized network of cortical and subcortical areas, it provides adequate cooperation between neural systems, which is crucial for multiple high-order functions such as perception, visuospatial attention, and emotional processing. Such a central role is made possible by a precise internal topographical organization, which is mirrored by anatomical connections as well as by the expression of neurochemical markers.

While being traditionally subdivided into sub-nuclei, each characterized by distinct connectional and morphological features, recent studies in both primate and human brains have highlighted that this topographical organization only marginally aligns with the conventional histological subdivision. Instead, it has been delineated in the context of continuous gradients of cortical connections along the dorsoventral and mediolateral axes. While this multi-gradient organization has been extensively documented in primate models, it remains relatively underexplored in the human brain.

The present work leverages high-quality, multi-modal structural and functional imaging data of 210 healthy subjects from the Human Connectome Project (HCP). Additionally, we incorporate a recently published whole-brain, large-scale, positron emission tomography (PET) atlas detailing 19 neurotransmitters and receptors distributed across the human brain. By applying diffusion embedding analysis to tractography, functional connectivity, and receptor co-expression data, we identify and characterize multiple topographically organized gradients of structural connections, functional coactivation, and molecular binding patterns. We demonstrate that such gradients converge on a shared representation along dorso-ventral and medio-lateral axes of the human pulvinar. This representation aligns with transitions in both structural and functional connectivity, spanning from lower-level to higher-order cortical regions. Moreover, it is paralleled by gradual changes in the expression of molecular markers associated with key neuromodulatory systems, including serotonergic, noradrenergic, dopaminergic, cholinergic, and opioid systems. We contend that our findings mark a significant stride towards a more comprehensive understanding of pulvinar anatomy and function, providing a nuanced characterization of its role in health and disease.

IMMATURE NEURON DISTRIBUTION AND DENSITY IN DOGS

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Brain structural plasticity is the ability of neural elements to perform adaptive changes. It includes synaptic plasticity, adult neurogenesis, and non-newly generated, "immature" neurons (INs). INs are prenatally generated, remain undifferentiated for long time (expressing immaturity markers, which allow their identification, e.g., doublecortin; DCX), and eventually mature and integrate in pre-existing functional circuits. INs are found in cortical and subcortical regions (amygdala, claustrum).

We previously showed a phylogenetic variation of cortical INs (cINs) in mammals spanning from small-brained, lissencephalic to large-brained, gyrencephalic species, revealing that these cells, while restricted to the paleocortex in laboratory rodents, extend to the entire neocortex in gyrencephalic species, with higher density in large-brained mammals. A notable interindividual variation was observed within heterogeneous animal groups, suggesting that individual life history might exert modulatory effects.

Here we pursue the IN study in mammals, considering cortical and subcortical regions of domestic dogs (*Canis lupus familiaris L*) to add a gyrencephalic species to our mapping, and to compare the IN density. Four one-year-old Beagle dogs, raised in the same environment at MarshallBio (Lyon, France), were studied employing a previously used methodology to obtain comparable results. Considering cINs, qualitative and quantitative analyses (DCX+ cells; using NeuroLucida software) were conducted on four anterior-posterior brain levels defined by corresponding neuroanatomical structures. Subcortical regions were examined using serial sections (spaced each other of 480 μ m) of the entire amygdala/claustrum length, allowing the counting of DCX+ cells, the determination of volumes, and their proportions with the whole brain. In cortical layer II, the INs form a monolayer, and the results are expressed as linear density (number of DCX+ cells/mm of layer II perimeter); in subcortical regions, being the cells more widespread, they are quantified as DCX+ cells/mm².

Cell quantification data on cortical layer II suggest that canine IN density is high, resembling that of other carnivores. Despite the analysis was conducted on animals maintained in the same environment, substantial inter-individual variation was observed, also suggesting the existence of genetic components.

SYNAPTIC DYSFUNCTION IN BRAIN DISORDERS: AN INTEGRATED STUDY ON PROTEIN ARCHITECTURE AND INTERACTION AT THE SYNAPSE

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Synapses play a crucial role in brain development connecting neurons into neural circuits and modifications. The alteration of their complex protein architecture can lead to synaptic pathologies or synaptopathies. Among the most studied proteins involved in synaptic alteration there are α -Synuclein (α -Syn) and Tau, that were recently demonstrated to interact in co-pathology experimental models. α -Syn is a protein widely expressed mainly at the level of presynaptic terminals and its contribution to some pathologies, such as Parkinson's disease (PD), defined them as synucleinopathies. On the other hand, Tau alteration has been recently linked to neuronal dysfunction, also involving synaptic compartment. In this study we focused our analysis on the synaptic expression of these two proteins in frontal and temporal cortex of human brain from control and PD subjects. These two cortical areas were chosen because of possible alterations of synaptic circuits correlated to the well-known cognitive impairment that appears in the last stages of PD. α -Syn general distribution in cortical gray matter showed a dot-like neuropilar signal, that in the examined cortical areas was generally more intense and different between layers in the temporal cortex if compared with the homogeneous frontal one. PD cortex showed a reduction of α -Syn-positive neuropil and the appearance of Lewy bodies and neurites mostly in layer V of temporal cortex. A quantitative analysis revealed that a high percentage of synapses contained α -Syn and were mostly glutamatergic especially in frontal cortex of both controls and PD. Finally, we deeply analyzed the synaptic localization and association of α -Syn and Tau. Tau distribution was finely granular in the neuropil, but also cytoplasmatic mainly in temporal cortex, where it further increased in PD, if compared with the frontal area. The discrimination of the synaptic component of Tau expression revealed its presence in about 55-75% of all the synapses, whereas 13-23% of total synapses contained both α -Syn and Tau, as assessed by means of a careful analysis with ArivisVision4D software after setting specific pipelines. Taken as a whole, our results offer new insights on the possible role of α -Syn, Tau and their association in synaptic dysfunction.

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SESSION II NEW FINDINGS AND TECHNIQUES IN NEUROANATOMY AND BEHAVIORAL STUDIES 2

COMPARATIVE EFFECTS OF FORCED SWIM AND TAIL PINCH ON THE LEVELS OF BDNF AND TRKB IN THE MESOCORTICOLIMBIC SYSTEM OF ROMAN RATS

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The outbred Roman Low- (RLA) and High-Avoidance (RHA) rats were psychogenetically selected for poor vs rapid acquisition of two-way active avoidance and represent two divergent phenotypes displaying reactive (RLA) vs proactive (RHA) coping styles when exposed to stressors. Different forms of stress-induced depression-like symptoms impair the signalling of brain neurotrophins like the Brain Derived Neurotrophic Factor (BDNF) and induce alterations of neuronal plasticity. Here, we use the Roman rats, submitted either to tail pinch (TP) or to forced swim (FS), to compare the impact of diverse aversive situations on the occurrence of BDNF and its receptor trkB in the VTA, nucleus accumbens (Acb), and areas of the prefrontal cortex (PFC) such as the infralimbic/prelimbic (IL/PL) and anterior cingulate (ACg). According to previous data, western blot analysis shows that BDNF levels markedly changed after FS as compared to controls and between the examined areas; thus, in the VTA and Acb core, FS elicited a significant increase of both BDNF- and trkB-like immunoreactivity (LI) in RHA but not RLA rats; in RLA rats, the basal levels of BDNF-LI in the IL/PL cortex and of trkB-LI in the ACg cortex were lower than those of RHA rats; moreover, BDNF- and trkB-LI in the IL/PL and ACg cortex were increased by FS in RLA rats but decreased in the RHAs. After TP, no changes occurred in the VTA, while in the Acb core the RHA rats showed a decrease of BDNF-LI, and the RLA showed an increase in both BDNF- and trkB-LI. By contrast, the shell showed an increase in BDNF and trkB-LI in the RHAs, but not in the RLAs. In the RHA rats, the TP induced a significant increase of BDNF-LI in both the IL/PL and ACg cortices; in the RLA only the IL/PL cortex showed a significant increase of BDNF. Immunoreactivity labelled neuronal cell bodies, proximal processes and varicose nerve fibers, with an uneven distribution in the VTA, Acb and PFC that support on a morphological ground the BDNF and trkB level changes observed with the WB.

These results provide compelling evidence that the genetic background influences the effects of stress on BDNF/trkB signaling and support the view that the same stressor may impact differently on the expression of BDNF in discrete brain areas. Interestingly, the TP stress appears to induce alterations in BDNF signaling that are not present during FS.

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ROLE OF SYNAPTIC PLASTICITY IN EPILEPTIC ENCEPHALOPATHIES DURING SLOW-WAVE SLEEP

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Epileptic encephalopathies (EEs) are a group of developmental seizure syndromes associated with cognitive decline and severe behavioral disorders. Some EEs, termed EEs with status epilepticus during sleep (ESES), feature abundant epileptiform activity, particularly during slow-wave sleep (SWS). While the epileptiform activity tends to remit around puberty, the neuropsychological deficits often persist into adulthood. These deficits may stem from alterations in synaptic plasticity mechanisms associated with memory consolidation during SWS.

In this study, we asked whether cortical synaptic connectivity is affected by spontaneous slow-wave discharges (SWDs), employing a putative animal model of ESES the AJ/Jax mouse. First, magnetic resonance imaging (MRI) was employed to study the brain of young mice in baseline conditions. Next, following extended training in the Rotarod, a motor learning task, mice underwent *in vivo* electrophysiological recordings and an *ex vivo* immunohistochemical study followed by the analysis of confocal microscopic images.

Structural MRI results showed that A/J Jax mice exhibited a higher volume of gray matter in the hippocampus compared to control (A/J OLA) mice. Results from EEG recordings confirm the early reports of spontaneous SWD episodes in A/J Jax mice. In contrast, no discharges were detected in any of the control mice. The results from Rotarod test indicate that motor performance improved significantly between day one and day two in OLA but not in JAX mice. These findings suggest that SWDs may play a role in the motor skill acquisition deficit. Preliminary histological analysis in the untrained animals did not show any significant difference between OLA and JAX in terms of presynaptic puncta in both glutamatergic and GABAergic neurons. However, the working hypothesis is that training promotes synaptogenesis in brain areas recruited during the prolonged execution of the motor task. Analyses aimed at detecting differences between trained and untrained animals are ongoing.

CHRONIC CONSTIPATION IN PARKINSON: MOLECULAR TRAIT

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Chronic constipation (CC) is a prodromal and severe symptom in

up to 80% of Parkinson's disease (PD) patients (PD/CC) usually refractory to laxative. The molecular mechanisms of PD/CC are still unclear, although changes of enteric nervous system and intestinal epithelial barrier (IEB) have been suggested as involved in its pathogenesis. In this study, functional and molecular traits of PD/CC, CC and Ctrl were analyzed and compared. 12 PD/CC (2 F; 51-80 yrs), 20 CC (15 F; 27-78 yrs) and 23 controls (Ctrls; 11 F; 32-74 yrs) were enrolled. PD was diagnosed according to the United Kingdom Parkinson's Disease Society Brain Bank clinical diagnostic criteria, whereas CC was established with Rome IV criteria. Ctrl were asymptomatic subjects. 10 PD/CC and 10 CC were functionally characterized by anorectal manometry (AM) and transit time (TT). Colonic biopsies were obtained in all subjects and tested for possible IEB abnormalities and vasoactive intestinal polypeptide (VIP) levels by RT-qPCR, immunoblot and immunofluorescence labelling. IEB markers assessed were claudin-4 (CLDN4), occludin-1 (OCCL-1) and zonula occludens-1 (ZO-1). Based on functional tests, PD/CC were clustered in patients with delayed TT and altered AM (60%), or with altered AM only (40%). The analysis of the specimens showed that CLDN4 mRNA was significantly increased in PD/CC vs Ctrl and CC, whereas a reduction of the protein was observed in PD/CC vs Ctrl and CC. The OCCL-1 mRNA was significantly increased in PD/CC vs Ctrl and CC. ZO-1 mRNA relative levels were consistent with those of OCCL-1. Notably, OCCL-1 and ZO-1 mRNAs were comparable in CC vs Ctrl. Immunofluorescence for OCCL-1 showed a decrease of structural organization in PD/CC vs CC and Ctrl. ZO-1 immunoreactivity pattern was conserved in PD/CC vs Ctrl, whereas it was compromised in CC. VIP mRNA levels were significantly higher in CC vs Ctrl and PD/CC, whereas a decreasing trend was observed in PD/CC vs Ctrl, confirmed by both immunofluorescence and immunoblot analysis that showed a 15% drop in VIP protein (PD/CC vs Ctrl). Our data show that PD/CC patients are characterized by transit and/or anorectal dysfunctions, ZO-1, OCCL-1, CLDN4 and VIP changes, thus supporting the role of an altered IEB as a contributory mechanism to the neuroenteric abnormalities. Our findings also suggest that IEB markers are subject to regulatory mechanisms presumably distinct in CC and PD/CC.

POSTNATAL GENISTEIN DIET ACTS ON ANXIOUS BEHAVIORS AND 5-HT SYSTEM IN MICE

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Genistein (GEN) is a phytoestrogen found in leguminous plants, particularly soy, which is used in postnatal weaning of infants, as soy milk. GEN's molecular structure similar, like that of estrogens, allows binding to their receptors (ERs) altering their estrogenic functions. Previous studies have shown that serotonergic (5-HT) system, which is strongly regulated by estrogen, is affected by postnatal GEN exposure, inducing an anxiolytic effect in males and anxiogenic in female animal models.

Therefore, the aims of this work are twofold: to understand which receptor is involved in altering the organizational effect of GEN in

the postnatal period and to evaluate how such exposure may affect the 5-HT system.

CD1 male and female mice were treated daily from PND5 to PND12 with corn oil (control group), GEN and GEN associated separately (MPP, ER α antagonist; PHTPP, ER β antagonist, and G15, GPR30 antagonist) or all together (mix group) with each of ERs antagonist. In adulthood the animals were tested with different behavioral tests to assess anxiety and stress state. Finally, they were sacrificed at PND90 to analyze the 5-HT system within the dorsal and median Raphe nucleus.

The behavioral tests showed an anxiogenic behavior in GEN and GEN + PHTPP only in males and a high locomotor activity along with anxiolytic behavior in all treated females. In 5-HT analyses, no significant differences were observed between males and females, but emerged that it ER α receptors was most affected by postnatal GEN treatment playing a crucial role in the pathways that regulate mood. The results show that the 5-HT system was most altered by treatment in the rostral part of the Raphe, suggesting that in that region the system is particularly sensitive to estrogen regulation. Thus, data obtained confirmed that postnatal GEN's administration has effects on estrogen receptors and on 5-HT system, altering the mood pathways.

SESSION III BRAIN TUMORS

TARGETING HUMAN HIGH-GRADE GLIOMAS INVASIVENESS

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High-grade gliomas (HGGs) are the most aggressive tumors of the central nervous system (CNS) and include astrocytoma grade III, IV isocitrate dehydrogenase mutant (IDH mut) and glioblastoma multiforme IDH wild type (GBM IDH wt). The tumors differ in age-incidence, and sensitivity to therapeutic protocols with a better outcome for the astrocytomas, making IDH mutation a relevant diagnostic marker.

HGGs are made up of astrocyte-like cells, whose migration is supported by resident microglia, astrocytes, and extracellular matrix (ECM) remodeling along with blood-brain barrier alteration. However, the IDH mutation in the tumor tissue leads to a better tolerance to chemo/radio and immuno-therapies. Despite the little progress, the knowledge regarding CNS remodeling and tumor invasiveness remains unknown.

In the present study, we aim to characterize the glial cells and ECM features in the tumor and peritumoral tissue from human astrocytoma IDH mut in acute slices. Human primary glioma cells tagged using lentiviral transduction were then injected into the organotypic cortex of peritumoral tissue (day 0) to investigate the astrocytoma invasiveness mechanisms. The activity of the slices was tested before and after the glioma cells injection through multi electrode array, and the tumor progression was studied at different time points until DIV 14 using pharmacological assays and morpho-molecular techniques.

Molecular targets related to astrocytes (Cx43, GFAP), microglia/macrophages (Iba1), and ECM (CD44) revealed differential glial morphology and ECM protein expression patterns in the tumor core and peritumoral tissue, with heterogenous profiles among the human specimens. High-grade astrocytomas IDH mut show a high level of Connexin 43 in the peritumoral tissue compared to other gliomas. Infiltrating glioma cells change their morphology with time and quickly invade the available gray matter by overexpressing Cx43. The selective blockage of Cx43 hemichannels modified the polarization and the morphology of glioma cells, indicating a role for the astrocytic Cx43 in orchestrating the CNS response to the tumor.

Our results shed light on the glial-tumor dynamic in the human astrocytoma peritumoral tissue with the identification of molecular targets that may be crucial for slowing down HGG's invasiveness of tumor microenvironment.

HEME OXYGENASE-1 INHIBITION INTERFERES WITH GLIOBLASTOMA PROGRESSION

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Glioblastoma multiforme (GBM) is classified as IV-grade astrocytoma and represents the deadliest brain cancer affecting adults with poor prognosis. It is a solid tumor with a high cell heterogeneity characterized by uncontrolled cell proliferation with consequent formation of hypoxic niches inside the cancer core. Microenvironmental hypoxia induces the transcription of hypoxic inducible factors (HIFs), including HIF-1 α , which in turn activates pathways making the tumor highly aggressive. More specifically, hypoxia regulates many downstream target genes including vascular endothelial growth factor (VEGF), responsible for aberrant neovascularization characterizing GBM progression, and it also modulates gene expression of many other factors with consequent upregulation of enzymes such as heme oxygenase-1 (HO-1).

HO-1 is the enzyme responsible for endogenous heme degradation and it plays a pivotal role in regulating redox homeostasis, especially in cancer cells, which can generate excessive ROS as a result of abnormally rapid proliferation. Its overexpression has been observed to be involved in the development of several types of cancer and has been widely associated with chemoresistance insurgence.

The HO-1 involvement in GBM progression has been previously suggested, but no evidence exists regarding its direct correlation with the hypoxic microenvironment.

In the present work, we have investigated for the first time the link between HO-1 expression and the hypoxic microenvironment of GBM. By culturing two different human glioblastoma cell lines, U87MG and A172 with different tumorigenic potential, in the presence of a hypoxic mimetic agent, deferoxamine (DFX), we have detected a concomitant overexpression of HIF-1 α and HO-1 24h after hypoxia exposure.

Subsequently, targeting hypoxia-induced HO-1 we have tested the effect of a novel azole-based HO-1 inhibitor (VP18/58) in GBM cells exposed to hypoxic insult.

Results have demonstrated that the HO-1 inhibitor treatment has counteracted GBM cell migration ability after 24h from DFX exposure and significantly reduced the expression levels of HO-1, HIF-1 α , and VEGF in the same experimental condition. Moreover, HO-1 inhibition significantly reduced HO-1 nuclear translocation following hypoxia exposure.

In conclusion, our data demonstrated that selected HO-1 inhibition counteracted cancer cell aggressiveness by modulating the HIF-1 α /HO-1/VEGF axis.

ROLE OF c-KIT RECEPTOR IN FETAL BRAIN AND GLIOBLASTOMA VASCULARIZATION

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In the early phases of angiogenesis in human normal developing brain and glioblastoma, an 'unconventional' mode of vessel growth guided by a specific type of pericyte nanostructures, called tunnelling nanotubes (TNTs) has been described. The precise role of pericyte-derived TNTs (P-TNTs), involved in the formation of dynamic vessel networks based on pericyte-endothelium interactions, has still to be ascertained along with the molecular mechanisms that regulate their function in angiogenesis. Several receptor tyrosine kinases (RTK)-based signaling pathways, involved in the canonical endothelial angiogenic vessel sprouting, have been extensively studied under normal and pathological conditions. Considering that among them, the class III c-KIT receptor has so far been underestimated as involved in pro-angiogenic signaling, this study aimed to deepen its possible role in P-TNT-driven angiogenesis. The analysis was carried out in human fetal brain and glioblastoma samples by confocal double immunofluorescence with c-KIT and the specific marker of pericytes, NG2, or with COL IV as a primary component of the vessel basal lamina. The observations showed a primary localization of c-KIT on endothelial cells and unveiled a different subcellular distribution of the receptor on normal *vs* glioblastoma vessels. Overall, the results indicate the c-KIT signaling as involved in P-TNT-driven angiogenesis and suggest this unconventional mode of vessel growth as a possible target for antiangiogenic therapeutic strategies.

SESSION IV NEURODEGENERATION AND NEUROINFLAMMATION

CLUSTERIN IN HUMAN BRAINS AFFECTED BY PARKINSON'S DISEASE

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Clusterin (Clu) is a ubiquitous extracellular chaperone involved in various biological functions, such as clearance of misfolded proteins, response to stresses, apoptosis, and aging. Clu has been implicated in neurodegeneration, since variations in its gene are the third major risk factor for Alzheimer's disease and it is involved in β -amyloid aggregation and clearance. Few studies have reported that Clu also modulated α -Synuclein (α Syn) aggregation and clearance in Parkinson's disease (PD) cells models, but whether this modulation is neuroprotective or neurotoxic is still controversial. Moreover, data regarding the involvement of Clu in PD human brains are currently lacking. The aims of this study are i) to comprehensively characterize the distribution of Clu in various regions of post-mortem human brains and ii) to investigate its potential association with α Syn pathology, using immunohistochemical techniques and high-resolution confocal microscopy. First, we examined Clu levels in neurons and astrocytes of substantia nigra pars compacta (SNpc), striatum, and entorhinal cortex of controls' and PD patients' brains. We observed high heterogeneity of Clu levels within neurons and astrocytes, especially among patients that could depend on patient-specific features. Despite that, we found a significant increase of "extracellular Clu" levels in the SNpc of PD patients. Then, we investigated the colocalization of Clu and α Syn in the abovementioned brain regions. Notably, for the first time, we uncovered Clu presence within Lewy Bodies. Our findings strongly suggest an association between Clu and PD pathophysiology and pave the way to mechanistic studies aimed at unravelling the role of Clu in the modulation of α Syn aggregation and toxicity, that would be important in the perspective of the development of a therapeutic strategy.

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NEUROPROTECTIVE EFFECTS INDUCED BY *BACOPA MONNIERI* AGAINST METHAMPHETAMINE AND MPP+ TOXICITY IN CATECHOLAMINE CELLS *IN VITRO*

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Methamphetamine (METH) and 1-methyl-4-phenylpyridinium (MPP+) are neurotoxins, which damage catecholamine neurons with substantial differences in their molecular and cellular mechanisms. In fact, while METH neurotoxicity mostly depends on oxidative species, MPP+ toxicity depends on the inhibition of mitochondrial activity. This explains why only a few compounds protect against both neurotoxins. Recently, evidence shows that phytochemicals may protect against neurodegeneration. This involves counteraction oxidative stress. Therefore in the present study we investigated whether: (i) natural extracts from *Bacopa Monnieri* (BM) may protect both METH and MPP+ toxicity; (ii) protection occurs along with suppression of reactive oxygen species (ROS); (iii) BM prevents mitochondrial alterations. The protective effects were measured in catecholamine cells by light and electron microscopy, with MitoTracker Red and Green as well as by ultrastructural morphometry of mitochondria. We found that BM dose-dependently protects against mitochondrial damage by suppressing mitochondrial crest destruction and matrix dilution and by increasing the amount of healthy and total mitochondria. This effect is related to the reduction of ROS formation. The present data provide evidence that BM protects catecholamine cells by exerting a powerful antioxidant action and preserving mitochondrial integrity, independently by the type of experimental toxicity.

ADIPOSE STEM CELLS FOR BLOOD-RETINAL BARRIER REPAIR

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In the last few decades, adipose-derived mesenchymal stem cells (ASCs) have been widely investigated in the field of regenerative medicine because of their multipotent differentiation ability. In fact, they can give rise not only to elements of mesodermal origin, but also to elements of different cell lines such as neurons or glial cells. In the present work, we tested a pericyte (PC) differentiation of ASCs in order to provide a tool to overcome the massive PC loss occurring in cases of diabetic retinopathy, characterized by the blood-retinal barrier (BRB) impairment due to altered interactions between endothelial cells and PCs. To this aim, pericyte-like ASCs (P-ASCs) were obtained by growing them in a specific PC medium. In addition, some samples of P-ASCs were cultured in high

glucose (HG) conditions to mimic the altered microenvironment of a diabetic eye. Their possible beneficial effects were assessed in co-cultures of P-ASCs and human retinal endothelial cells (HRECs). Results obtained by immunofluorescence techniques show that, compared to native ASCs, the presence of P-ASCs induced an increased endothelial expression of junction proteins (VE-cadherin and ZO-1), suggesting an improved BRB integrity, as also indicated by higher values of trans-endothelial electrical resistance. Moreover, by three-dimensional co-cultures carried out in Matrigel, it was possible to demonstrate that P-ASCs were preferentially positioned in the same location as native PCs, i.e. around the typical tubular, vessel-like structures formed by HRECs. It can be concluded that P-ASCs may represent a valuable tool to develop therapeutic strategies to counteract BRB disruption in case of diabetic retinopathy.

THIOCTIC ACID AND CDP-CHOLINE MODULATE THE NEUROINFLAMMATION IN LPS-STIMULATED MICROGLIA CELLS AND HIPPOCAMPUS OF HYPERTENSIVE RATS

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Brain diseases may occur as a consequence of neuroinflammatory cascades, including alterations in the cross-talks between glial cells and neurons due to the activation of microglia and astrocytes. The aim of the study was to investigate whether (+)-thioctic acid (TIO) and CDP-choline (CDP) alone or in association could block the inflammatory response in lipopolysaccharide (LPS)-stimulated BV2 microglia cells and in the hippocampus of 24-week-old spontaneously hypertensive rats (SHR). A murine microglial cell line was incubated with LPS and different concentrations of both compounds for 24 h. Following treatments, the cell viability assay did not show significant changes. LPS promoted morphological alterations, an increase in ionized calcium-binding adapter molecule 1, and interleukin-1 beta levels accompanied by nuclear translocation of nuclear factor-kappa B. These changes were reversed after the treatments with both TIO and CDP. The results of *in vitro* experiments were consistent with those obtained in the hippocampus of SHR rats treated for four weeks with TIO and CDP, alone or in combination. On the other hand, treatment with TIO and CDP attenuated gliosis and microglial activation. Moreover, the expression levels of interleukin-1 beta and nuclear factor-kappa B were decreased. These findings suggest that the use of an antioxidant compound associated with a cholinergic neurotransmission enhancer could represent an approach for treating brain disorders characterized by neuroinflammation and vascular impairment.

COMPARATIVE ANTI-INFLAMMATORY EFFECT OF EPA AND DHA DERIVATIVES ON OECs EXPOSED TO LPS

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Neurodegenerative diseases are characterized by neuroinflammation, a symptom with growing interest directed towards the development of active drugs for the reduction or elimination of its negative effects. The anti-inflammatory and potential neuroprotective activity in some neural cells of Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), belonging to the class of ω -3 polyunsaturated fatty acids, is largely reported. Most of the observed biological activities of these fatty acids are maintained, and in some case enhanced, in the corresponding amide derivatives or oxygenated metabolites. While free acids EPA and DHA are commercially available, the corresponding amides were suitably prepared by aminolysis of the ethyl esters of EPA and DHA with ethanolamine in the presence of immobilized lipase from *Candida antarctica* (Novozym 435) and molecular sieves in tert-butyl methyl ether.

Our study aims to elucidate the protective effect of both EPA and DHA, as well as of the corresponding N-ethanolamides EPA-EA and DHA-EA, on Olfactory Ensheathing Cells (OECs) exposed to lipopolysaccharide (LPS)-induced neuroinflammation for 24 h. OECs are glial cells located in the olfactory system, which is the first to show a deficit in neurodegenerative diseases. To verify the anti-inflammatory effect of these compounds on OEC cultures and on their morphological features, the expression of some cytoskeletal proteins, such as Vimentin and Glial Fibrillary Acid Protein (GFAP), was evaluated by immunocytochemical procedures. In addition, MTT test was carried out to establish the non-toxic concentrations and the optimal time of exposure. Mitotoxicity and cytotoxicity levels, in all experimental groups, were detected by using the HCS Mitochondrial Health Kit, while apoptosis was determined in by staining with the TUNEL Alexa Fluor Imaging assay.

Our results showed a decrease of GFAP and Vimentin expression in OECs treated with EPA or DHA acids or EPA-EA or DHA-EA and stressed with LPS when compared with OECs exposed to LPS alone. While a protective role on cell morphology was predominantly observed for EPA and DHA, the amides EPA-EA and DHA-EA mainly showed anti-inflammatory effects, superior to those of free acids. These results highlight that all the tested compounds have anti-inflammatory activity on LPS-exposed OECs and may provide an innovative tool to contrast neuroinflammation, which plays a key role in several neurodegenerative diseases.

SESSION V NEURODEGENERATION AND NEUROINFLAMMATION 2

SYNUCLEINOPATHIES PREDICTORS AND WHERE TO FIND THEM

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Synucleinopathies are complex, multifaceted neurodegenerative diseases characterized by the accumulation of pathogenic aggregates of α -synuclein, ultimately leading to neuronal death. The differential diagnosis of these diseases is still a challenge, especially in early stages, and is currently based on the evaluation of neurological symptoms, assisted by neuroimaging techniques. At the time of diagnosis, brain injury is already beyond the "event horizon", thus it become a necessity to identify risk groups and predict the pathologies at the prodromal stages, to ensure patients early preventions and personalized interventions. In this field, giving the emerging evidence of α -synuclein pathology also in the peripheral nervous system, we took advantage of skin biopsies, a simple and non-invasive model, to identify reliable biomarkers for synucleinopathies. We studied the distribution of α -synuclein oligomers, a toxic and aggregation-prone strand, within the sudomotor autonomic fibers of idiopathic (iPD) and GBA1-associated (GBA-PD) Parkinson's disease patients. Compared to controls, both iPD and GBA-PD samples displayed a significant increase in the oligomeric burden. The good sensitivity, specificity, and positive predictive value (82%, 86% and 89%, respectively) of the quantitative score we reported, endorse the hypothesis that α -synuclein oligomers could constitute a reliable diagnostic biomarker for both iPD and GBA-PD. In the attempt to discriminate between iPD and GBA-PD, we observed no difference in oligomers distribution between the two subpopulations. Nevertheless, an increased synaptic density was found in GBA-PD compared to both iPD and controls, which may suggest different pathophysiological mechanisms underlying the two diseases, but also a common pathway that ultimately leads to the formation of oligomers. On this background, a new project started this year, aimed to develop a strategy for prediction of phenoconversion from pre-clinical to manifest pathology [DEEPEN-iRDB]. For this study, the Luxembourg National Centre for Excellence in Research on Parkinson enrolled about a hundred of patients affected by synucleinopathies (iPD, GBA-PD, MSA, DLB) together with healthy controls and iRBD patients, known to evolve to synucleinopathies with a very high incidence. The goal is to identify clinical and molecular fingerprints to implement correct diagnosis, to define prognosis at early disease stage and to develop tailored interventions.

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ROLE OF PACAP-ADNP AXIS ON SOD1-G93A MOTOR NEURONS

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Amotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease characterized by progressive degeneration of upper and lower motor neurons. Mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) account for approximately 20% of familial ALS cases. The pathological mechanism underlying the toxicity induced by mutated SOD1 is still unknown. However, overproduction of reactive oxygen species (ROS) has been observed in the spinal cord and motor cortex of both patients as well as animal models. Moreover, in an *in vitro* model of the disease, it has been documented that mutated SOD1 impairs the expression of the nuclear factor erythroid 2-related factor 2 (Nrf2), involved in the antioxidant response.

The protective effect of pituitary adenylate cyclase-activating peptide (PACAP) has been demonstrated in various neurological disease, including ALS. It was observed that some of its effects are mediated through the stimulation of an intracellular factor known as activity-dependent protein (ADNP).

To date, the role of PACAP-ADNP axis on mutated SOD1 motor neurons degeneration has not been explored. The aim of the study was to investigate whether the protective effect of PACAP against apoptotic cell death induced by growth factor deprivation is mediated by ADNP activation counteracting the oxidative insult.

Our data revealed that PACAP is able to prevent cells death following growth factors deprivation by activating ADNP expression. Furthermore, we have also demonstrated that PACAP/ADNP axis counteracted ROS formation by inducing translocation of the Nrf2 from cytoplasm to the nucleus.

In conclusion, this study provides new insights regarding the protective role of PACAP-ADNP axis in ALS.

LABELLED NANOVESICLES FROM ADIPOSE MESENCHYMAL STEM CELLS TARGET INFLAMED LYMPH NODES IN CHRONIC EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Adipose mesenchymal stem cells (ASC) represent a promising therapeutic approach in neurological disorders like Multiple Sclerosis (MS). Recently, several lines of evidence indicate that most biological activities of ASC are mediated by the release of soluble factors in form of micro and nanovesicles. Indeed, we have recently demonstrated that ASC-derived nanovesicles (ASC-NVs) ameliorate clinical and pathological aspects of experimental autoimmune encephalomyelitis (EAE).

Despite this evidence, the precise action mechanisms and molecular/cellular target of NVs are unknown. For this purpose, we injected intravenously (i.v.) NVs loaded with ultra-small superparamagnetic iron oxide nanoparticles (USPIO-NVs) in EAE mice. Histochemical analysis and transmission electron microscopy were analyzed 48 h post-injection in lymph nodes and other tissues of EAE mice. Noteworthy, the TEM analysis of EAE mice (but not PBS-injected controls) showed the presence of labelled NVs in their lymph nodes. We then performed flow cytometry on cells extracted *ex vivo* from EAE mice to assess the cellular target of NVs. We showed that most of ASC-NVs were located in macrophages and dendritic cells. To our knowledge this is the first direct evidence of the migration of ASC-NVs after i.v. injection in reactive lymph nodes; this result together with our previous demonstration of a potent anti-inflammatory action exerted by ASC-NVs in EAE lymph nodes may indicate a causal correlation. These data give us important information on the homing and cellular targets of ASC-NVs which may help the development of NV-based therapy in MS.

SESSION VI PERIPHERAL SYSTEM AND GUT-BRAIN AXIS

TARGETING GUT-BRAIN AXIS IN ALZHEIMER'S DISEASE

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Several studies highlighted the relevant role of microbiota-gut-brain (MGB) axis in the pathophysiology of Alzheimer's disease (AD) and related intestinal symptoms. In this context, the modulation of gut microbiota is emerging as a suitable additional therapeutic option, targeting MGB axis, to halt or slow down the cognitive impairment and intestinal symptoms associated with AD. The aim of the present study was to evaluate the putative beneficial effect of mixture of probiotics (MP) in counteracting central and peripheral morphological and functional alterations in a spontaneous murine model of AD. Senescence-accelerated mouse prone 8 (SAMP8) mice (4 months old) and control SAMR1 strain were treated orally with MP 1×10^9 CFU/mouse/day or placebo for two months (up to 6 months of aged) to evaluate the effects of probiotics during the earliest stages of AD before the full development of brain pathology. Cognitive functions and *in vitro* colonic motility were assessed. Then, the following parameters were evaluated by immunohistochemistry and ELISA techniques: 1) brain and colonic interleukin (IL)-1 β levels; 2) alterations of the intestinal epithelial barrier (plasma LBP levels, acid/neutral mucin ratio and claudin-1). SAMP8 mice showed cognitive impairment and colonic dysmotility as well as an increase in brain and colonic IL-1 β . In addition, SAMP8 animals displayed an increase in plasma LBP levels and intestinal acid mucins along with a reduction in intestinal claudin-1, as compared with SAMR1 mice. Intake of MP counteracted cognitive impairment, colonic dysmotility, the increase in central and peripheral IL-1 β levels in SAMP8 mice. MP also restored colonic acid mucins and reduced plasma LBP levels.

In conclusion, the MP alleviates cognitive decline and restores colonic motility, by preventing gut barrier impairments and decreasing gut and brain inflammation in AD mice in the prodromal phases of the disease, via MGB axis. Therefore, dietary supplementation with MP can represent a useful therapeutic approach to counteract the morphological and functional alterations underlying neurodegeneration and intestinal dysfunction associated with AD.

MORPHOLOGICAL MODULATION OF COLONIC ENTERIC PLEXI IN MICE MODEL OF COLITIS AND THE POSSIBLE EFFECTS OF BACTERIAL STRAIN SUPPLEMENTATION

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Inflammatory bowel diseases (IBD) are gastrointestinal disorders associated with altered intestinal permeability, which causes a degeneration of the enteric plexi of the enteric nervous system (ENS). Treatments for IBD show poor efficacy. Many studies have identified probiotic supplementation as a possible method for alleviating clinical symptoms. The potential properties of *Pediococcus acidilactici* 46A (Pa) were evaluated on a murine model of Dextran sulfate sodium (DSS)-induced colitis as models of chemically induced IBD. Colitis was induced in 8-week-old mice, administering 2.5% (w/v) DSS in drinking water for 7 days. Pa was supplemented orally (1×10^8 CFU daily) for 10 days before DSS administration. General conditions, body weight loss, stool characteristics, and occult blood were monitored to evaluate the clinical progression of colitis. Histological damage, neurodegeneration, and pro-inflammatory cytokines expression were detected on proximal and distal colon sections and the histological index scoring was evaluated. Pa in the pretreated mice was able to reduce the colitis severity while not affecting weight loss, compared to the DSS group. Defects of enteric glial cells (EGCs) function and localization, barrier integrity dysfunction, and immune cell infiltrations were observed in colitis-induced groups and a positive improvement was evidenced in Pa-supplemented groups. Morphological modification of neurons of the myenteric plexus was assessed by evaluating HuC/D pan-neuronal marker, then colonic nitergic and cholinergic pathways were focused, and a neurodegeneration was appreciated in DSS-mice. These results demonstrate that Pa seems to counteract colonic mucosal degeneration and neuronal alteration. However, further studies are needed to demonstrate the use of specific bacterial strains to manage intestinal disorders and correlated ENS modulation in IBD.

PERIPHERAL NERVE STIMULATION PRESERVES PERIPHERAL NERVE INJURY INDUCED MORPHOLOGICAL CHANGES IN THE TRIGEMINAL SYSTEM

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Peripheral nerve injury (PNI) triggers a complex cascade of neurobiological events within the somatosensory system, the full extent of which remains to be fully elucidated. Previous studies have primarily focused on specific components of the ascending system, with limited attention given to comprehensively exploring the entire somatosensory system and understanding the morpho-

functional changes that occur following PNI. In addition, peripheral nerve stimulation (PNS) has emerged as an alternative therapy for managing pain resulting from PNI. Our group studied morphological changes in the ascending sensory trigeminal tract following axotomy of the trigeminal nerve and peripheral chronic electrical stimulation in rats. Our results showed significant drop in GABAergic and modulatory neuronal populations in the entire trigeminal ascending system and pain-modulatory limbic axis. However, PNS demonstrated a neuroprotective role as it prevented PNI-induced alteration in cellular populations. Unilateral loss of GABAergic and ACh neurons following PNI causes a loss of inhibitory tone and may explain the principal alterations which are followed by chronic pain disorders. Moreover, PNS emerges a convenient tool to address fundamental morphological reactions following injury. Although our study demonstrates the therapeutic capacity of PNS, the mechanisms are not yet fully elucidated.

CIPN AND ITS MECHANISM OF DEVELOPMENT

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect of various cancer chemotherapy treatments. Typically, CIPN manifests in patients as numbness, tingling, altered sensation, usually associated with neuropathic pain and, therefore the adjustment of chemotherapy dosages or even discontinuation of the treatment. Although various chemotherapeutic agents induce neuropathy, they do so by targeting different cellular processes and having different mechanisms. The pathomechanism by which chemotherapeutics damage the nervous is multifactorial and involves microtubule disruption, oxidative stress and mitochondrial damage, altered ion channel activity, myelin sheath damage, DNA damage, immunological processes and neuroinflammation. Neuroinflammation is one of the host defensive mechanisms and occurs as one of the most common pathological outcomes in most neurological and neurodegenerative diseases make it the promising target. Several CIPN preclinical studies suggest critical neuro-immune interactions as a mechanism of neurotoxicity.

In this study we analyzed the presence of neuroinflammatory marker proteins in rats treated with chemotherapy drugs, in order to identify the inflammatory components in pathology of CIPN development. Expression of inflammasome protein NLRP3, interleukin IL6 and chemokine CCL2 was analyzed in peripheral nervous tissue after chemotherapy with Paclitaxel, Oxaliplatin and Bortezomib. All three proteins showed a significant increase in the caudal nerve in Paclitaxel treatment over 4 weeks. Treatment with Oxaliplatin did not give any increase in protein expression, while Bortezomib showed a significant increase in caudal nerve only for CCL2. Immunohistochemical staining represents infiltration of macrophages in caudal nerve in rats treated with Paclitaxel and Bortezomib but not Oxaliplatin. Neurophysiology and dynamic tests showed that rats treated with Paclitaxel and Bortezomib develop allodynia, reduce nerve conducting velocity and nerve potential amplitude. Rats treated with Oxaliplatin showed only reduction of nerve amplitude. Despite all three chemotherapy treatments resulting in neuropathy development, we were able to

demonstrate distinct pathological processes and biomolecular patterns in disease progression. This finding suggests that each of the three chemotherapies operates through a different mechanism. Specifically, only Paclitaxel treatment exhibited activation of neuroinflammation.

ACELLULAR PIG NERVE GRAFT TO REPAIR MEDIAN NERVE INJURY: A PRELIMINARY STUDY ON RAT

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When a peripheral nerve injury with large defect occurs, end to end suture is not possible and the use of conduit is not enough to achieve nerve regeneration and functional recovery of target organ. Allograft could be an alternative, but nerves from donors frequently cause immunogenic response; for this reason, several authors are looking for the correct way to decellularize nerves preserving both the extracellular matrix (ECM) and basal lamina that represent the key elements used by Schwann cells during the regenerative process. 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.

The first aim of the present study was to test the efficacy of a decellularization method currently used to decellularized horse tendons in order to evaluate its possible application to create efficient nerve graft. The second aim was to evaluate the ability of the acellular porcine nerve graft obtained through the decellularization procedure to repair a median nerve lesion in rat.

To investigate ability of the decellularization protocol to remove immunogenic cellular components of the nerve tissue and to preserve the basal lamina and extracellular matrix, morphological analysis has been performed comprising Masson's Trichrome staining, immunofluorescence, high resolution light microscopy and transmission electron microscopy (TEM).

Morphological analysis was also used to study the ability of the porcine acellular graft to support nerve regeneration.

Four weeks after injury, regenerating fibers have colonized the graft suggesting a promising use for repairing severe nerve lesions.

OXALIPLATIN NEUROTOXICITY: MORPHO-FUNCTIONAL APPROACH

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Oxaliplatin (OHP) chemotherapy (CHT) is seriously limited by neurotoxic side effects for whom there is no treatment; this unmet clinical need is in part due to an uncompleted pathogenetic knowledge and, therefore, robust preclinical models are needed to advance patients' care. OHP-induced peripheral neurotoxicity (OIPN) has a peculiar profile: it comprises an acute syndrome and a chronic sensory axonopathy. Acute OIPN is characterized by transient cold-induced paresthesia and cramps, lasting 2-3 days after each administration; acute OIPN has been attributed to a transient ion channel dysfunction. The worse acute OIPN is, the more severe the chronic neuropathy that ensues. Therefore, an OIPN model should be able to reproduce both conditions.

We designed an *in vivo* study to this aim: we compared a control group with a treated group (OHP 3 mg/Kg twice a week over 4 weeks, iv). Nerve excitability testing (NET) was used to assess acute OIPN. Behavioural test, nerve conduction studies (NCS), and neuropathology were used to characterise chronic OIPN; the latter included: morphological/morphometrical assessments of the caudal nerve and dorsal root ganglia (DRG); intraepidermal nerve fiber density (IENFD); spinal cord immunohistochemistry for the transient receptor potential vanilloid type-1 (TRPV1) receptor. Data were collected at the end of treatment and 6 weeks after.

NET allowed us to show that acute OIPN ensued as soon as the first administration and it did not persist after CHT (no NET alterations 1 week after CHT completion). Behavioural tests, NCS, nerve/DRG morphological/morphometrical analysis, and IENFD showed that a mild sensory neuronopathy/axonopathy had ensued at CHT completion and nearly completely resolved at follow-up. Densitometric analysis of TRPV1 immunolabeling in the dorsal horn of the spinal cord at the end of treatment showed an increased density of TRPV1 staining in OHP animals (in lamina I and inner lamina II). This difference was maintained at follow-up.

We showed that acute OIPN (*i.e.*, alterations of ion channels) is transient and chronologically related to OHP administration: it resolves after chemotherapy completion and it does not correspond to neuropathic pain and/or small fiber neuropathy; NET was normal 1 week after CHT completion, whereas IENFD and spinal cord immunohistochemistry showed alterations even 6 weeks after. Acute and chronic OIPN are distinct entities to be carefully and separately considered in future research.

POSTERS SESSION

IN VITRO ANALYSIS OF AN INNOVATIVE BIOMATERIAL

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The objective of the present study is to evaluate biocompatibility and biomimeticity of an innovative membrane with the aim to apply it for repairing somatic and autonomic peripheral nerves in case of traumatic or iatrogenic lesions. Starch-derived (GLUCIDEX[®]) hyper-crosslinked polymers with suitable mechanical properties were electrospun as membrane and tested, *in vitro* using immortalized Schwann Cells (RT4-D6P2T cells), for cell survival and proliferation to evaluate the biocompatibility and biomimetic nature of the scaffolds.

RT4-D6P2T cells were cultured i) in direct contact with the membrane, to investigate the interaction with the substrate and ii) in the presence of membrane dissolution products, to test the effect on cell proliferation and organization.

- i) Concerning to the adhesion assays, the actin cytoskeleton results more organized in the control group, however, after 24 h, the density and the area occupied by RT4-D6P2T increased.
- ii) Several analyzes were conducted using the dissolution products of Glucidex[®] membranes; the proliferation assay revealed that, after 1, 4 and 7 days of culture, cells maintain proliferative behavior under all conditions tested although a slight decrease, compared to the control, is observed at the first two time points. The actin cytoskeleton profile revealed that cells cultured in conditioned medium have a high organization and generate membrane protrusions, lamellipodia, correlated to cell migration, an important feature of glial cells in support of peripheral nerve regeneration.

Investigating apoptosis and the specific cellular alterations due to Bax, pro-apoptotic protein, and Bcl-2, anti-apoptotic protein, our study revealed that the dissolution products of the membrane are not related with cell death, contrarily, they are associated with good survival. Further investigations are underway to deepen the effect of the dissolution products on expression of gene involved in the regulation of nerve regeneration by Schwann cells.

SATELLITE GLIAL CELLS IN CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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Chemotherapy-induced peripheral neuropathy (CIPN) is a frequent side effect caused by many of the most commonly used chemotherapeutic agents, including anti-tubulins (paclitaxel, PTX) and platinum derivatives (cisplatin, CDDP). Due to incomplete understanding of the molecular mechanisms of CIPN, to date no

effective therapy is available. Sensory neurons into dorsal root ganglia (DRG) have been investigated as principal targets of neurotoxicity so far. In this study, we focus on a possible novel target of CIPN, investigating the changes of satellite glial cells (SGCs) in the DRG and their crosstalk with neurons following repeated administration of PTX and CDDP in rats.

Morpho-functional analyses were performed to verify the features of CIPN. Qualitative and quantitative immunohistochemistry, 3D-immunofluorescence, immunoblotting, and transmission electron microscopy analyses were also performed to detect alterations in SGCs and their interconnections.

We demonstrated that after 4 weeks of PTX, but not CDDP treatment, SGCs were strongly activated. A similar activation remained after 4 weeks of follow up, when the painful component of neuropathy, but not the nerve damage, was resolved. In addition, non-physiological connections between SGCs and/or SGC-neuron were evident in PTX rats: we observed activated SGCs surrounding different adjacent neurons and an increase in the intimate contact between SGCs and their associated neurons where a complex and peculiar pattern of glial cytoplasmic projections was present. Moreover, PTX increased the expression of Connexin43 with perineuronal localization and the expression of the adhesion molecule L1-CAM in the cytoplasm and plasma membrane of neurons. We conclude that SGCs may act as principal actors in PTX-induced peripheral neurotoxicity, paving the way for the identification of new druggable targets for CIPN treatment and prevention.

THE ROLE OF PACAP IN AN *IN VITRO* MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is an incurable and multifactorial neurodegenerative disease induced by the synergistic action of genetic and environmental factors. It is characterized by the loss of motor neurons (MNs), but not all MNs undergo degeneration: neurons of the oculomotor nucleus, which regulate eye movements, are less vulnerable compared to hypoglossal nucleus MNs. The adenylate cyclase-activating polypeptide 1 (ADCYAP1) gene, encoding for pituitary adenylate cyclase-activating polypeptide (PACAP), was found to significantly up-regulated in the oculomotor versus hypoglossal nucleus suggesting that it could play a trophic effect on MNs in ALS. By using a motor neuron-like hybrid cell line (NSC-34) expressing human SOD1 G93A as an *in vitro* model of ALS, we investigated the role of PACAP following growth factors deprivation. Our results showed that PACAP increases cell viability and prevents epidermal growth factor (EGF) deprivation-induced cell death in NSC-34 cells through EGFR transactivation mediated by protein kinase A stimulation. Overall these data that a deeper characterization of mechanisms involved in PACAP/EGFR axis activation in G93A SOD1 mutated neurons may allow identifying new targets for ALS therapy.

THE BBB PERMEABILITY: HOW MUCH BLOOD FLOW-INDUCED SHEAR STRESS AFFECT IT

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The blood-brain barrier (BBB) is the well-known physiological wall that allow the selective influx and efflux of molecules between the brain parenchyma and the blood circulation. The protagonists of this mechanism are the endothelial cells, assisted by the presence of pericytes and astrocytes. On the other hand, cerebral blood flow is also strictly regulated and its increase can induce shear stress. Indeed, in the past years, neurodegenerative disorders were associated to blood pressure variability. In the present research we evaluated the shear stress effect on rat brain endothelial cell line (RBE4), a widely used BBB *in vitro* model, in order to investigate the putative role of increasing flow in tight junction dislocation. To mimic blood flow in our *in vitro* model, we used the LiveBox2 (LB2) instrument (IVTech S.r.l., Lucca, Italy) that allow to set-up a millifluidic flow, ranging from 50 to 500 μ l/min. Briefly, the RBE4 cells were gently seeded on the cover slip of LB2 chamber system, and allowed to growth at least for 24 h. The day after, in order to induce the medium flow on the chamber system, the system was connected to the pump and the appropriate flow rate was set-up. The system was left for 3 days at 37°C, 5% CO₂ in humidified atmosphere, then the system was opened and the cells were fixed in cold methanol for 20 min. at 4°C. Immunofluorescent staining for zonula occludens-1 (ZO-1) was performed in order to evaluate the tight junction dislocation. Our results clearly demonstrated that shear stress affect the ZO-1 localization starting from 100 μ l/min flow rate. Such a deleterious effect can be hypothesized as a possible mechanism that induces an increase in barrier permeability and therefore allows the entry of harmful substances that alter the brain parenchyma.

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FUNCTIONALLY INDEPENDENT SUBUNITS OF THE ARCULATE FASCICULUS AND THEIR CONTRIBUTION TO HIGHER-ORDER PROCESSING

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Despite being one of the most studied white matter bundles of the human brain, the anatomical and functional organization of the arcuate fasciculus is still a matter of debate. While earlier anatomo-clinical models of language processing considered the arcuate fasciculus as a unique entity, recent evidence has highlighted the importance of distinct tract segments, each with its specific functional relevance in language comprehension and production.

Herein, we employed track-weighted dynamic functional connectivity, a recently developed hybrid imaging technique, to analyze the structure and function of the arcuate fasciculus. By mapping time-windowed functional connectivity, sampled from resting-state functional MRI, back on the underlying white matter anatomy, reconstructed by tractography, we were able to gather information on the spontaneous functional activity of the arcuate fasciculus in a large cohort of healthy subjects. By decomposing the multivariate signal, we found that the arcuate fasciculus may be subdivided, according to dynamic changes in functional connectivity at the streamline endpoints, into two independent components arranged in a dorsoventral and mediolateral topographical organization. Aiming at identifying white matter connectivity patterns that might be related to cognitive processes and at quantitatively estimating their behavioral profiles, we first applied an unsupervised, hard parcellation algorithm to these independent components retrieving spatially segregated subunits within the arcuate fasciculus, and, subsequently, quantified their functional involvement using a meta-analytic approach. We were able to identify three clusters with distinct courses, cortical termination, and functional implications: the ventral segment of the arcuate fasciculus being related to auditory and phonological processing, the intermediate segment to language functions in general and particularly to semantic processing, and the dorsal segment to other higher-order processes, such as social cognition. Our findings provide the first data-driven evidence for the morpho-functional segregation of the arcuate fasciculus and may contribute to shed new light on the anatomy, organization, and functional contribution of this clinically relevant white matter tract.

POSTNATAL ESTROGEN RECEPTOR ANTAGONIZATION HAS ORGANIZATIONAL EFFECT ON KISS SYSTEM

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Mammalian reproduction is orchestrated by the interaction of many factors, including estradiol (E2). During critical periods of development, this hormone programs and stabilizes many hypothalamic systems that control reproduction, acting through three different receptors: ER α , ER β and GPR30.

To understand the role of these estrogen receptors on organizational effect of E2 in both sexes, we treated male and female CD1 mice from post-natal day (PND) 5 to PND12 with subcutaneous injections of vehicle (corn oil), E2 and E2 associated with selective antagonist of estrogen receptors (MPP; PHTPP; G15) alone or together (mix). We analyzed, during the development, different physiological parameters related to reproduction (puberty onset, estrus cycle) and behavior (Y-maze, sexual behavior). In adulthood, we have immunohistochemically evidenced the expression of the Kisspeptin system (kiss) within different hypothalamic nuclei, as a key system in the regulation of reproductive behavior. In general, postnatal treatment with E2 induces sexually dimorphic effects on sexual behaviors, which are much more pronounced in females. A dominant role in sexual preference is given by the two

nuclear receptors (ER α and ER β): postnatal antagonization leads to loss of preference in both sexes. The kisspeptin system showed no alteration in treated males, whereas in females treatment with E2 alone significantly reduced kiss-ir in all nuclei analyzed. The results showed a clear role of the ER α receptor in controlling kiss organization in females only. In conclusion, our data demonstrate that E2 has a strong organizational role on reproductive behavior, acting primarily on nuclear estrogen receptors.

NEURAL-LIKE DIFFERENTIATION OF ADIPOSE STEM CELLS

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Various strategies can be adopted to induce a neural-like differentiation of adipose-derived mesenchymal stem cells (ASCs). This is of great interest to develop therapeutic strategies for the treatment of a number of human pathologies, including neurodegenerative diseases. Other than adding inductive chemical molecules to the culture medium, good results can be obtained by using a conditioned medium (CM) from other cells such as neural cells. In previous investigations, a neural-like ASC differentiation was achieved using CM from Schwann cells or Olfactory Ensheathing Cells (OECs), to mimic as close as possible a physiological neural microenvironment. In this work, a CM from Neural Progenitor Cells (NPCs) was tested and compared to results obtained by using an OEC-CM or a neural basal medium (NBM), specifically designed for NPCs. To this purpose, the fluorescent immunocytochemical expression of typical neural markers (β 3-Tubulin, Synapsin and Neuron-specific enolase) was detected after 1 and 7 days of treatment. A sample of native ASCs cultured in the basal growth medium served as control. Results obtained confirmed that, compared to controls, a neural-like ASC differentiation could be induced by using OEC-CM. Similar outcomes were observed when ASCs were exposed to the neural basal medium. A much more increased expression of neural markers was observed by growing ASCs in NPC-CM. Moreover, also cell morphology changed during the differentiation processes, since bigger cell bodies were observed, featuring cytoplasmic elongations. Overall, these protocols represent further strategies to induce a neural-like ASC differentiation, aimed at developing ASC-based therapeutical approaches.

NEUROPROTECTIVE EFFECT OF EXTRACELLULAR VESICLES DERIVED FROM ADIPOSE STEM CELLS DIFFUSED THROUGH AN EPITHELIUM ON INJURED NEURONAL CELLS

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Adipose mesenchymal stem cells (ASCs) represent a feasible and valid alternative to other sources of mesenchymal stem cells in the treatment of several neurological disorders. Currently, the scientific community brings a growing body of evidences indicating that ASCs exert their neuroprotective and immunomodulatory effects by a paracrine mechanism through the release of extracellular vesicles (EVs). Indeed, EVs are considered important mediators in intercellular communication as they can transfer their cargo (proteins, miRNAs and mRNAs) to nearby cells promoting nerve regeneration, neuronal protection, synaptic plasticity and remyelination in different pathophysiological contexts, recapitulating the effect of origin cells. However, the passage of therapeutic agents through the physiological barriers could represent a limiting factor to treat CNS disorders. For this purpose, the use of ASC-EVs, due to their small dimension represent a promising therapeutic approach in particular in view of their potential feasible and translational administration. The aim of this study is to evaluate the ASC-EVs neuroprotective effects after their passage through an epithelial barrier. For this purpose, we developed an *in vitro* model of epithelium form nasal-derived cells, evaluating ASC-EVs neuroprotective effect after their passage through the epithelial barrier on an oxidative stress-induced model of neuronal cells. Fluorescent labelled ASC-EVs were used to investigate ASC-EVs preferential crossing route of the epithelium and subsequent uptake by damaged neurons. The results showed that ASC-EVs are able to rescue injured cells from oxidative damages after their passage through the epithelium. These results pave the way to use ASC-EVs as a novel treatment and in particular for the development of innovative and targeted delivery system of ASC-EVs for several neurodegenerative diseases.

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EXTRACELLULAR VESICLES FROM ADIPOSE STEM CELLS: THEIR EFFECT ON A MICROGLIAL MODEL OF ALS

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the progressive degeneration of upper and lower motor neurons (MNs) in the brain and spinal cord leading to

paralysis and early death. Increasing evidence indicates that neuroinflammation plays an important role in ALS pathogenesis and disease progression. Neuroinflammatory responses, primarily driven by activated microglia and astrocytes, and followed by infiltrating peripheral immune cells, contribute to exacerbate MNs death. In particular, the role of microglia in ALS remains unclear, partly due to the lack of a model system that is able to completely recapitulate the complexity of ALS pathology. Here we developed and characterize a microglial cells line, SIM-A9 expressing human mutant protein Cu⁺/Zn⁺ superoxide dismutase1 (SIM-A9hSOD1(G93A)) as *in vitro* model mimicking microglia activity in ALS. The expression of hSOD1(G93A) in SIM-A9 cells is able to induce their metabolic activity, causing polarization into a pro-inflammatory phenotype and enhancing reactive oxygen species production, which can lead to activation of cell death processes and apoptosis. Afterwards we used our microglia cellular model as an experimental set up to investigate the therapeutic action of extracellular vesicles from adipose mesenchymal stem cells (ASC-EVs). ASC-EVs represents an innovative therapeutic treatment for ALS due to their neuroprotective and immunomodulatory properties. However, their capacity to modulate inflammatory microglia in ALS is not clear. Here we demonstrate that the treatment with ASC-EVs on microglial cells is able to modulate the inflammatory microglia reducing their metabolic activity, polarizing their phenotype toward an anti-inflammatory one, and decreasing the reactive oxygen species production.

EFFECTS OF MTOR INHIBITION IN IPSC-DERIVED MOTOR NEURONS FROM PATIENTS WITH C9ORF72 REPEAT EXPANSION

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In the present study we analyzed a cell model derived from iPSC, which were promoted to differentiate into motor neurons (iPSC-MNs). The use of iPSC-MNs is now more and more important in dissecting at cellular level the cell pathology occurring in a variety of disorders. This is specifically important in neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS). Therefore, we profited from these iPSC-MNs, which were obtained from patients carrying a repeat expansion in C9orf72, a common genetic cause of familial ALS, to test whether specific cell pathways, such as the autophagy machinery and the formation and release of exosomes, were altered. In neurodegeneration, autophagy impairment may impair in clearance of pathological aggregates, which can be transmitted from cell to cell. Therefore, in this context, intracellular pathological proteins and exosomes, or other cell-to-cell communication pathways, could promote the spreading of pathological elements, contributing to disease progression. In detail, the gene coding C9ORF72 protein is involved in the autophagy machinery, interacting with Unc-51 Like Kinase 1 (ULK1) in autophagy initiation. In fact, lithium administered to patients carrying either ULK1 and C9orf

72 mutation, delays disease course. This suggests that autophagy plays a key role in the degeneration of motor neurons. According to this evidence, in the present study, iPSC-MNs were treated with rapamycin, which works as autophagy activator by inhibiting the mTOR complex. In these cells, we analyzed the expression of TDP-43 protein, that contributes to the formation of pathological aggregates in C9orf-associated ALS and the expression of two exosomal markers: ALIX and TSG-101. These are involved in exosome biogenesis from multi-vesicular bodies. Rapamycin significantly reduces the massive amount of TDP-43, which accumulates within C9orf72 iPSC-MNs both within the cytosol and nucleus. Concomitantly, rapamycin suppresses TSG-101 and ALIX within C9orf72 iPSC-MNs. These findings suggest a novel approach, which based on mTOR inhibition, in the course of autophagy-dependent specific ALS phenotype to improve modify the disease course.

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γ -PGA PROTECTIVE EFFECT IN A CELLULAR PD MODEL

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The accumulation of α -Synuclein aggregates is a common neuropathological hallmark of Parkinson's disease (PD), a neurodegenerative movement disorder, affecting more than 6 million people worldwide. Neurons of different brain areas are highly susceptible to the pathology, but recently emerging evidence has shown that astrocytes also significantly contribute to PD pathogenesis. Astrocytes have a dual role, on one side they are involved in the clearance of extracellular α -Synuclein aggregated species, while on the other hand they acquire inflammatory and altered metabolic properties thus inducing detrimental effects that can potentially exacerbate the damage. Therefore, identifying glial specific mechanisms, that contributes to the pathology progression could be crucial for PD treatment. On this basis the aim of the present study was to test the effect of α -Synuclein pre-formed fibrils (PFFs) on primary culture of astrocytes and to investigate the potential protective role of poly-gamma-glutamic acid (γ -PGA), a biodegradable, non-toxic, and non-immunogenic biopolymer. γ -PGA, produced through fermentation by *Bacillus* species, is known to be a gut stabilizer, highly consumed in Japan and it has also been proposed to exert an antioxidant and anti-inflammatory property and to alleviate neuronal cell death and memory deficit. Our preliminary results, obtained through immunohistochemical methods, show that γ -PGA treatment is able to significantly reduce PFFs induced inflammation in primary astrocyte cultures derived from the cortex of P1-P2 mice. Collectively, high resolution images also showed that, upon treatment with γ -PGA, the dis-

tribution of PFFs is significantly reduced. Finally, we tested the ability of γ -PGA to interfere with α -Synuclein aggregation, confirming with RT-QuIC analysis that the polymer is able to significantly delay it. Overall, these data suggest that γ -PGA might be an attractive candidate to alleviate both aggregation and the inflammatory phenotype known to be involved in the exacerbation of PD pathology.

PATHOLOGICAL REMODELING OF GUT BARRIER IN OBESITY

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Changes in the gut microbiota, alterations in intestinal mucosal permeability, chronic mild enteric inflammation, and neuroplastic changes in the enteric nervous system have been proposed to be involved in the development of obesity and related bowel disturbances. In particular, gut barrier impairments have been proposed to be at the crossroads between alterations in enteric bacteria and the triggering of neurogenic/immune-inflammatory responses in obesity and that such alterations could represent early events in obesity. However, whether gut barrier remodeling represents a prodromal event in obesity before weight gain, metabolic alterations, and systemic inflammation remains unclear. To examine morphologic changes in the gut barrier in a mouse model of high-fat diet (HFD) since the earliest phases of diet assumption.

C57BL/6J mice were fed with standard diet (SD) or HFD for 1, 2, 4, or 8 weeks. Body weight and epididymal fat weight were evaluated at all time points. Plasma interleukin-1beta (IL-1 β), IL-6 and resistin levels were assessed by ELISA. Remodeling of intestinal epithelial barrier, inflammatory infiltrate, and collagen deposition in the colonic wall was assessed by histochemistry and immunofluorescence analysis.

Obese mice displayed increased body and epididymal fat weight along with increased plasma resistin, IL-1 β , and IL-6 levels after 8 weeks of HFD. Starting from 1 week of HFD, mice displayed (1) a decreased claudin-1 expression in lining epithelial cells, (2) an altered mucus in goblet cells, (3) an increase in proliferating epithelial cells in colonic crypts, (4) eosinophil infiltration along with an increase in vascular P-selectin, and (5) deposition of collagen fibers. HFD intake is associated with morphologic changes in the large bowel at mucosal and submucosal levels. In particular, the main changes include alterations in the mucous layer and intestinal epithelial barrier integrity and activation of mucosal defense-enhanced fibrotic deposition. These changes represent early events occurring before the development of obesity that could contribute to compromise the intestinal mucosal barrier and functions, opening the way for systemic dissemination of pathological bacteria and inflammatory mediators.