International Symposium on Neurobiology

Joint Meeting

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University of Surrey

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Car: A4 highway, exit Cinisello Balsamo/Sesto S. Giovanni, then direction Milano

Train: Milano Greco FS railway station (www.trenitalia.it)

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Contact us

Dr. Elisabetta Donzelli

School of Medicine and Surgery

via Cadore 48 – 20900 Monza (MB)

+39 02 6448 8119

elisabetta.donzelli@unimib.it

http://www.neuroscienze.medicina.unimib.it

Program

08.45 – **Welcome and symposium presentation** (A. Biondi, J. McFadden)

09.10 – **Metabolomics - a tool for neuroscience research** (D. Skene)

09.40 – **G-protein coupled heterodimers as novel targets in neurological pathologies** (P. McCormick)

10.00 – Mesenchymal Stem Cell – derived Extracellular Vesicles as possible modulators of the neuronal GABAergic Excitatory/Inhibitory shift (G. Desiato)

10.15 – CR4056, an imidazoline-2 receptor ligand as a novel analgesic drug (E. Comi)

10.30 – **coffee break**

11.00 – **Central and peripheral nervous system models in experimental neuroscience** (G. Cavaletti)

11.30 – PET molecular imaging in animal models of neuroinflammation and neurodegeneration (RM. Moresco)

11.45 – Hindering Alzheimer's disease progression in vivo by multifunctional liposomes (S. Mancini)

12.00 – From the lungs to the brain: the fantastic voyage of nanoparticles targeting beta-amyloid (Aβ) (G. Sancini)

12.15 – **Contribution of sleep and circadian rhythms to brain function** (DJ. Dijk)

12.45 – **lunch**

14.00 –**Maximising plasticity in the human brain** (A. Sterr)

14.30 – Expertise-induced neural plasticity: Muscular effort coding in action representation in ballet dancers and naïve (A. Orlandi)

14.45 – nAChR contribution to brain development and epilepsy (S. Brusco)

15.00 – **posters view** (presentation of UNIMIB and UoS labs and research interests)

15.30 – **Language learning from a brain perspective** (B. Opitz)

16.00 – **From bench to bed: translational research in neurodegenerative diseases** (C. Ferrarese)

16.30 – Epigenetic dysfunction in amyotrophic lateral sclerosis: looking at the interface of the genes-environment dualism (L. Tremolizzo)

16.45 – **Symposium closure** (MG. Valsecchi)

Abstracts selected for

ORAL PRESENTATION

- Mesenchymal Stem Cell – derived Extracellular Vesicles as possible modulators of the neuronal GABAergic Excitatory/Inhibitory shift (*G. Desiato*)
- CR4056, an imidazoline-2 receptor ligand as a novel analgesic drug (*E. Comi*)
- PET molecular imaging in animal models of neuroinflammation and neurodegeneration (*RM. Moresco*)
- Hindering Alzheimer's disease progression in vivo by multifunctional liposomes (*S. Mancini*)
- From the lungs to the brain: the fantastic voyage of nanoparticles targeting beta-amyloid (Aβ) (*G. Sancini*)
- Expertise-induced neural plasticity: Muscular effort coding in action representation in ballet dancers and naïve (*A. Orlandi*)
- nAChR contribution to brain development and epilepsy (*S. Brusco*)
- Epigenetic dysfunction in amyotrophic lateral sclerosis: looking at the interface of the genes-environment dualism (*L. Tremolizzo*)

Mesenchymal Stem Cell – derived Extracellular Vesicles as possible modulators of the neuronal GABAergic Excitatory/Inhibitory shift

Genni Desiato[1,2], Davide Pozzi[2], Michela Matteoli[2,3] and Silvia Coco[1]

[1]School of Medicine and Surgery, University of Milano-Bicocca, Monza
[2]Laboratory of Pharmacology and Brain Pathology, Humanitas Clinical and Research Center, Milan;
[3]CNR, Institute of Neuroscience, Milan

During brain development, the transition of the GABA signalling from excitatory to inhibitory represents a critical process. Literature suggests that mesenchymal stem cells (MSCs) can sustain neuronal morphological and functional growth and also can enhance the GABAergic transmission and their action is especially mediated through extracellular vesicle (EVs) release. Our project aims at investigating whether MSCs by means of EVs might alter the GABA switch and, possibly, clarifying the molecular mechanism beyond this modulation.

Primary bone marrow-derived Sca1+, CD105+, CD73+, CD106+ MSCs and primary hippocampal neurons (HNs) cultures were established. EVs were isolated by differential centrifugation steps and visualized by Nanosight. HNs were fed with MSC -conditioned medium (CM), or co-cultured with MSCs, or treated with MSC-derived EVs from the very early stage of neuronal growth. Functional approaches, such as Calcium and Chloride live imaging, were employed to monitor the level of neuronal maturation; qRT-PCRs were performed to clarify if MSCs can modulate the expression of neuronal genes involved in the GABA switch.

Preliminary data revealed that the GABA switch is completed within 7 days in vitro in control HNs and confirmed that GABA signalling itself is involved in this process. NKCC1 and KCC2 were reciprocally regulated during the normal development, as revealed by qRT-PCR. Interestingly, Chloride imaging showed that GABA signalling is altered at 7 DIV in HNs cultured with MSC-CM and qRT-PCRs indicated that also at mRNA level, some modifications could be found. Intriguingly, MSC-derived EVs seemed to affect the neuronal development in EV-type related manner, and modulate the Chloride concentration within the neurons.

Results from these studies will allow us to clarify MSC mechanisms of action in such a critical process, which is crucial for the normal brain development, and whose alteration is involved in the pathogenesis of several neurodevelopmental disorders, such as autism and epilepsy.

CR4056, an imidazoline-2 receptor ligand as a novel analgesic drug

Eleonora Comi[1,2], Marco Lanza[1], Flora Ferrari[1], Gianfranco Caselli[1], Lucio Claudio Rovati[1]

[1]Rottapharm Biotech, Monza, Italy
[2]PhD program in Neuroscience, XXIX PhD cycle, University of Milan-Bicocca, Monza, Italy

Aim: Pain is the earliest and most disabling symptom of osteoarthritis (OA). The chronic use of first-line pharmacological treatments to handle OA pain is frequently associated with side effects. CR4056, an imidazoline-2 receptor ligand, is a promising analgesic drug that has been reported to be effective in several animal models of pain.

The aims of this study were to compare the progression of OA pain and evaluate the efficacy of CR4056, in comparison with naproxen, in two well-established rat models of OA.

Methods: Knee OA was induced either by single intra-articular injection of 1 mg/50 µl monoiodoacetate (MIA) or by medial meniscal tear (MMT) in the right knee of male rats.

In both OA models, pain behaviour was assessed as mechanical allodynia and hyperalgesia, static and dynamic hind paw weight bearing (HPWD) asymmetry. Moreover, microglia activation was evaluated in ipsilateral dorsal horn of L4 spinal cord.

CR4056 (6 and 20 mg/kg) and 10 mg/kg naproxen were administered as acute and sub-acute treatments.

Results: MIA model was characterized by the significant development of primary mechanical hyperalgesia, mechanical allodynia and asymmetry in both static and dynamic HPWD, while after MMT surgery only a significant asymmetry in static HPWD and secondary hyperalgesia occurred. In MIA model, CR4056 significantly and dose-dependently reduced both allodynia and hyperalgesia, after acute and sub-acute treatment, whereas naproxen was effective after sub-acute treatment only. In MMT model, CR4056 and naproxen promoted a significant anti-hyperalgesic effect after acute treatment only, while only CR4056 repeated treatment significantly reduced static HPWD asymmetry. Sub-acute treatment with CR4056 and naproxen reversed MIA-induced microglia activation, while no difference in microglia activation was detected between MMT and sham group.

Conclusions: The relative contribution of peripheral and central pain mechanisms differs between MIA and MMT model. Moreover, CR4056 may represent a novel effective treatment for OA pain.

PET molecular imaging in animal models of neuroinflammation and neurodegeneration

Sara Belloli[1,3], Silvia Valtorta[2,3], Isabella Raccagni[2,3], Valentina Murtaj [2,3], Paolo Rainone[1,3], Cristina Monterisi[2,3], Elia Turolla[2,3], Sergio Todde[2,3] and Rosa Maria Moresco[2,3]

[1]Institute of Molecular Bioimaging and Physiology (IBFM), CNR, Segrate (MI), Italy;
[2]School of Medicine and Surgery and Tecnomed Foundation, University of Milano-Bicocca, Monza, Italy;
[3]Milan Center for Neuroscience (NeuroMi), Milan, Italy.

Positron emission tomography (PET) represents a unique tool for the non-invasive assessment of molecular processes attending brain functions. PET allows the in vivo imaging and quantification of a number of molecular events related to neurobiology or neuropathology of the brain of living subjects. Depending on the molecular target of radiopharmaceuticals, PET imaging offers the advantage of measure different biological properties of brain like modifications in energy consumption, presence of amyloid deposition, microglia activation or the integrity of selected population of neurons. Moreover, the use of PET allows to image the increase of glucose metabolism or cell proliferation in the brain, thus characterizing tumour using radiopharmaceuticals as [18F]FDG and [18F]FLT. Advances in detector technology have led to a consistent improvement in spatial resolution of PET (1-2 mm), enabling the application of tomographs for preclinical investigations in small experimental animal models. Using this technique, we have characterized in longitudinal studies the integrity of selected population of neurons and the activation of microglial cells in different preclinical models of neurodegenerative or neuroinflammatory disorders like Huntington's and Parkinson's disease or Multiple Sclerosis. Furthermore, using the glucose analogue [18F]FDG, we are currently evaluating the involvement of regional brain metabolism in behavioural or cognitive disorders like stress or mental retardation. Finally, we are studying the potential use of [18F]FLT as early prognostic marker of treatment response in mice model of glioblastoma.

Key words: Inflammation, neurodegeneration, PET

Hindering Alzheimer's disease progression in vivo by multifunctional liposomes

Simona Mancini[1], Claudia Balducci[2], Edoardo Micotti[2], Daniele Tolomeo[2], Gianluigi Forloni[2], Massimo Masserini[1], Francesca Re[1].

[1]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.
[2]Department of Neuroscience, IRCCS-Mario Negri Institute for Phamarcological Research, Milano, Italy.

A central role in Alzheimer's disease (AD) is played by Amyloid-β (Aβ) peptide, progressively depositing as plaques in the brain of diseased individuals and directly or indirectly leading to neuronal degeneration, cognitive dysfunction and memory loss, other features of the disease. Since Aβ alterations are thought to take place decades before the appearance of the first signs of dementia, this preclinical phase is considered the most promising period for successful disease-modifying therapies, which are still lacking. In the present study, we evaluated the possibility to delay the progression of Alzheimer's disease (AD) *in vivo* by means of an early intervention using multi-functional liposomes previously designed for AD therapy and already proved effective on AD mouse models displaying evident amyloid plaques and cognitive deficit. Liposomes, administered weekly to pre-symptomatic 5-month-old APP/PS1 mice for 7 months, prevented the onset of long-term recognition memory impairment and hindered brain Aβ deposition. A peripheral 'sink' activity contributed to the low Aβ content found out in the brain of treated mice, as suggested by the increase of Aβ levels in the liver. Strikingly, all these impressive effects were maintained up to 3 months after treatment discontinuation, when a modification in the levels of Aβ transporters on the BBB was also observed in the brain of previously treated animals. Together, these findings promote multi-functional liposomes as a valuable approach, potentially suitable for delaying relevant features of AD.

References:

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From the lungs to the brain: the fantastic voyage of nanoparticles targeting beta-amyloid (Aβ)

[1,2,3,4]Sancini G., [1,2,3] Dal Magro R., [1,2,3,5]Re F., [1,3,4]Donzelli E.

[1]School of Medicine and Surgery, University of Milano-Bicocca, Italy
[2] "NanoMiB"- Milano-Bicocca Nanomedicine Center, University of Milano-Bicocca, Italy
[3] "NeuroMI" - Milan Center of Neuroscience, University of Milano-Bicocca, Italy
[4] Neuroscience PhD Program, University of Milano-Bicocca, Italy
[5] PhD Program in Translational and Molecular Medicine, University of Milano-Bicocca, Italy

The brain is always confronted with the dilemma of the protection from noxious substances from the blood and the delivery of vital metabolites. Endothelial cells, forming together with other cells the blood-brain barrier (BBB), are known as the "gatekeepers" of this trafficking. On the one hand, the protection from toxic molecules is achieved by the obstruction of the paracellular pathway with tight junctions, that fuse brain capillary endothelia into a continuous tubular cell layer. On the other hand, vital molecules are transported from the blood by mean of active trans-cellular mechanisms. Recent applications in nanomedicine focus on nanoparticles (NP) as they are promising tools for site-specific delivery of drugs and diagnostic agents, through the possibility to functionalize their surface with target-specific ligands. Treatment options for Alzheimer's disease (AD) are limited because of the inability of drugs to cross the BBB. Previously, we showed that intraperitoneal administration of liposomes functionalized with phosphatidic acid and an ApoE-derived peptide (mApoE-PA-LIP) reduces brain beta-amyloid (Aβ) burden and ameliorates impaired memory in AD mice. Among the different administration routes, pulmonary delivery is a field of increasing interest not only for the local treatment of airway diseases but also for the systemic administration. We investigated lung administration as an alternative, non-invasive NP delivery route for reaching the brain. Our results show that mApoE-PA-LIP were able to cross the pulmonary epithelium in vitro and reach the brain following in vivo intratracheal instillations. Lung administration of mApoE-PA-LIP to AD mice significantly decreased total brain Aβ (–60%; $p < 0.05$) compared to untreated mice. These results suggest that pulmonary administration could be exploited for brain delivery of NP designed for AD therapy.

Contact info:

Giulio Sancini
Contact number: + 39 02 64488310
Mobile: +39 3478917267
e-mail: giulio.sancini@unimib.it
LinkedIn account: https://www.linkedin.com/in/giulio-sancini-6436b136

Expertise-induced neural plasticity: Muscular effort coding in action representation in ballet dancers and naïve

Orlandi A, Arno E, D'Incà S, Proverbio AM

NeuroMI, Dept. of Psychology, University of Milano-Bicocca, Milan

It is known that the observation and imagination of human actions requiring a great muscular effort (i.e. running) lead to an increased heart and respiratory rate. In addition TMS evidences have been provided of a greater muscle-specific cortical excitability during the observation of heavy vs. light objects lifting, while electrophysiological studies have shown an increase in late ERP potentials during observation of dynamic vs. static gestures. In the present study, the neural correlates of perceiving effortful vs. effortless movements belonging to a specific repertoire were investigated, by means of the EEG/ERP technique. 15 professional female ballet dancers and 15 female *naïve* controls with no experience whatsoever with dance, gymnastic or martial arts were recruited. They were shown hundreds of short video clips displaying a male dancer performing a standard step from ballet technique. Particularly, the movement could be either effortful or relatively effortless. Participants were asked to observe each clip and to imagine themselves physically executing the same movement. Regardless of the effort of actions, the perception of the moving body in the videos elicited to a bilateral body related N190 in dancers, whilst a larger component was found on the right than left hemisphere in controls. Furthermore, the observation of effortful compared to effortless movements resulted in a greater P300 over frontal sites. Such difference was enhanced in professional that controls, probably because of their visuomotor expertise with the specific steps. It can be assumed that a resonance process occurred allowing them to better codify the required effort. Moreover, a greater P300 was found over central sites elicited by effortless movements in both groups of participants, probably indexing a categorization process. Finally, a Late Positivity over occipito-parietal sites was observed in response to perception of effortful stimuli in both groups, suggesting a greater amount of visual kinematic information related to effortful movements.

nAChR contribution to brain development and epilepsy

Brusco Simone, Becchetti Andrea

University of Milano-Bicocca, Department of Biotechnology and Bioscience

The nocturnal frontal lobe epilepsy (NFLE) comprises a large group of partial epilepsies with heterogeneous origin. Approximately 12% of the families affected by the autosomal dominant form of NFLE (ADNFLE) carry mutations on genes coding for subunits of the heteromeric neuronal nicotinic receptors (nAChRs). This is consistent with the widespread expression of nAChRs, and particularly α4β2, in the mammalian brain. Attacks arise in the frontal lobe, usually during stage 2 of sleep, and are characterized by clusters of complex and stereotyped hyperkinetic seizures.

Besides directly promoting hyperexcitability in mature networks through cell depolarization and/or altered neurotransmitter release, mutant nAChRs could determine the pathogenetic process during early developmental phases, by affecting synaptic remodeling. These alterations can lead to an unbalance between excitatory and inhibitory transmission in prefrontal cortex (PFC), therefore facilitating the epileptic fits.

By using a murine strain which conditionally expresses β2-V287L, a mutant nAChR subunit linked to ADNFLE, we found that the mutant nAChRs modify the balance between excitation and inhibition in the adult brain, by increasing the nicotine-dependent glutamate release.

We are currently investigating the effect of β2-V287L in early developmental stages, during which its expression is crucial for the epileptic phenotype to manifest. By patch-clamp recordings, we observed that mutant nAChRs did not interphere with the GABAergic excitatory/inhibitory transition during early developmental stages (which is known to play a role in synaptic remodelling), but affected the development of the glutamatergic system.

We aim to shed new light on the nAChR contribution to brain development and its role in the establishment of an epileptic phenotype, besides its direct effect on excitability in mature prefrontal networks. In this way, we should be able to identify a temporal window for early pharmacological treatment during the pathogenetic process.

Epigenetic dysfunction in amyotrophic lateral sclerosis: looking at the interface of the genes-environment dualism

L. Tremolizzo,[1,2] A. Arosio,[1,2] E. Conti,[1,2] G. Sala,[1,2] S. Barabino,[3] A. Loffreda,[3] C. Lunetta,[4] F. Gerardi,[4] C. Ferrarese[1,2]

[1]School of Medicine and Surgery
[2]Milan Center for Neuroscience (NeuroMI)
[3]Dept. of Biotechnology and Biosciences, University of Milano-Bicocca;
[4]NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milano; Italy

Amyotrophic lateral sclerosis is a neurodegenerative disorder which pathogenesis is still largely unknown. Genetic bases for the disease have been significantly elucidated and novel genes appear every year, offering new hopes of understanding the dysfunctional processes involved in this disease. However, in most of ALS cases is not possible to find a mutation, implying a potential role for the control of transcription via epigenetic mechanisms. Epigenetics refers to the study of heritable changes in gene function that do not entail a change in the sequence of the DNA. DNA methylation and chromatin remodelling are the most studied epimutations, but often non-coding RNA are included in this perspective.

We report here data suggesting that: (a) the panorama of chromatin structure is altered in ALS both in the CNS and peripheral tissues; (b) micro-RNA, short non-coding regulatory RNA, are differentially expressed in different models of ALS, including peripheral lymphomonocytes obtained from patients.

The epigenetic hypothesis of ALS pathogenesis offers a new point of view for understanding this disease, possibly leading to unravel new mechanisms amenable of dedicated treatments.

Abstracts selected for

POSTER PRESENTATION

1. Advanced neurophysiology – a translational approach to neuropathy and neuropathic pain. (*Alberti P, Fumagalli G, Monza L, Cavaletti G*).
2. Alterations of mitochondrial dynamics in human peripheral blood mononuclear cells (PBMC) as potential peripheral biomarkers of Parkinson's disease. (*Aprea F, Sala G, Zoia CP, Riva C, Brighina L, Martorana F, Alberghina L, Colangelo AM, Ferrarese C*).
3. Valproic acid modulates WNT signaling pathway and affects cell migration through the impairment of epithelial-mesenchymal transition program in cancer stem cells from glioblastoma multiforme. (*Bazzoni R, Riva G, Cadamuro M, Cilibrasi C, Bentivegna A*).
4. Monitoring Neuroinflammation with the TSPO tracer [18F]VC701, after LPS systemic administration in adult and aged mice. (*Belloli S, Murtaj V, Monterisi C, Masiello V, Moresco RM*).
5. Characterization of an outward current induced by a VGF-derived peptide in microglia. (*Binda A, Rizzi L, Molteni L, Bresciani E, Locatelli V, Torsello A, Rivolta I*).
6. Acute variations of cytokine levels after antipsychotic treatment in drug-naïve subjects with a first-episode psychosis: A meta-analysis. (*Capuzzi E, Bartoli F, Crocamo C, Carrà G, Clerici M*).
7. Neurotoxicity and photon activation therapy effect of new heavy metal-based anticancer complexes.(*Ceresa C, Nicolini G, Semperboni S, Gandin V, Requardt H, Santini C, Pellei M, Bravin A, Cavaletti G*).
8. Multimodal experimental approach to the study of human neurological diseases (*Chiorazzi A, Meregalli C, Canta A, Carozzi VA, Oggioni N, Rigolio R, Pozzi E, Monza L, Fumagalli G, Alberti P, Marmiroli P, Cavaletti G*).

9. The one-carbon metabolism disruption in anorexia nervosa of the restricting type. (*Conti E, Tremolizzo L, Corbetta F, Bomba M, Neri F, Ferrarese C, Nacinovich R*).

10. Interaction between cholinergic, immune and inflammatory systems in Alzheimer's disease: the IMMUNAD project. (*Conti E, Tremolizzo L, Grana D, Zoia CP, Arosio A, Cereda D, Stefanoni G, Villa C, Combi R, Aliprandi A, Salmaggi A, Appollonio I, Ferrarese C*).

11. Beta-amyloid-induced peripheral chemotactic response: what role in Alzheimer's disease? (*Grana D, Conti E, Stefanoni G, Zoia CP, Bossi M, Aliprandi A, Appollonio I, Ferrarese C, Tremolizzo L*).

12. Burning Mouth Syndrome: current concepts. (*Lauritano D, Zucchinelli L, Greco G, Borgia R*).

13. Tubulin polymerization and mitochondria transport involvement in Bortezomib peripheral neurotoxicity. (*Malacrida A, Meregalli C, Semperboni S, Rodriguez-Menendez V, Nicolini G, Miloso M, Cavaletti G*).

14. Altered regulation of autophagy in fibroblasts from patients with sporadic and LRRK2 mutant Parkinson's disease. (*Marinig D, Riva C, Zoia C, Ferrarese C, Sala G*).

15. Biological and protective properties of Mesenchymal Stem Cells (*Monfrini M, Donzelli E, Scuteri A*).

16. Incubation with low doses of oxaliplatin modifies the electrophysiological properties of dorsal root ganglion neurons. (*Pastori V, Monza M, Cavaletti G, Becchetti A, Lecchi M*).

17. Does music enhance memory? An electrical neuroimaging study. (*Proverbio AM, De Benedetto F*).

18. Isolation and characterization of neutrophils from Multiple Sclerosis patients (*Rigolio R, Fusco ML, Malacrida M, Cogo M, Foti M, Avezza F, Cavaletti G*).

19. In vitro and ex vivo study of the autophagy-lysosome pathway in neurodegenerative diseases. (*Sala G, Riva C, Marinig D, Sirtori R, Brighina L, Zoia C, Arosio A, Tremolizzo L, Ferrarese C*).

20. Compound heterozygosis in the SCN1A gene in a family showing GEFS+ and IGE. (*Villa C, Sancini G, Binini N, Grioni D, Dal Magro R, Toselli M, Combi R*).

21. Study of the role of the CRH in Autosomal dominant nocturnal frontal lobe epilepsy. (*Villa C, Volpe B, Chisci E, Ferini-Strambi L, Giovannoni R, Combi R*).

22. SiTraNeD: signal transduction in peripheral models of neurodegenerative diseases. (*Zoia CP, Bazzini C, Conti E, Tremolizzo L, Ferrarese C*).

Poster n. 1

Advanced neurophysiology – a translational approach to neuropathy and neuropathic pain.

Alberti P.[1,2], Fumagalli G.[1,2], Monza L.[2,3], Cavaletti G.[1,2]

1. Experimental Neurology Unit and PhD program in Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.
2. Milan Center for Neuroscience, Milan, Italy
3. PhD program in Translational and Molecular Medicine, DIMET, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy.

Peripheral Nervous System (PNS) can be affected by different diseases due to many causes: traumatic, inherited, autoimmune, toxic, and metabolic ones. PNS dysfunction is characterized by signs and very disabling symptoms such as: numbness and tingling in feet and/or hands, lack of coordination, and muscle weakness or paralysis. Neuropathic pain is frequently present. Since there are so many causing factors, peripheral neuropathies are a rather common condition; in the clinical setting, they are still difficult to be diagnosed and overall treated. In the Experimental Unit, we specifically work on animal models of peripheral neuropathies; we investigate Chemotherapy Induced Peripheral Neurotoxiciy (CIPN). Many commonly employed anticancer drugs can cause a long-lasting or even permanent neuropathy: platinum drugs, taxanes, vinca alkaloids and proteasome inhibitors. CIPN is a relevant clinical condition which still awaits a cure. This lack is also due to the absence of definite pathophysiological information; animal models can be useful in this regard. However, at the bench side, innovative methods to promptly translate data to bedside are still warranted. In this regard, neurophysiological testing is the ideal candidate. We apply an innovative approach to CIPN animal models, to enhance a translational approach. We match behavioral and histopathological findings with advanced neurophysiological techniques. We are implementing these techniques in our rat and mouse models: nerve conduction studies, nerve excitability testing, microneurography, electrophysiological analysis of wide dynamic range neurons in the spinal dorsal horn. Thus, we can characterize our models also with functional analysis of PNS dysfunction. Neurophysiology can be performed both in humans both in animals: data obtained with these techniques have a high translational potential and can be promptly transferred to clinical trials.

Poster n. 2

Alterations of mitochondrial dynamics in human peripheral blood mononuclear cells (PBMC) as potential peripheral biomarkers of Parkinson's disease

Federica Aprea[1,5], Gessica Sala[2,6], Paola Paci[4,5], Chiara Paola Zoia[2,6], Chiara Riva[2,6], Laura Brighina[3,6], Francesca Martorana[1,5], Lilia Alberghina[1,5], Anna Maria Colangelo[1,5,6], Carlo Ferrarese[2,3,6]

1. Lab. of Neuroscience "R. Levi-Montalcini", Dept. of Biotechnology and Biosciences, University of Milano-Bicocca, Milano, Italy
2. Lab. of Neurobiology, School of Medicine and Surgery, University of Milano-Bicocca
3. Dept. of Neurology, San Gerardo Hospital, Monza
4. IASI-CNR Viale Manzoni 30, Rome, Italy
5. SYSBIO Centre of Systems Biology, University of Milano-Bicocca, Milano, Italy.
6. NeuroMI, Milan Centre for Neuroscience, University of Milano-Bicocca, Milano, Italy

Aims Diagnosis of Parkinson's disease (PD) and other neurodegenerative disorders occurs at late stages of diseases. Early diagnosis of neurodegenerative pathologies is fundamental for effective therapeutic intervention. Based on the well-known role of oxidative stress in PD, we aimed to identify potential biomarkers of PD pathology in a peripheral tissue, such as blood. Among others, mitochondrial dysfunction and oxidative stress are known to be a major cause of dopaminergic neurons vulnerability. Therefore, we aimed to evaluate mitochondrial protein levels in peripheral blood mononuclear cells (PBMC) from PD and healthy control subjects.

Materials and Methods A total number of 18 subjects was analyzed in this study, including 9 PD patients (3 females and 6 males) and 9 control subjects. Healthy controls were age- and sex-matched volunteers. All samples were analyzed by western blot analysis for protein levels of oxidative stress-sensor (DJ-1), mitochondrial fission-fusion proteins (P-Drp-1, Opa-1 and Mfn-2), proteins controlling mitophagy (PINK-1 and Parkin) and biogenesis regulator (mtTFAM).

Results and Conclusions After determination of protein levels in PBMC samples from PD patients and healthy controls, we applied a multivariate analysis to identify patterns of mitochondrial protein abnormalities in PD patients as compared to healthy controls, as well as their age-dependent distributions in females versus male subjects. Current data in three-dimensional plots revealed that alterations of most mitochondrial proteins were irrespective of sex and age. Interestingly, preliminary data also indicated that Parkin alterations were mainly present in female patients. While this study was performed on a limited number of subjects, further analyses on extended number of patients will be of utmost relevance to substantiate current results and potential development of mitochondrial protein changes as peripheral biomarker of PD.

Poster n. 3

Valproic acid modulates WNT signaling pathway and affects cell migration through the impairment of epithelial-mesenchymal transition program in cancer stem cells from glioblastoma multiforme.

Bazzoni R[1], Riva G[1,2,3], Cadamuro M[1], Cilibrasi C[1,2,3], Bentivegna A[1,3]

1. School of Medicine and Surgery, University of Milano-Bicocca, via Cadore 48, 20900 Monza, Italy
2. PhD Program in Neuroscience, University of Milano-Bicocca
3. NeuroMI, Milan center of Neuroscience

Glioblastoma multiforme (GBM) is the most frequent and aggressive primary brain tumor, as patients die within 15 months after diagnosis. The failure of current therapies is due to the presence within tumor of a subpopulation of cells with stem-like properties, called glioma stem cells (GSCs), which are particularly resistant to chemo- and radio-therapy.

Increasing evidence suggests the role of epigenetic aberrations in the development and/or progression of several types of tumors. The potential reversibility of epigenetic alterations represents an attractive approach to "reset" the abnormal cancer epigenome by using epigenetic drugs such as Valproic acid (VPA), a histone deacetylase inhibitor. VPA, an anticonvulsant and mood-stabilizing drug, has proved to have numerous potent antitumor effects in numerous *in vitro* and *in vivo* glioma studies.

The WNT signaling is an evolutionary conserved pathway involved in stem cells regulation of self-renewal, differentiation and migration. It is frequently dysregulated in cancers through either genetic or epigenetic alterations and could represent a pharmacological target to defeat the GSC compartment.

In this study, we highlighted the VPA ability to modulate the expression of several WNT molecular pathway-related genes on seven GSC lines by Real-Time PCR. Then, by Western blot we evaluated, after 96 h VPA treatment, the protein levels of nuclear and cytoplasmic β-catenin, the most important effector of such pathway, and of Twist1 and Snail1, two activators of epithelial-mesenchymal transition. VPA was able to modify heterogeneously the expression of these proteins. Furthermore, we observed that this drug also induced a heterogeneous effect on cell motility and invasive behavior of GSCs.

Data obtained suggest the possibility to use VPA in the context of a GSC-targeted therapy, in order to eradicate completely the GSC compartment, also in combinations with other drugs. Further investigations into the VPA effects on GBM are required in order to benefit patient prognosis.

Poster n. 4

Monitoring Neuroinflammation with the TSPO tracer [^{18}F]VC701, after LPS systemic administration in adult and aged mice.

Sara Belloli[1,3], Valentina Murtaj [2,3], Cristina Monterisi[2], Masiello Valeria[4] and Rosa Maria Moresco[2,3]

[1]Institute of Molecular Bioimaging and Physiology (IBFM), CNR, Segrate (MI), Italy
[2]Department of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[3]Milan Center for Neuroscience (NeuroMi), Milan, Italy
[4]Nuclear Medicine Department, San Raffaele Scientific Institute, Milan, Italy

Neuroinflammation is widely studied in many neurodegenerative diseases, including Alzheimer's and Parkinson's disease, in which age play a crucial role. Aging process may be considered the result of some environmental and genetic factors. Aim of the study is monitoring brain inflammation and its modulation by age after systemic administration of LPS. To this end, we evaluated microglia activation performing *ex vivo* biodistribution with the Translocator protein (TSPO) specific radiotracer [^{18}F]VC701, and cytokines expression by RT-PCR. [^{18}F]VC701 biodistribution was measured in wt animals 6 hours and 3 months after systemic injection of LPS/vehicle in young and aged C57BL/6 male mice. Two hours after tracer injection, animals were sacrificed and specific brain regions collected for gamma counting and RT-PCR. Peripheral challenge with LPS induced a significant increase of [^{18}F]VC701 uptake in cortex and cerebellum of aged mice 6 hours after toxin administration. On the contrary, no inflammatory response was observed in young animals and no significant differences were observed in basal [^{18}F]VC701 uptake of young and old mice. Measurements of cytokines transcripts confirmed these data, showing higher of IL-1β and TNF-α RNAs only in aged mice treated with LPS. Considering the later time point (3 months), no significant increase of tracer uptake or cytokines expression was observed in aged and young treated mice, showing a return of neuroinflammation to basal levels. Finally, LPS failed to modify other inflammatory targets like IL-4 or Arg1 in both types of animals. Results of this study confirmed an exaggerated response of the aged brain to peripheral inflammatory challenge that can be detect using TSPO radioligand like [^{18}F]VC701.

Key words: Inflammation, aging, LPS, PET

Poster n. 5

Characterization of an outward current induced by a VGF-derived peptide in microglia.

Anna Binda[1], Laura Rizzi [1], Laura Molteni[1], Elena Bresciani[1], Vittorio Locatelli[1], Antonio Torsello[1], Ilaria Rivolta[1]

[1] Dipartimento di Medicina e Chirurgia, Università di Milano Bicocca, Via Cadore, 48, 20900 Monza (MB)

VGF, a granin-like protein, is known to be processed in an incompletely characterized panel of neuropeptides. Among VGF-derived peptides, TLQP-21, which spans from residue 556 to 576 of the precursor sequence, induces an increase in free calcium cytoplasmic levels that control physiological activities in microglial cells. Ion channels are often modulated by intracellular calcium increase, so we evaluated whether JMV-5656, a TLQP-21 derivative with overlapping biological activity, could affect membrane currents in a model of murine N9 microglia cell line. We performed patch-clamp experiments on N9 cells superfused with an extracellular solution containing 10 μM of JMV-5656 peptide and we observed an about 3 fold increase in the total outward currents in the 77% of cells tested. The effect was time and concentration dependent. Several ion channels blockers or modulators were applied to identify the outward currents activated by JMV-5656 superfusion. Data seemed to suggest an involvement of intermediate-conductance Ca2+-activated potassium (IK) channels. In order to support this hypothesis, we performed experiments in the presence of selective inhibitors of IK channels and, further, cells were transfected with siRNA targeting IK channels. All the data collected suggested that JMV-5656-induced intracellular calcium release leads to an increased outward potassium current, that, at least partially, involves the recruitment of IK channels.

Poster n. 6

Acute variations of cytokine levels after antipsychotic treatment in drug-naïve subjects with a first-episode psychosis: A meta-analysis

Enrico Capuzzi[a], Francesco Bartoli[a], Cristina Crocamo[a], Giuseppe Carrà[a,b], Massimo Clerici[a]

[a]School of Medicine and Surgery, University of Milano Bicocca, via Cadore 48, 20900 Monza (MB), Italy.
[b]Division of Psychiatry, University College London, 6th Floor, Maple House, 149 Tottenham Court Road, London W1T 7NF, UK.

Many studies have reported an association between schizophrenia and immunological abnormalities. However, antipsychotics may induce immunomodulatory effects, influencing plasma cytokines. In order to distinguish these influences, we carried out a systematic review and meta-analysis exploring the acute effect of antipsychotic therapy on candidate cytokines plasma levels (IL-1β, IL-2, IL-6, IL-17, IFN-γ, and TNF-α) among drug-naïve subjects with first episode psychosis.

We searched main Electronic Databases, identifying eight studies meeting our inclusion criteria. Pre and post-treatment plasma cytokines values were used to estimate standardized mean differences. Heterogeneity was estimated using the I^2 index. Leave-one-out analysis was performed. IL-2 (p=0.023) and IL-6 (p=0.012) levels showed a significant decrease after four weeks of antipsychotic treatment. Leave-one-out analysis confirmed these findings. IL-1β had high between-study heterogeneity. However, leaving out one study, significant after treatment decrease was found. IL-6 and IL-2, and possibly IL-1β, could be considered state markers, decreasing after antipsychotic treatment, whilst TNF-α, IL-17, and IFN-γ might be considered trait markers. Options for novel treatments in FEP, involving cytokine-modulating agents, should be further studied.

Poster n. 7

Neurotoxicity and photon activation therapy effect of new heavy metal-based anticancer complexes.

Ceresa C.[1], Nicolini G.[1], Semperboni S.[1], Gandin V. [4], Requardt H.[2], Santini C.[3], Pellei M.[3], Bravin A.[2], Cavaletti G.[1]

(1) Experimental Neurology Unit, School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy. (2) Biomedical Beamline (ID17), European Synchrotron Radiation Facility (ESRF), Grenoble, France. (3) School of Science and Technology - Chemistry Division, University of Camerino, Camerino, Italy. (4) Department of Pharmaceutical and Pharmacological Sciences, University of Padova, Padova, Italy.

Cisplatin is one of the most efficient metal-based anticancer agents, targeting several solid tumours. Despite its efficacy, cisplatin treatment is still limited by severe side effects such as neuro-, hepato- and nephro-toxicity and by resistance phenomena, only partially overcome by new platinum drugs (i.e. oxaliplatin and carboplatin). The development of alternative metal-based compounds would be useful. These complexes are particularly interesting for cancer treatment because they can be used in combination with synchrotron radiation (SR) for Photon Activation Therapy (PAT) applications.

Activity and neurotoxicity of different water soluble complexes based on copper ($[Cu(PTA)_4]PF_6$ and $[Cu(thp)_4]PF_6$) and gold ($[Au(PTA)_4]PF_6$ were investigated *in vitro*.

Atomic absorption spectroscopy experiments demonstrated a dose-dependent manner internalization of the compounds in cancer cells and neurons.

Neurotoxicity was evaluated using embryonic rat dorsal root ganglia (DRG) explants. After 48 hours of treatment, both copper-based compounds were not neurotoxic even at higher concentrations with respect to the IC_{50} while $[Au(PTA)_4]PF_6$ was neurotoxic at lower concentrations than IC_{50} in human cancer cells.

Since the ubiquitin-proteasome system has been identified as target drugs in cancer cells, we evaluated their ability to hinder the proteasome machinery in DRG neurons. Both not neurotoxic copper-based compounds do not inhibit DRG neurons proteasome activity. On the contrary, the neurotoxic complex $[Au(PTA)_4]PF_6$, induces a significant inhibition of proteasome activity even at concentration lower than the IC_{50}. An increase in a-tubulin polymerization was also observed in $[Au(PTA)4]PF_6$ treated neurons while both copper-based compounds did not induce a significant microtubule stabilization.

Finally, PAT experiments demonstrated cell death increase and DNA damage in SR/$[Cu(PTA)_4]PF_6$ treated cells with respect to single treatments.

Our results, together with the low IC_{50} of the copper compounds compared to the one observed for cisplatin, suggest them as promising chemotherapeutic drugs suitable for PAT applications and provide support to further *in vivo* studies.

Poster n. 8

Multimodal experimental approach to the study of human neurological diseases

Chiorazzi A[1], Meregalli C[1], Canta A[1], Carozzi VA[1], Oggioni N[1], Rigolio R[1], Pozzi E[1,2], Monza L[1,3], Fumagalli G[1,2], Alberti P[1,2], Marmiroli P[1], Cavaletti G[1]

[1]School of Medicine and Surgery, Experimental Neurology Unit, University of Milano-Bicocca, Monza (MB), Italy; [2]PhD Program in Neuroscience, University of Milano-Bicocca; [3]PhD Program in Translational and Molecular Medicine, University of Milano-Bicocca

Peripheral neuropathies are a heterogeneous group of disorders characterized by alterations of the peripheral nerve, related to its structure and / or function. The causes of peripheral neuropathy are several, as well as the clinical features. Peripheral neuropathies can be clinically divided in polyneuropathy(of toxic, metabolic or inflammatory origin); mononeuropathies (mostly of traumatic origin); multineuropathies (mostly of vascular origin).

Chemotherapy-Induced Peripheral Neurotoxicity (CIPN) is a frequent, potentially severe and dose-limiting side-effect of cancer treatment. Having regard to its clinical relevance, preclinical and clinical studies have extensively investigated CIPN searching for effective strategies to limit its severity or to treat CIPN-related side effects on the peripheral nervous system.

Diabetes is a metabolic disease with increasing incidence worldwide and with important social and economic effects. The disease is characterized by hyperglycemia, which may be caused by an alteration of insulin production secondary to degeneration of pancreatic beta cells (type-1), or by an altered body response to insulin (type-2). Peripheral neuropathy is one of the most debilitating side effect during diabetes.

Basic researchers are involved in the preclinical investigation on CIPN and diabetic neuropathy in order to achieve better understanding of its pathogenesis necessary to identify suitable druggable targets for neuroprotective pharmacological intervention.

Increased life expectancy and improved survival rates are often associated with long-term treatment-related neurological complications that severely compromise the quality of life and the functional status of patients. For these reasons also the study of Central Nervous System (CNS) disorders is important. As an example, multiple sclerosis (MS) is the most frequent inflammatory demyelinating disorder of the CNS characterized by a heterogeneous clinical course. Animal models of MS, such as Experimental Autoimmune Encephalomyelitis (EAE), were developed to dissect the pathogenesis of this complex disease and to investigate the efficacy of potential new therapies and treatments.

Poster n. 9

The one-carbon metabolism disruption in anorexia nervosa of the restricting type

E. Conti,[1] L. Tremolizzo,[1] F. Corbetta,[2] M. Bomba,[2] F. Neri,[2] C. Ferrarese,[1] R. Nacinovich[2]

[1]Neurology and [2]Child and Adolescent Neuropsychiatry, School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza, Italy

Epigenetic dysfunctions are potential culprits in a complex disorder such as anorexia nervosa of the restricting type (ANr). DNA methylation is the best-studied epimutation, able to induce profound changes in gene expression without altering the underlying base sequence. Aim of this project consisted in approaching various targets within the one-carbon metabolism in AN, especially when considering that the methylation potential is granted by the dietary intake of methionine, an essential amino acid. Whole-blood global DNA methylation was significantly reduced in ANr patients and related to serum steroid hormone levels, while leptin was markedly downregulated. About a third of patients displayed increased serum homocysteine and significantly increased vitamin B12 levels correlating with disease severity. Beta-amyloid-40 was unchanged albeit correlating with homocysteine levels.

Our data suggest an impairment of the one-carbon metabolism at different levels and more work is needed for elucidating this matter. In particular, we are now considering the expression and activity of DNA methylating and demethylating enzymes in ANr patients.

Poster n. 10

Interaction between cholinergic, immune and inflammatory systems in Alzheimer's disease: the IMMUNAD project

E. Conti,[1] L. Tremolizzo,[1] D. Grana,[1,2] C.P. Zoia, [1] A. Arosio,[1] D. Cereda,[1] G. Stefanoni,[1] C. Villa,[1] R. Combi,[1] A. Aliprandi,[3] A. Salmaggi,[3] I. Appollonio, [1] C. Ferrarese[1]

[1]School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI), [2]PhD Program in Neuroscience, University of Milano-Bicocca, Monza; [3]"Manzoni" hospital, Lecco; Italy

According to the amyloid cascade hypothesis, beta-amyloid (Abeta) aggregation represents the core mechanism in the pathogenesis of Alzheimer's disease (AD). Neuroinflammation plays a key role as well, sustaining a response against Abeta aggregates, eventually damaging the surrounding neurons. The inflammatory reflex is a neural circuit regulating a response against Abeta aggregates. The afferent arc of this reflex is activated by cytokines and paralleled by the invasion of peripheral mononuclear cells into the CNS. The efferent arc is a vagal cholinergic pathway projecting onto a7nAChR expressed by macrophages and shutting off the inflammasome that promotes the maturation of inflammatory cytokines.

In this project, we focused on the role of TSPO, a biomarker of neuroinflammation in PET studies that marks activated microglia and regulates monocyte chemotaxis and blood-brain barrier crossing. Our ex vivo data on peripheral blood mononuclear cells (PBMC) show a mild decrease of TSPO expression in agitated AD patients, while the TSPO ligand, the diazepam binding inhibitor (DBI), is increased in AD patients versus controls. Chemotaxis assays were conducted both on THP-1 cells and on monocyte cultures obtained from patients, showing an increase of the Forward Migration Index following exposure to Abeta. An increase of a7nAChR mRNA levels was also shown in AD PBMC versus controls and preliminary data indicate that the dup-a7nAChR mRNA levels (a duplicated receptor expressing a dominant negative effect) are increased as well. Finally, anticholinesterase drug treatment (Donepezil) increases GATA-3 expression in AD PBMC, increasing the production of auto-antibodies directed against Abeta.

In conclusion, we report here data suggesting that: (1) the TSPO/DBI system might be dysregulated in AD and in agitated patients explaining a different propensity to chemotaxis toward Abeta deposition sites; (2) the cholinergic system in AD monocytes might be altered, potentially explaining differences in clinical expression and drug response of our patients.

Poster n. 11

Beta-amyloid-induced peripheral chemotactic response: what role in Alzheimer's disease?

D. Grana,[1,2] E. Conti,[1] G. Stefanoni,[1] C.P. Zoia,[1] M. Bossi,[1] A. Aliprandi,[3] I. Appollonio,[1] C. Ferrarese,[1] L. Tremolizzo,[1]

[1]School of Medicine and Surgery and Milan Center for Neuroscience (NeuroMI)
[2]PhD program in Neuroscience, University of Milano-Bicocca, Monza;
[3]"Manzoni" Hospital, Lecco; Italy

Introduction: The key event of Alzheimer's Disease (AD) is considered the aggregation and deposition of the β-amyloid peptide (Abeta). Peripheral mononuclear cells penetrate into the CNS to help resident microglia with Abeta removal, but eventually might represent a further source of damage supporting the inflammatory milieu.

Methods: Time-lapse microscopy was performed in Ibidi µ-slide chambers. Photos were taken every 4 min for 18 h and data were analysed using ImageJ tools. Boyden chambers had inserts with pores of 8 µm (for THP-1) or 5 µm (for human monocytes) and they were left at 37 °C for 6 h (THP-1) or 90 min (human monocytes). Subsequently, filters were fixed and exposed to a modified-Giemsa staining. We took 10 random fields photos per filter and we calculated the mean number of migrated cells for each treatment. The chemotactic index was expressed normalizing each treatment to vehicle-treated.

Results: Time-lapse microscopy recordings of THP-1 cells showed a clear tendency in migration when exposed to MCP-1 (10 ng/ml) or 125 nM Abeta ($p=0.03$). Boyden chambers experiments confirmed these results with a 2-fold increased migration rate following exposure to 125 pM Abeta. The same results were obtained on monocytes obtained from controls ($p<0.05$). Finally, monocytes obtained from AD patients displayed an increased propensity to migration in response to 125 pM Abeta versus matched controls (2-fold change, n=3). Lastly, we exposed monocytes to Donepezil (acetylcholinesterase inhibitor and nAchRa7 agonist) and methyllycaconitine (nAchRa7 antagonist): both prevented the increase in Abeta-induced monocyte migration ($p<0.03$).

Conclusions: All together these findings show clear chemoattractant properties of Abeta, suggesting that an alteration of monocyte migration is operative in AD patients. Further studies are needed to expand these preliminary results. Ongoing experiments are now considering the specific role of molecular players selectively involved in the chemotactic response such as CCR2 and TSPO.

Poster n. 12

Burning mouth syndrome: current concepts.

D. Lauritano, L. Zucchinelli, G. Greco, R. Borgia.

University of Milan-Bicocca. Department of Medicine and Surgery.

BMS is a relatively common chronic intraoral pain disorder in peri-/postmenopausal women, classically characterised by intractable burning that may be associated with dysgeusia and xerostomia. Its pathogenesis is related to a complex interplay of central and/or peripheral neural pain pathways. The aetiology of BMS is multifactorial and the secondary form of BMS should be diligently sought and treated. The aetiopathogenesis of BMS is complex: local, systemic, and/or psychological factors are involved in generating the painful oral burning symptoms. BMS has also been found to be associated with peripheral nerve damage and dopaminergic system disorders. A major challenge for the clinician is the treatment of BMS: identifying the possible causative factors is the first step, because treatment or elimination of these factors can lead to significant clinical improvement in the painful oral burning symptoms. However, this condition is often idiopathic; in this case, drug therapy should be instituted. Additionally, psychotherapy and behavioural feedback may help to eliminate BMS symptoms. In conclusion, further research is required to determine the true efficacy of current management strategies for patients with this disorder.

Poster n. 13

Tubulin polymerization and mitochondria transport involvement in Bortezomib peripheral neurotoxicity

Malacrida A.[1,2], Meregalli C.[2], Semperboni S.[1,2], Rodriguez-Menendez V.[2], Nicolini G.[2], Miloso M.[2], Cavaletti G.[2]

[1]PhD Program in Neuroscience, University of Milan Bicocca, Monza, Italy
[2]School of Medicine and Surgery, University of Milan Bicocca, Monza, Italy

Bortezomib (BTZ) is a proteasome inhibitor widely used for multiple myeloma treatment. BTZ induced peripheral neuropathy (BIPN) is the most frequent adverse effect. It is a disabling condition and often requires BTZ doses reduction or treatment total discontinuation. BIPN is due to degeneration of sensory neuron axons, but the mechanisms underlying this event are not yet clear. Homeostatic tubulin polymerization in microtubules is finely regulated by tubulin post-translational modification and by interactions with microtubule associated proteins (MAP). Axonal transport of mitochondria along microtubules is controlled by specialized motor and docking proteins. The correct mitochondrial movement along the axons is essential for metabolic state and synaptic activity. Alterations of these two processes could be associated with axonal degeneration and BIPN.

In this work, we evaluated *in vitro* the effects of different concentrations of BTZ on neurons isolated from adult mice dorsal root ganglia. Tubulin polymerization, acetylation and MAP2 expression were assessed by western blot analysis. With live imaging technique, we quantified the axonal transport of mitochondria, while the number of depolarized mitochondria was measured by loss in tetramethylrodamine methyl ester staining (TMRM).

72h BTZ treatment induced an increase in tubulin polymerization and in MAP2 expression levels. Tubulin acetylation were instead reduced by BTZ treatment. After 24h of BTZ treatment, we observed a dose-dependent reduction of mitochondria transport speed, but no alterations were observed on the direction of the movement. None of BTZ doses induced a change in the levels of mitochondria depolarization.

In summary, we have developed an *in vitro* model of BIPN which demonstrates that BTZ-induced transport impairments are already present before tubulin polymerization. The polymerization instead occurs after 72h of treatment and could be due to an increase in MAP2 activity, but not to acetylation post-translational modification. Continuous alterations of axonal transport of mitochondria and tubulin polymerization could play a critical role during BIPN onset.

Poster n. 14

Altered regulation of autophagy in fibroblasts from patients with sporadic and LRRK2 mutant Parkinson's disease

Daniele Marinig[1,2], Chiara Riva[1], Chiara Zoia[1], Carlo Ferrarese[1,3], Gessica Sala[1]

[1]Lab. of Neurobiology, School of Medicine and Surgery, Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca; [2]PhD program in Neuroscience, University of Milano-Bicocca; [3]Dept. of Neurology, San Gerardo Hospital, Monza, Italy.

Alterations of the intracellular catabolic mechanisms have already been observed in Parkinson's disease (PD). The evidence that the major constituent of Lewy bodies, alpha-synuclein, is selectively targeted by the chaperone-mediated autophagy (CMA) has raised great interest in the study of such mechanism in PD. Moreover, the PD-related mutation G2019S on the gene LRRK2 is able to block the multimerization of the lysosomal receptor lamp2A leading to accumulation of CMA substrates.

Aim: to study the effect of mutant LRRK2 on autophagy in primary fibroblast cell lines from G2019S mutant PD patients, obtained from "Parkinson Institute Biobank", Milan, as compared to sporadic PD patients (sPD) and healthy controls.

Methods: the protein expression of the two key-regulators of CMA, hsc70 and lamp2A, as well as the macroautophagy markers, LC3, beclin1 and p62 were measured in fibroblasts under basal conditions and after 24 hours exposure to autophagy inducers (serum starvation, rapamycin) or inhibitors (ammonium chloride, 3-methyladenine). The effects on autophagy parameters of the molecular chaperone drug ambroxol were tested.

Results: no difference in hsc70, lamp2A or beclin1 basal levels was observed in G2019S or sPD fibroblasts with respect to controls, while G2019S showed a significant increase of LC3-II and p62. Serum starvation and rapamycin resulted in a decrease of hsc70, while rapamycin significantly increased lamp2A levels in control fibroblasts. 3-methyladenine increased lamp2A levels in control fibroblasts, while ammonium chloride resulted in an accumulation of LC3-II and p62 in all cell lines. Ambroxol increased the levels of p62 and LC3-II in all cell lines.

Discussion: these findings indicate that mutant LRRK2 fibroblasts display a macroautophagy alteration under basal condition and that both sporadic and G2019S fibroblasts have a different susceptibility to autophagy modulators. Moreover, the ambroxol-induced accumulation of p62 and LC3-II indicates that this drug is involved in macroautophagy modulation.

Keywords: Autophagy, Parkinson's disease, LRRK2, fibroblasts

Poster n. 15

Biological and protective properties of Mesenchymal Stem Cells

Marianna Monfrini, Elisabetta Donzelli, Arianna Scuteri

Experimental Neurology Unit (ENU), School of Medicine and Surgery, University of Milano-Bicocca and Milan Center for Neuroscience (NeuroMi), Milan, Italy

Stem cells play fundamental roles in the body representing the endogenous precursors of many cells. Among adult stem cells, Mesenchymal Stem Cells (MSCs) obtained from bone marrow are the best characterized. They can be isolated basing on their adhesion to culture plastic, obtaining after few in vitro passages a homogenous population of cells with a fibroblastic-like morphology. Given their ability to differentiate *in vitro* into different cell lineages (mainly adipogenic, osteogenic and chondrogenic) they are useful both as *in vitro* models to study the differentiation processes and as therapeutic tools for tissue regeneration.

In our laboratory, we characterize and use MSCs derived from human, mouse and rat bone marrow. A single marker allowing for an unequivocal identification of the MSCs is still lacking, so for their identification and characterization, a set of surface markers and the differentiative capabilities into the major mesengenic cell types are evaluated.

Besides differentiation potential, MSCs can exert important protective functions due to their immunomodulatory properties and to the ability to support cellular survival. We demonstrated in several models their multi-sided protective potential. In fact, we observed that *in vitro* MSCs protect sensory neurons from both the effects of *in vitro* aging and the toxicity induced by chemotherapeutics. *In vitro* we showed also a protective effect towards pancreatic islets. *In vivo* in a diabetes model the co-transplantation of MSCs and pancreatic islets reduced the minimum number of pancreatic islets required to ensure glycemic control and ameliorated diabetic neuropathy symptoms. The administration of MSCs abrogated the relapse phase in rats with Chronic Relapsing-Remitting EAE, a model of the immune-mediated chronic disease Multiple Sclerosis (MS).

Taken together our findings show that MSCs represent both an interesting model to study biological key aspects of the differentiation processes, and promising therapeutic tools for both neurological and non-neurological diseases.

Poster n. 16

Incubation with low doses of oxaliplatin modifies the electrophysiological properties of dorsal root ganglion neurons

Valentina Pastori[1], Laura Monza[2], Guido Cavaletti[2], Andrea Becchetti[1], Marzia Lecchi[1]

[1] Department of Biotechnology and Bioscience and Milan Center for Neuroscience, University of Milano-Bicocca, Piazza della Scienza 2, 20126 Milano, Italy
[2] Experimental Neurology Unit and Milan Center for Neuroscience, School of Medicine and Surgery, University of Milano-Bicocca, Via Cadore 48, 20900 Monza, Italy

Platinum derivatives, which are used for the treatment of solid cancer, cause peripheral neuropathy as side effect. Oxaliplatin, a third generation platinum-based chemotherapy, is a principal component of the treatment for colorectal cancer. It frequently causes both acute and chronic neurotoxicity, respectively characterized by cold hyperalgesia and loss of sensory and motor function. Whereas acute neuropathy typically resolves within a week, increasing cumulative doses cause chronic neuropathy in 20-50% of patients. Oxaliplatin was shown to modulate Na^+ and K^+ channels in several types of neurons suggesting that acute neuropathy may be related to effects on ion channel properties. However very high concentrations were used in the experiments and the effects of lower doses, reached in the patients, are still to be elucidated.

Since dorsal root ganglia (DRG) neurons are the main target of cisplatin-induced peripheral neuropathy, we incubated differentiated F11 cells (rat DRG neurons x mouse neuroblastoma N18TG-2 cell line) for 24 and 48 hours in 7,5 uM oxaliplatin, and we investigated the electrophysiological properties by the patch-clamp technique. Compared to control differentiated cells, treated F11 showed depolarized resting membrane potentials, significantly decreased firing frequencies, and increased sodium current densities. On the contrary, potassium currents through delayed rectifier channels were not significantly affected by the treatment.

These results suggest that prolonged oxaliplatin-incubation affects DRG neuron electrical activity by depolarizing membrane potentials probably through a main action on voltage-dependent Na^+ channels.

Poster n. 17

Does music enhance memory? An electrical neuroimaging study

Proverbio AM[1], De Benedetto F.[1]

[1]Milan-Mi Center for Neuroscience, Department, of Psychology, University of Milano-Bicocca

The aim of the present study was to investigate how auditory background can affect concurrent cognitive processes, and in particular memory for faces. Previous studies show that music listening alters the way in which we perceive the world. Reported effects of background music go toward both facilitatory and interfering consequences on concurrent cognitive tasks.

An investigation using event related potentials (ERPs) was designed on to the evidence of a preliminary study, in order to investigate the neural mechanism of memory encoding for faces during listening to classical music (Čajkovskij), environmental sounds (rain) or silence. Participants were 15 healthy non-musician university students, engaged in an old/new memory task (involving the study of about 400 unknown faces, followed by a recognition phase). Faces were better recalled if learning occurred in silence or during listening to music, as compared to listening to rain.

Results indicate that listening to music enhances memory recollection of faces. Listening to music led to a better encoding of the visual stimulus (as compared to listening to rain), as indexed by an increased Anterior Negativity. A swLORETA analysis showed the main involvement of Superior Temporal Gyrus (STG) in integrating audio-visual information at about 400-500 after stimulus onset. Only music listening activated the right inferior frontal gyrus, that might be involved in the processing of familiar musical pieces.

Poster n. 18

Isolation and characterization of neutrophils from Multiple Sclerosis patients.

Roberta Rigolio[1,2], Maria Letizia Fusco[3], Alessio Malacrida[1,2,4], Martina Cogo[3], Maria Foti[2,5], Federica Avezza[1], Guido Cavaletti[1,2,3,4]

[1]Experimental Neurology Unit (ENU), School of Medicine and Surgery, University of Milano-Bicocca; [2]Milan Center for Neuroscience (NeuroMi), Milan; [3]Neuroimmunology Clinic, S. Gerardo Hospital, Monza, Italy; [4] PhD program in Neuroscience, University of Milano-Bicocca, Monza, Italy; [5]School of Medicine and Surgery, University of Milano-Bicocca

For a long time Neutrophils have been mainly considered short life circulating blood cells which are the first defence line towards pathogens and danger signals.

In order to perform this defence role, Neutrophils are naturally prone-to be activated-cells and this characteristic make them a type of challenging cells to be isolated and managed for different kind of analysis.

On the other hand, in the last few years their reputation has been gradually evolved. In fact, they have been demonstrated not to be so short term life and to be able to react shaping the adaptive immune response through different signals.

Multiple Sclerosis is a bona fide autoimmune disease of the Central Nervous System (CNS) mainly involving adaptive immunity, while little attention has been generally paid to the innate immunity. Nevertheless, in the last few years Neutrophils have been tentatively studied showing increased life span together with activation state in MS patient as well as to be relevant for the T cells infiltrating the CNS in the animal model of MS.

The final aim of our work is to define the epigenetic signature in neutrophils, i.e. differential miRNA expression, that results to be relevant in MS patients and which can contribute to dissect the neutrophil role and contribution in MS inflammatory compartment.

In order to run accurate epigenetic analysis we need to work with pure neutrophils whose activation status corresponds to that detected in blood stream.

Here the preliminary results on neutrophils isolation from MS patients and age-matched healthy control subjects are presented.

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Poster n. 19

In vitro and ex vivo study of the autophagy-lysosome pathway in neurodegenerative diseases

Gessica Sala[1], Chiara Riva[1], Daniele Marinig[1,2], Riccardo Sirtori[1], Laura Brighina[3], Chiara Zoia[1], Alessandro Arosio[1], Lucio Tremolizzo[1,3], Carlo Ferrarese[1,3]

[1]Lab. of Neurobiology, School of Medicine and Surgery, NeuroMI Milan Center for Neuroscience, University of Milano-Bicocca; [2]PhD program in Neuroscience, University of Milano-Bicocca; [3]Dept. of Neurology, San Gerardo Hospital, Monza, Italy.

Many neurodegenerative diseases including Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) belong to the large category of proteinopathies, conditions characterized by the presence of proteinaceous inclusions within and/or outside the degenerating neurons. The identification of such aggregates supports the view that misfolded proteins represent a basic requirement for the neurodegenerative process and provided input to verify the existence of possible dysfunctions of the biological systems influencing neuronal protein homeostasis. Considering the post-mitotic nature of neurons, a proper activity of the intracellular protein degradation systems appears to be crucial to ensure the maintenance of cell homeostasis and to prevent the onset of the neurodegeneration. The ubiquitin-proteasome system (UPS) and the autophagy-lysosome pathway (ALP), mainly represented by macroautophagy and chaperone-mediated autophagy (CMA), are the two major protein catabolic systems that cooperate in maintaining the protein quality control. Alterations of both UPS and ALP have been reported in patients with PD, AD and ALS, supporting a role for autophagy dysfunction in neurodegeneration.

We used different pharmacological or genetic in vitro models of neurodegenerative diseases to study the molecular mechanisms involved in ALP dysfunction and to identify new possible neuroprotective compounds. The deriving results could lead to a better understanding of the pathogenesis of neurodegenerative diseases and to the identification of new therapeutic targets. The in vitro study was paralleled by an ex vivo study in cells obtained from patients (lymphomonocytes or fibroblasts) with PD, AD and ALS aimed at investigating the existence of possible systemic autophagy alterations useful to identify new peripheral disease biomarkers for early diagnosis, personalized therapy, or monitoring of drug efficacy in clinical trials.

Poster n. 20

Compound heterozygosis in the *SCN1A* gene in a family showing GEFS+ and IGE

C. Villa[a], G. Sancini[a], N. Binini[b], D. Grioni[c], R. Dal Magro[a], M. Toselli[b], R. Combi[a]

[a]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[b]Dept. of Biology and Biotechnology "L. Spallanzani", University of Pavia, Pavia, Italy
[c]Dept. of Pediatric Neurology, San Gerardo Hospital, Monza, Italy

Background: mutations in the Nav1.1 Na+ channel encoding gene (*SCN1A*) causing either loss or gain of function have been frequently found in patients affected by genetic epilepsy with febrile seizures plus (GEFS+).

Aims: to search for mutations in *SCN1A* in a family affected by different genetic epilepsies and to investigate their possible functional effects.

Methods: genomic DNA was isolated from peripheral blood. All exons and the exon-intron boundaries of *SCN1A* gene were amplified and then sequenced. Functional studies were performed by *in vitro* mutagenesis, cloning, transient transfections and electrophysiological analyses.

Results: a compound heterozygosis for two missense mutations (p.Arg1525Gln and p. Thr297Ile) was identified in the family members affected by GEFS+ or IGE (idiopathic generalized epilepsy). The p.Arg1525Gln mutation was not previously reported yet and was predicted to be pathological, whereas the p.Thr297Ile was already identified in patients showing SMEI (severe myoclonic epilepsy in infancy) even if *in silico* prediction tools suggested that it is benign. Functional studies revealed that the Nav1.1 channels expressing either the p.Arg1525Gln or both mutations were characterized by a significant shift in the activation curve towards more positive potentials whereas no significant differences were observed in both activation and inactivation kinetics of Nav1.1 Na+ channels expressing only the p.Thr297Ile.

Conclusions: the p.Arg1525Gln represents a novel mutation in *SCN1A* gene altering the channel properties and having a dominant effect on the p.Thr297Ile that is instead benign even if it was already reported in more severe cases of epilepsy.

Poster n. 21

Study of the role of the CRH in Autosomal dominant nocturnal frontal lobe epilepsy

C. Villa[a], B. Volpe[a], E. Chisci[a], L. Ferini-Strambi[b], R. Giovannoni[a], R. Combi[a]

[a]School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[b]Dept. of Clinical Neurosciences, Sleep Disorders Center, University Vita-Salute San Raffaele, Milan, Italy

Background: autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an autosomal dominant childhood-onset focal epilepsy characterized by the presence of clusters of nocturnal frontal lobe seizures, whose underlying genes have been partially discovered. In a large cohort of ADNFLE Italian patients, our group reported an unknown missense mutation (p.Pro30Arg) in the coding region of the *corticotropin releasing hormone* (*CRH*) gene. Through *in vitro* studies, we demonstrated that this mutation causes an abnormal retention of the immature form of the protein into both the endoplasmic reticulum and Golgi apparatus, thus reducing the level of mature-CRH promptly secreted by the cells in response to a stressful signal.

Aims: a direct role of *CRH* mutations in NFLE pathogenesis has still to be demonstrated, so more complex models are necessary to verify this hypothesis. To reach this goal, we recently plan to develop a novel transgenic mouse model carrying the p.Pro30Arg using the CRISPR/Cas9 genome editing technique. Our approach consisted in substituting the *Chr* murine coding sequence with the human orthologous one, through the co-injection in cultured cells of a donor vector with a plasmid encoding both newly designed and *Crh* targeted single guide RNAs (sgRNAs) and the Cas9.

Results: we generated a donor vector containing the human *CRH* coding sequence flanked by left and right murine homology regions of *Crh* gene, allowing the CRISPR/Cas9-mediated site-specific integration into the first intron of *Chr* locus of the human sequence. Four sgRNAs were designed using the CRISPR design tool (http:// with the highest quality scores and the lowest number of off-targets sites and cloned into a Cas9 expression plasmid pSpCas9 in order to test the feasibility of this CRISPR/Cas9-mediated gene modification *in vitro*.

Conclusions: this study will allow to evaluate the role of CRH in the ADNFLE onset and to unravel the underlying pathogenetic mechanism.

Poster n. 22

SiTraNeD: signal transduction in peripheral models of neurodegenerative diseases

C.P. Zoia[1], C. Bazzini[1], E. Conti[1], L. Tremolizzo[1,2], C. Ferrarese[1,2]

[1]School of Medicine and Surgery, Milan Center for Neuroscience (NeuroMI), University of Milano-Bicocca, Monza; [2]Dept. of Neurology, San Gerardo Hospital, Monza, Italy

Soluble oligomers are the toxic species of beta Amyloid (Aβ), and they have been shown to produce cognitive deficits in the absence of plaques (Gandy, et al., 2010) in patients with Alzheimer's Disease (AD). These oligomers lead to neurodegeneration and memory impairment via critical cell signaling pathways, i.e. Kinases (Petersen et al., 2013). They are used extensively to transmit signals and regulate complex processes in cells. Phosphorylation of molecules can enhance or inhibit their activity and modulate their ability to interact with other molecules. The addition and removal of phosphoryl groups provides the cell with a means of control because various kinases can respond to different conditions or signals. Mutations in kinases that lead to a loss-of-function or gain-of-function can cause cancer and disease in humans, including neurological disorders. Kinases are critical in metabolism, cell signaling, protein regulation (i.e. metabolism and catabolism, proteasome system and autophagy pathway), cellular transport, secretory processes, and many other cellular pathways. In primary fibroblasts and/or lympho-monocytes from AD patients and aged healthy subjects (HC), molecular mechanisms involvement in Aβ-oligomers toxicity have been investigated.

We observed Ras and GSK3 signaling pathways involvement in AD severity, APP metabolism and molecular regulation of glutamate transport, in fibroblasts from sporadic AD patients, from prodromal to severe cases, with respect to Parkinson's Disease and HC cells.

We are investigating the involvement of kinase signaling in apoptosis, mitophagy and autophagy regulation in AD cells. Studies about modulation of Aβ-oligomers toxicity, in presence of drugs or natural molecules, are in progress.

These signaling pathways may help to clarify the disease pathogenic mechanisms and progression, to obtain potential molecular targets, and to check specific disease drugs.